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CHAPTER 24

Natural Products (Secondary Metabolites)

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Introduction

Natural products have primary ecological functions.

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as **secondary metabolites,** often are differentially distributed among limited taxonomic groups within the plant kingdom. Their functions, many of which remain unknown, are being elucidated with increasing frequency. The **primary metabolites,** in contrast, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic roles that are essential and usually evident.

Although noted for the complexity of their chemical structures and biosynthetic pathways, **natural products** have been widely perceived as biologically insignificant and have historically received little attention from most plant biologists. Organic chemists, however, have long been interested in these novel phytochemicals and have investigated their chemical properties extensively since the 1850s. Studies of natural products stimulated development of the separation techniques, spectroscopic approaches to structure elucidation, and synthetic methodologies that now constitute the foundation of contemporary organic chemistry. Interest in natural products was not purely academic but rather was prompted by their great utility as dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and drugs. Recognition of the biological properties of myriad natural products has fueled the current focus of this field, namely, the search for new drugs, antibiotics, insecticides, and herbicides. Importantly, this growing appreciation of the highly diverse biological effects produced by natural products has prompted a reevaluation of the possible roles these compounds play in plants, especially in the context of ecological interactions. As illustrated in this chapter, many of these compounds now have been shown to have important

adaptive significance in protection against herbivory and microbial infection, as attractants for pollinators and seed-dispersing animals, and as allelopathic agents (allelochemicals that influence competition among plant species). These ecological functions affect plant survival profoundly, and we think it reasonable to adopt the less pejorative term "plant natural products" to describe secondary plant metabolites that act primarily on other species.

The boundary between primary and secondary metabolism is blurred.

Based on their biosynthetic origins, plant natural products can be divided into three major groups: the terpenoids, the alkaloids, and the phenylpropanoids and allied phenolic compounds. All terpenoids, including both primary metabolites and more than 25,000 secondary compounds, are derived from the five-carbon precursor isopentenyl diphosphate (IPP). The 12,000 or so known alkaloids, which contain one or more nitrogen atoms, are biosynthesized principally from amino acids. The 8000 or so phenolic compounds are formed by way of either the shikimic acid pathway or the malonate/acetate pathway.

Primary and secondary metabolites cannot readily be distinguished on the basis of precursor molecules, chemical structures, or biosynthetic origins. For example, both primary and secondary metabolites are found among the diterpenes (C_{20}) and triterpenes (C_{30}). In the diterpene series, both kaurenoic acid and abietic acid are formed by a very similar sequence of related enzymatic reactions (Fig. 24.1); the former is an essential intermediate in the synthesis of gibberellins, i.e., growth hormones found in all plants (see Chapter 17), whereas the latter is a resin component largely restricted to members of the Fabaceae and Pinaceae. Similarly, the essential amino acid proline is classified as a primary metabolite, whereas the C_6 analog pipecolic acid is considered an alkaloid and thus a natural product (Fig. 24.1). Even lignin, the essential structural polymer of wood and second only to cellulose as the most abundant organic substance in plants, is considered a natural product rather than a primary metabolite.

In the absence of a valid distinction based on either structure or biochemistry, we return to a functional definition, with primary products participating in nutrition and essential metabolic processes inside the plant, and natural (secondary) products influencing ecological interactions between the plant and its environment. In this chapter, we provide an overview of the biosynthesis of the major classes of plant natural products, emphasizing the origins of their structural diversity, as well as their physiological functions, human uses, and potential biotechnological applications.

24.1 Terpenoids

Terpenoids perhaps are the most structurally varied class of plant natural products. The name terpenoid, or terpene, derives from the fact that the first members of the class were

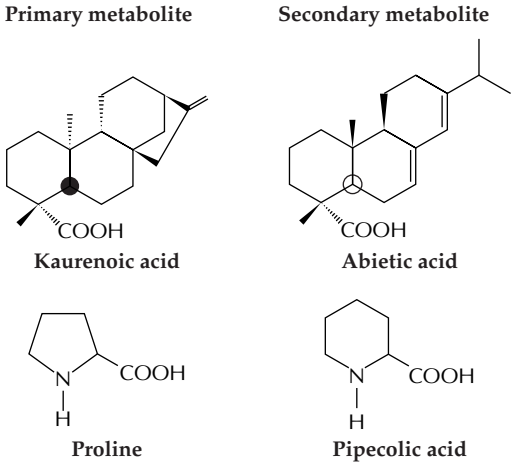


Figure 24.1
Kaurenoic acid and proline are primary metabolites, whereas the closely related compounds abietic acid and pipecolic acid are considered secondary metabolites.

isolated from turpentine ("terpentin" in German). All terpenoids are derived by repetitive fusion of branched five-carbon units based on isopentane skeleton. These monomers generally are referred to as isoprene units because thermal decomposition of many terpenoid substances yields the alkene gas isoprene as a product (Fig. 24.2, upper panel) and because suitable chemical conditions can induce isoprene to polymerize in multiples of five carbons, generating numerous terpenoid skeletons. For these reasons, the terpenoids are often called isoprenoids, although researchers have known for well over 100 years that isoprene itself is not the biological precursor of this family of metabolites.

24.1.1 Terpenoids are classified by the number of five-carbon units they contain.

The five-carbon (isoprene) units that make up the terpenoids are often joined in a "head-to-tail" fashion, but head-to-head fusions are also common, and some products are formed by head-to-middle fusions (Fig. 24.2, lower panel). Accordingly, and because extensive structural modifications with carbon–carbon bond rearrangements can occur, tracing the original pattern of isoprene units is sometimes difficult.

The smallest terpenes contain a single isoprene unit; as a group, they are named **hemiterpenes** (half-terpenes). The best known hemiterpene is isoprene itself, a volatile product released from photosynthetically active tissues. The enzyme isoprene synthase is present in the leaf plastids of numerous C_3 plant species, but the metabolic rationale for the light-dependent production of isoprene is unknown (acclimation to high temperatures has been suggested). Estimated annual foliar emissions of isoprene are quite substantial (5×10^8 metric tons of carbon), and the gas is a principal reactant in the NOx radical–induced formation of tropospheric ozone (see Chapter 22, Fig. 22.37).

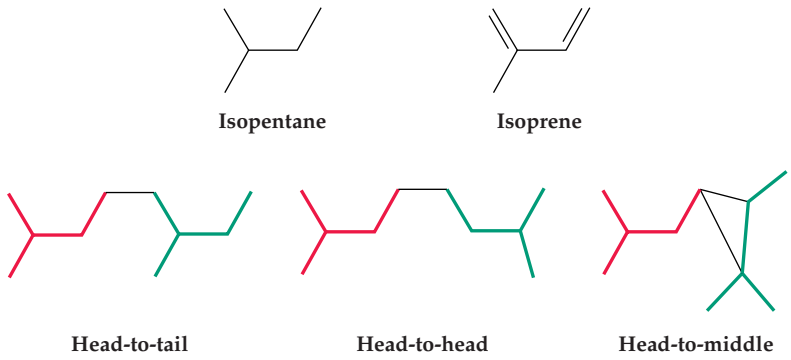


Figure 24.2
Terpenes are synthesized from C_5 units. The upper panel shows the structures of the isopentane skeleton and isoprene gas. The lower panel shows how different patterns of isoprene unit assembly yield a variety of different structures. For example, the triterpene squalene is formed by head-to-head fusion of two molecules of farnesyl diphosphate (FPP), which itself is the product of the head-to-tail fusion of isopentenyl diphosphate (IPP) and geranyl diphosphate (GPP) (see Fig. 24.7). The monoterpene pyrethrin I (see Fig. 24.10) results from a head-to-middle fusion of two C_5 units.

C_{10} terpenoids, although they consist of two isoprene units, are called **monoterpenes;** as the first terpenoids isolated from turpentine in the 1850s, they were considered to be the base unit from which the subsequent nomenclature is derived. The monoterpenes are best known as components of the volatile essences of flowers and of the essential oils of herbs and spices, in which they make up as much as 5% of plant dry weight. Monoterpenes are isolated by either distillation or extraction and find considerable industrial use in flavors and perfumes.

The terpenoids that derive from three isoprene units contain 15 carbon atoms and are known as **sesquiterpenes** (i.e., one and one-half terpenes). Like monoterpenes, many sesquiterpenes are found in essential oils. In addition, numerous sesquiterpenoids act as phytoalexins, antibiotic compounds produced by plants in response to microbial challenge, and as antifeedants that discourage opportunistic herbivory. Although the plant hormone abscisic acid is structurally a sesquiterpene, its C_{15} precursor, xanthoxin, is not synthesized directly from three isoprene units but rather is produced by asymmetric cleavage of a C_{40} carotenoid (see Chapter 17).

The **diterpenes,** which contain 20 carbons (four C_5 units), include phytol (the hydrophobic side chain of chlorophyll), the gibberellin hormones, the resin acids of conifer and legume species, phytoalexins, and a host of pharmacologically important metabolites, including taxol, an anticancer agent found at very low concentrations (0.01% dry weight) in yew bark, and forskolin, a compound used to treat glaucoma. Some gibberellins have only 19 carbon atoms and are considered norditerpenoids since they have lost 1 carbon through a metabolic cleavage reaction (see Chapter 17).

The **triterpenes,** which contain 30 carbon atoms, are generated by the head-to-head joining of two C_{15} chains, each of which constitutes three isoprene units joined head-to-tail. This large class of molecules includes the brassinosteroids (see Chapter 17), the phytosterol membrane components (see Chapter 1), certain phytoalexins, various toxins and feeding deterrents, and components of surface waxes, such as oleanolic acid of grapes.

The most prevalent **tetraterpenes** (40 carbons, eight isoprene units) are the carotenoid accessory pigments which perform essential functions in photosynthesis (see Chapter 12). The **polyterpenes,** those containing more than eight isoprene units, include the prenylated quinone electron carriers (plastoquinone and ubiquinone; see Chapters 12 and 14), long-chain polyprenols involved in sugar transfer reactions (e.g., dolichol; see Chapters 1 and 4), and enormously long polymers such as rubber (average molecular mass greater than 10^6 Da), often found in latex.

Natural products of mixed biosynthetic origins that are partially derived from terpenoids are often called **meroterpenes.** For example, both cytokinins (see Chapter 17) and numerous phenylpropanoid compounds contain C_5 isoprenoid side chains. Certain alkaloids, including the anticancer drugs vincristine and vinblastine, contain terpenoid fragments in their structures (see Fig. 24.34). Additionally, some modified proteins include a 15- or 20-carbon terpenoid side chain that anchors the protein in a membrane (see Chapter 1).

24.1.2 A diverse array of terpenoid compounds is synthesized by various conserved reaction mechanisms.

At the turn of the 20th century, structural investigations of many terpenoids led Otto Wallach to formulate the "isoprene rule," which postulated that most terpenoids could be constructed hypothetically by repetitively joining isoprene units. This principle provided the first conceptual framework for a common structural relationship among terpenoid natural products (Box 24.1). Wallach's idea was refined in the 1930s, when Leopold Ruzicka formulated the "biogenetic isoprene rule," emphasizing mechanistic considerations of terpenoid synthesis in terms of electrophilic elongations, cyclizations, and rearrangements. This hypothesis ignores the precise character of the biological precursors and assumes only that they are "isoprenoid" in structure. As a working model for terpenoid biosynthesis, the biogenetic isoprene rule has proved essentially correct.

Despite great diversity in form and function, the terpenoids are unified in their common biosynthetic origin. The biosynthesis of all terpenoids from simple, primary metabolites can be divided into four overall steps: *(a)* synthesis of the fundamental precursor IPP; *(b)* repetitive additions of IPP to form a series of prenyl diphosphate homologs, which serve as the immediate precursors of the different classes of terpenoids; *(c)* elaboration of these allylic prenyl diphosphates by specific terpenoid synthases to yield terpenoid skeletons; and *(d)* secondary enzymatic modifications to the skeletons (largely redox reactions) to give rise to the functional properties and great chemical diversity of this family of natural products.

24.2 Synthesis of IPP

24.2.1 Biosynthesis of terpenoids is compartmentalized, as is production of the terpenoid precursor IPP.

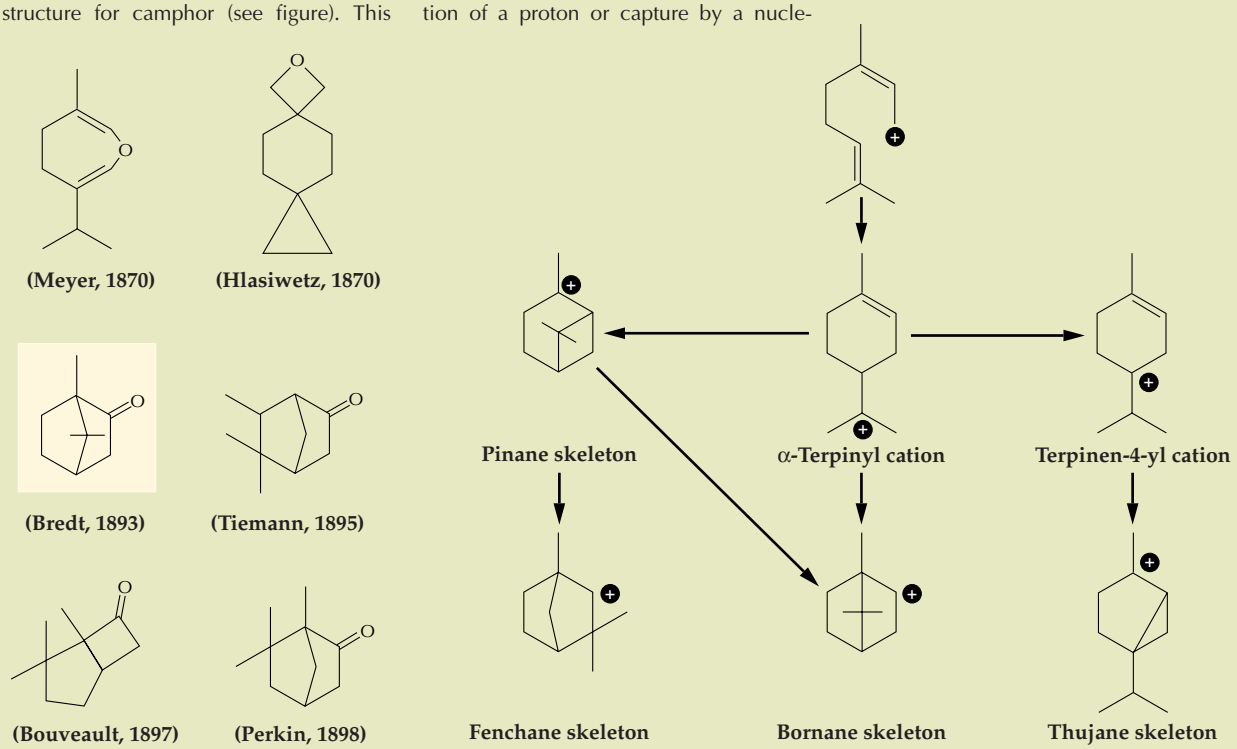
Although terpenoid biosynthesis in plants, animals, and microorganisms involves similar classes of enzymes, important differences exist among these processes. In particular, plants produce a much wider variety of terpenoids than do either animals or microbes, a difference reflected in the complex organization of plant terpenoid biosynthesis at the tissue, cellular, subcellular, and genetic levels. The production of large quantities of terpenoid natural products as well as their subsequent accumulation, emission, or secretion is almost always associated with the presence of anatomically highly specialized structures. The glandular trichomes (Fig. 24.3A, B) and secretory cavities of leaves (Fig. 24.3C) and the glandular epiderms of flower petals generate and store or emit terpenoid essential oils that are important because they encourage pollination by insects. The resin ducts and blisters of conifer species

Box 24.1

Early investigators formulated rules for identifying and naming isoprenoid structures.

In the late 1800s, chemists struggled to define the structures of the monoterpenes. The mixed results achieved by these efforts are illustrated by the numerous structures proposed for camphor ($C_{10}H_{16}O$; see structures at left of figure, which include the names of the proposers and the dates proposed). Chromatographic purification techniques and spectroscopic methods for structure elucidation were not available to these early chemists, who relied on the preparation of crystalline derivatives to assess purity and on chemical degradation studies to determine structures. Systematic study of the monoterpenes led the German chemist Otto Wallach to recognize that many terpenoid compounds might be constructed by joining isoprene units, generally in a repetitive head-to-tail fashion, as in Bredt's correct proposed structure for camphor (see figure). This concept, known as the **isoprene rule,** earned Wallach the Nobel Prize in Chemistry in 1910.

By the 1930s, faced with a bewildering array of terpenoid substances, Leopold Ruzicka and his contemporaries sought to develop a unifying principle that could rationalize the natural occurrence of all of the known terpenoids, even those that did not strictly fit Wallach's isoprene rule. Ruzicka's ingenious solution to the problem was to focus on reaction mechanisms and ignore the precise character of the biological precursor, assuming only that it had a terpenoid structure during reaction. He hypothesized the involvement of electrophilic reactions that generated carbocationic intermediates, which underwent subsequent C_5 addition, cyclization, and in some cases skeletal rearrangement before elimination of a proton or capture by a nucleophile to yield the observed terpenoid products. This proposal, which Ruzicka called the **biogenetic isoprene rule,** can be stated simply: A compound is "isoprenoid" if it is derived biologically from an "isoprenoid" precursor, with or without rearrangements. Ruzicka's concept differs from Wallach's in its emphasis on biochemical origin rather than structure. The great strength of the biogenetic isoprene rule lay in its use of mechanistic considerations to classify the bulk of known terpenoids, including structures that did not strictly follow Wallach's isoprene rule. Application of the biogenetic isoprene rule to the origin of several of the common monoterpene skeletons is illustrated in the right panel of the figure (note the bornane skeleton from which camphor is derived). Ruzicka was awarded the Nobel Prize in Chemistry in 1939.



(Fig. 24.3D) produce and accumulate a defensive resin consisting of turpentine (monoterpene olefins) and rosin (diterpenoid resin acids). Triterpenoid surface waxes are formed and excreted from specialized epidermis, and laticifers produce certain triterpenes and polyterpenes such as rubber. These specialized structures sequester natural products away from sensitive metabolic processes and thereby prevent autotoxicity. Most structures of this type are nonphotosynthetic and must therefore rely on adjacent cells to supply

the carbon and energy needed to drive terpenoid biosynthesis.

A more fundamental, and perhaps universal, feature of the organization of terpenoid metabolism exists at the subcellular level. The sesquiterpenes (C_{15}), triterpenes (C_{30}), and polyterpenes appear to be produced in the cytosolic and endoplasmic reticulum (ER) compartments, whereas isoprene, the monoterpenes (C_{10}), diterpenes (C_{20}), tetraterpenes (C_{40}), and certain prenylated quinones originate largely, if not exclusively, in the plastids. The evidence now indicates that the biosynthetic pathways for the formation of the fundamental precursor IPP differ markedly in these compartments, with the classical acetate/mevalonate pathway being active in the cytosol and ER and the glyceraldehyde phosphate/pyruvate pathway operating in the plastids. Regulation of these dual pathways may be difficult to assess, given that plastids may supply IPP to the cytosol for use in biosynthesis, and vice versa. Mitochondria, a third compartment, may generate the ubiquinone prenyl group by the acetate/mevalonate pathway, although little is known about the capability of these organelles for terpenoid biosynthesis.

24.2.2 Hydroxymethylglutaryl-CoA reductase, an enzyme in the acetate/mevalonate pathway, is highly regulated.

The basic enzymology of IPP biosynthesis by way of the acetate/mevalonate pathway is widely accepted (Fig. 24.4). This cytosolic IPP pathway involves the two-step condensation of three molecules of acetyl-CoA catalyzed by thiolase and hydroxymethylglutaryl-CoA synthase. The resulting product, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), is subsequently reduced by

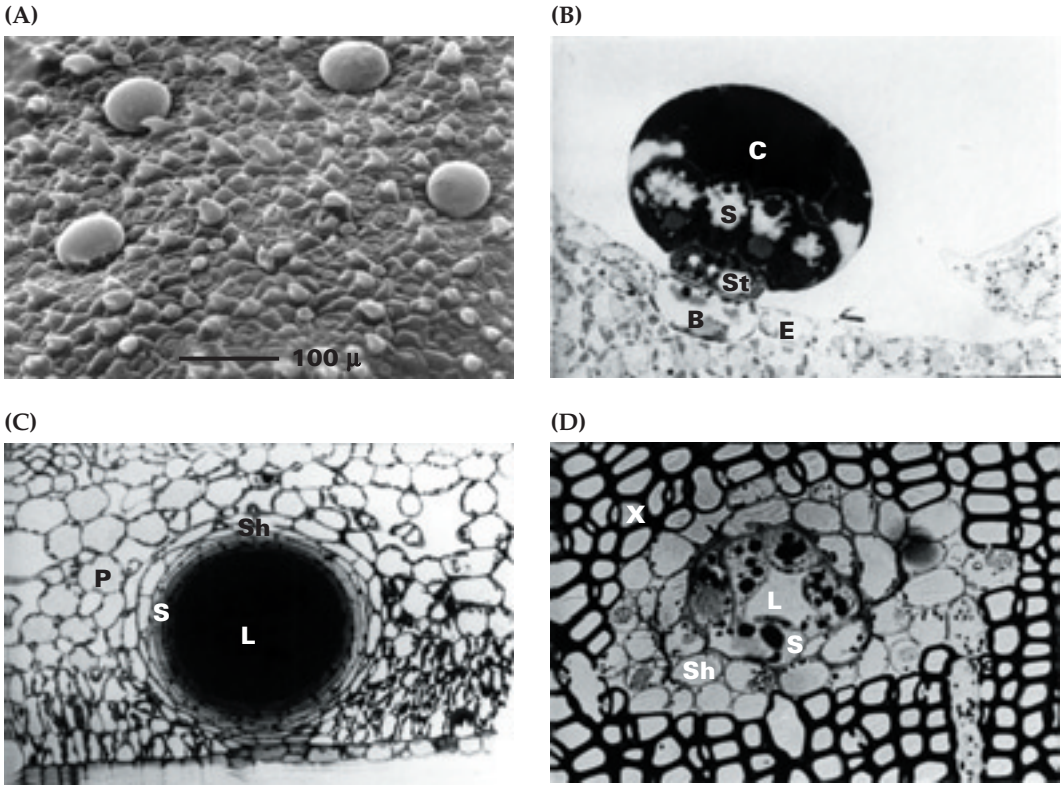


Figure 24.3
(A) Scanning electron micrograph of the leaf surface of thyme. The round structures are peltate glandular trichomes, in which monoterpenes and sesquiterpenes are synthesized. (B) Light micrograph of a glandular trichome from spearmint, shown in longitudinal section. C, subcuticular space; S, secretory cells; St, stalk; B, basal cell; E, epidermal cell. (C) Light micrograph of a secretory cavity in a lemon leaf, shown in cross-section. L, lumen; Sh, sheath cells; P, parenchyma cell. (D) Light micrograph of a resin duct in wood of Jeffrey pine, shown in cross-section. X, secondary xylem.

HMG-CoA reductase in two coupled reactions that form mevalonic acid. Two sequential ATP-dependent phosphorylations of mevalonic acid and a subsequent phosphorylation/elimination-assisted decarboxylation yield IPP.

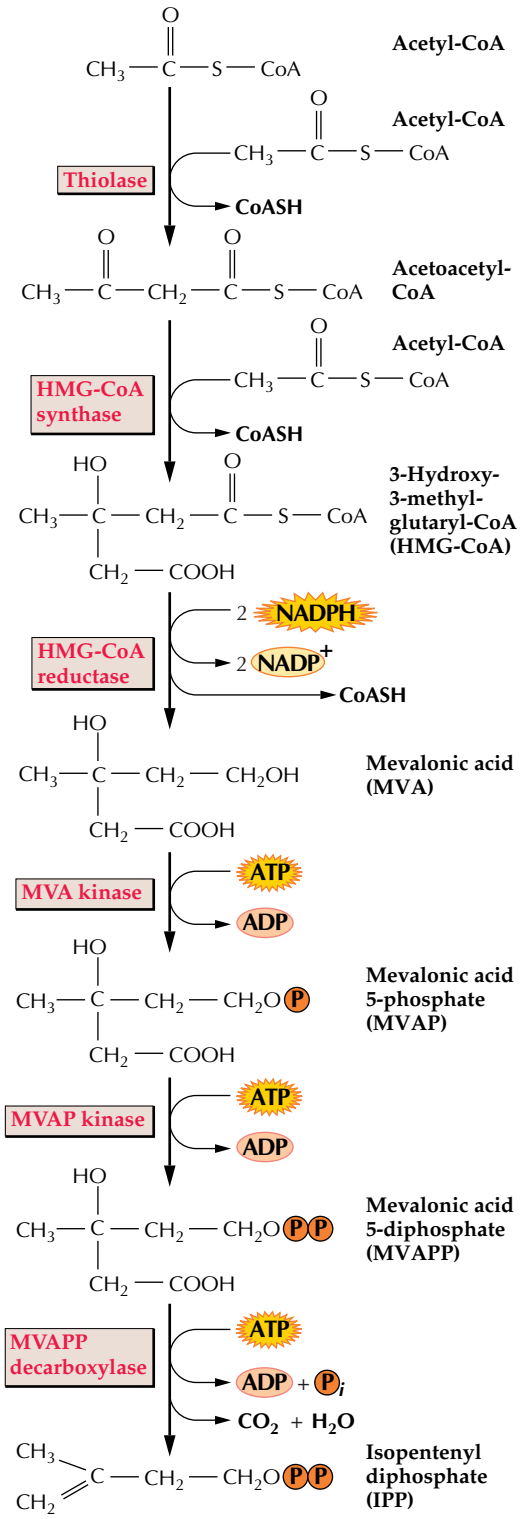


Figure 24.4
The acetate/mevalonate pathway for the formation of IPP, the basic five-carbon unit of terpenoid biosynthesis. Synthesis of each IPP unit requires three molecules of acetyl-CoA.

HMG-CoA reductase is one of the most highly regulated enzymes in animals, being largely responsible for the control of cholesterol biosynthesis. Accumulated evidence indicates that the plant enzyme, which is located in the ER membrane, is also highly regulated. In many cases, small gene families, each containing multiple members, encode this reductase. These gene families are expressed in complex patterns, with individual genes exhibiting constitutive, tissue- or development-specific, or hormone-inducible expression. Specific HMG-CoA reductase genes can be induced by wounding or pathogen infection. The activity of HMG-CoA reductase may be subject to posttranslational regulation, for example, by a protein kinase cascade that phosphorylates and thereby inactivates the enzyme. Allosteric modulation probably also plays a regulatory role. Proteolytic degradation of HMG-CoA reductase protein and the rate of turnover of the corresponding mRNA transcripts may also influence enzyme activity. Researchers have not arrived at a unified scheme that explains how the various mechanisms that regulate HMG-CoA reductase facilitate the production of different terpenoid families. The precise biochemical controls that influence activity have been difficult to assess in vitro because the enzyme is associated with the ER membrane. A model proposed to rationalize the selective participation of HMG-CoA reductase in the biosynthesis of different mevalonate-derived terpenoids is shown in Figure 24.5.

24.2.3 In plastids, IPP is synthesized from pyruvate and glyceraldehyde 3-phosphate.

The plastid-localized route to IPP involves a different pathway, demonstrated in green algae and many eubacteria as well as plants. In this pathway, pyruvate reacts with thiamine pyrophosphate (TPP) to yield a two-carbon fragment, hydroxyethyl-TPP, which condenses with glyceraldehyde 3-phosphate (see Chapter 12, Fig. 12.41, for similar TPP-mediated C_2 transfers catalyzed by transketolase). TPP is released to form a five-carbon intermediate, 1-deoxy-D-xylulose 5-phosphate, which is rearranged and reduced to form 2-*C*-methyl-D-erythritol 4-phosphate and subsequently transformed to yield IPP (Fig. 24.6, upper pathway). The

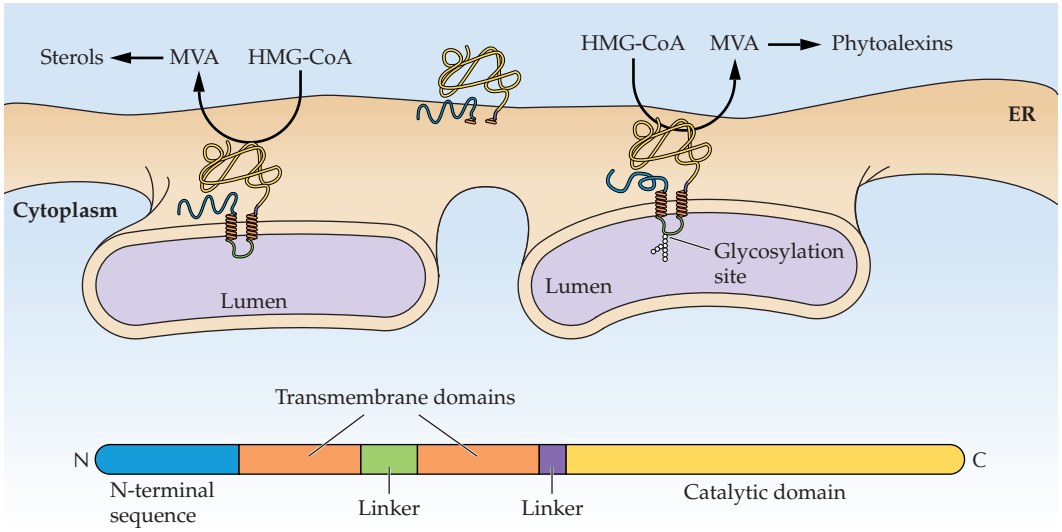


Figure 24.5
Model for the membrane topology of HMG-CoA reductase (HMGR). The protein includes a highly variable hydrophilic N-terminal sequence (blue), a conserved membrane anchor (orange), a highly variable linker sequence (green and purple), and a highly conserved, cytosol-exposed, C-terminal catalytic domain (yellow). Isoforms of HMGR that are associated with elicitor-induced synthesis of sesquiterpenoid phytoalexins contain an N-linked glycosylation site exposed to the ER lumen. Differences in N-terminal sequences and extent of glycosylation may affect targeting of HMGR to various ER domains and to other organelles of the endomembrane system (see Chapters 1 and 4). ER, endoplasmic reticulum; MVA, mevalonic acid.

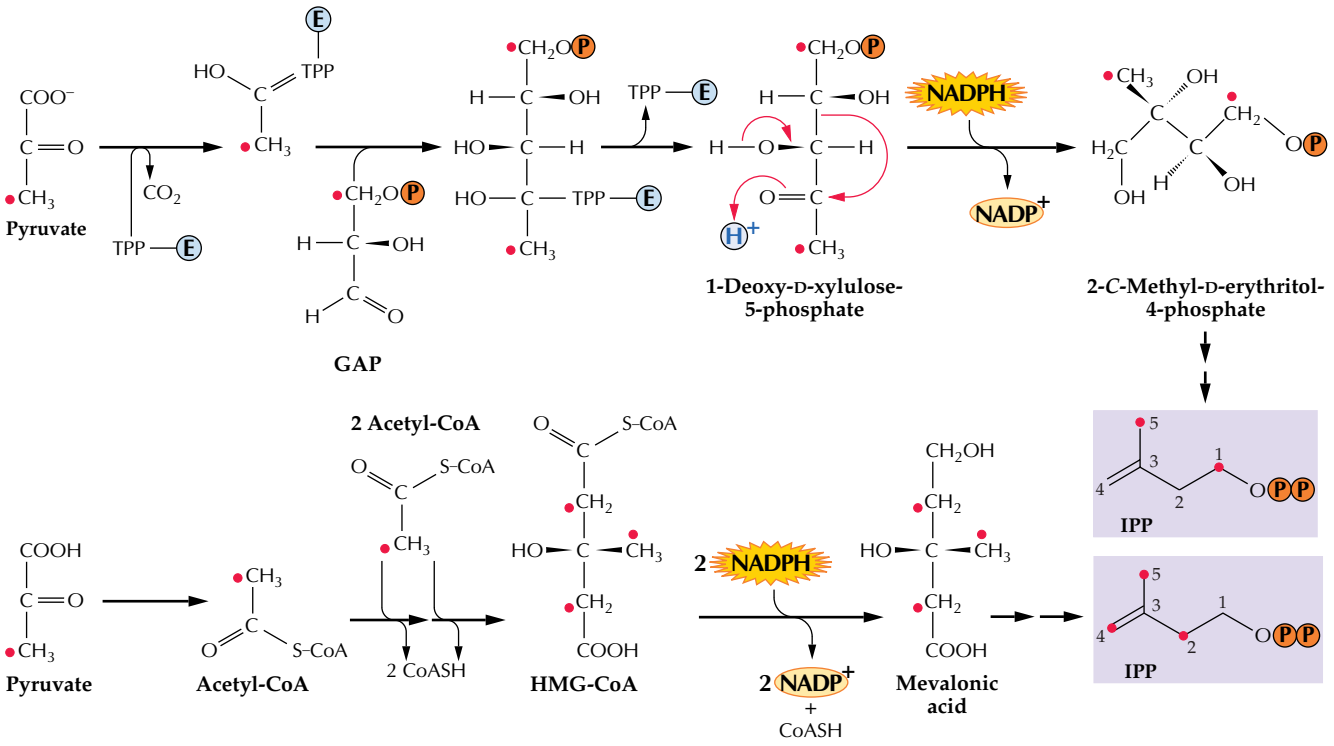


Figure 24.6
Feeding studies distinguish two pathways of isoprenoid biosynthesis. When glucose isotopically labeled at C-1 is transformed by glycolytic enzymes and pyruvate dehydrogenase, the label subsequently appears in the methyl groups of pyruvate and acetyl-CoA and in C-3 of glyceraldehyde 3-phosphate (GAP). IPP synthesized from labeled pyruvate and GAP by the plastid-localized pathway will be labeled at C-1 and C-5 (upper panel), whereas IPP formed from labeled acetyl-CoA by way of the cytosolic acetate/mevalonate pathway will be labeled at C-2, C-4, and C-5 (lower panel).

discovery of this new pathway for IPP formation in plastids suggests that these organelles, presumed to have originated as prokaryotic endosymbionts, have retained the bacterial machinery for the production of this key intermediate of terpenoid biosynthesis.

The details of the glyceraldehyde 3-phosphate/pyruvate pathway and the enzymes responsible have not yet been fully defined. However, products of the two IPP biosynthesis pathways can be easily distinguished in experiments that utilize [1-^{13}C]glucose as a precursor for terpenoid biosynthesis. Nuclear magnetic resonance (NMR) spectroscopy (see Chapter 2, Box 2.2) can be used to determine the ^{13}C-labeling pattern of each isoprene unit in a terpenoid compound, allowing researchers to infer the labeling pattern of the corresponding IPP units (Fig. 24.6).

24.3 Prenyltransferase and terpene synthase reactions

Prenyltransferase enzymes generate the allylic diphosphate esters geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP). Reactions that these compounds undergo (often cyclizations), which are catalyzed by terpene synthases, yield a wide variety of terpenoid compounds. Both prenyltransferases and terpene synthases utilize electrophilic reaction mechanisms involving carbocationic intermediates, a feature of terpenoid biochemistry. Enzymes in both groups share similar properties and contain conserved sequence elements, such as an aspartate-rich DDxxD motif involved in substrate binding, which may participate in initiating divalent metal ion–dependent ionizations.

24.3.1 Repetitive addition of C_5 units is carried out by prenyltransferases.

IPP is utilized in a sequence of elongation reactions to produce a series of prenyl diphosphate homologs, which serve as the immediate precursors of the different families of terpenoids (Fig. 24.7). Isomerization of IPP by IPP isomerase produces the allylic isomer dimethylallyl diphosphate (DMAPP),

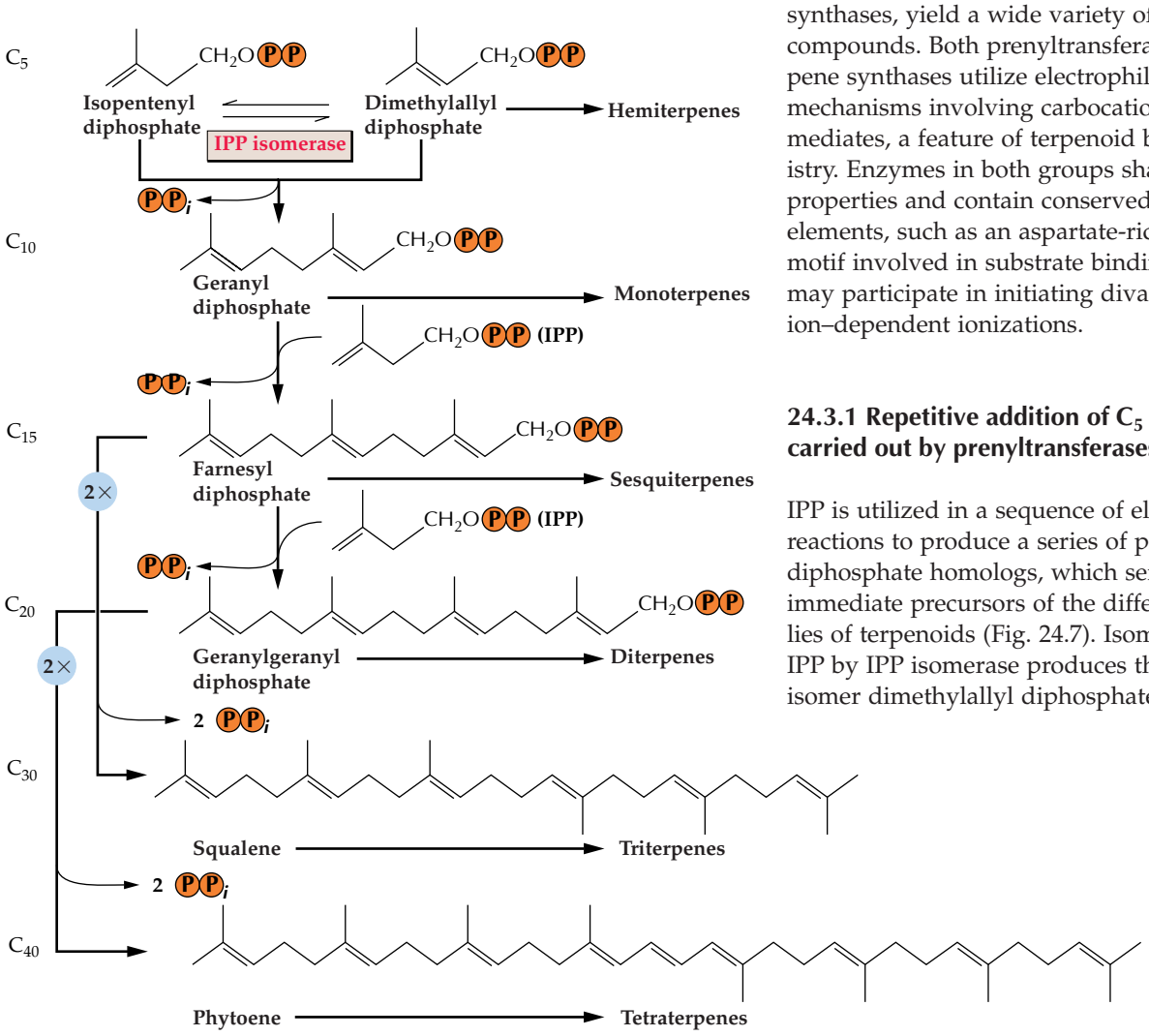


Figure 24.7
The major subclasses of terpenoids are biosynthesized from the basic five-carbon unit, IPP, and from the initial prenyl (allylic) diphosphate, dimethylallyl diphosphate, which is formed by isomerization of IPP. In reactions catalyzed by prenyltransferases, monoterpenes (C_{10}), sesquiterpenes (C_{15}), and diterpenes (C_{20}) are derived from the corresponding intermediates by sequential head-to-tail addition of C_5 units. Triterpenes (C_{30}) are formed from two C_{15} (farnesyl) units joined head-to-head, and tetraterpenes (C_{40}) are formed from two C_{20} (geranylgeranyl) units joined head-to-head.

which is considered the first prenyl diphosphate. Because DMAPP and related prenyl diphosphates contain an allylic double bond, these compounds can be ionized to generate resonance-stabilized carbocations. Once formed, a carbocation intermediate of *n* carbons can react with IPP to yield a prenyl diphosphate homolog containing *n* + 5 carbons. Thus, the reactive primer DMAPP undergoes condensation with IPP to yield the C_{10} intermediate GPP. Repetition of the reaction cycle by addition of one or two molecules of IPP provides FPP (C_{15}) or GGPP (C_{20}), respectively. Each prenyl homolog in the series arises as an allylic diphosphate ester that can ionize to form a resonance-stabilized carbocation and condense with IPP in another round of elongation (Fig. 24.8).

The electrophilic elongation reactions that yield C_{10}, C_{15}, and C_{20} prenyl diphosphates are catalyzed by enzymes known collectively as prenyltransferases. GPP, FPP, and GGPP are each formed by specific prenyltransferases named for their products (e.g., farnesyl diphosphate synthase). The new allylic double bond introduced in the course of the prenyltransferase reaction is commonly in the trans geometry, although this is not always the case: The transferase responsible for rubber biosynthesis introduces cis-double bonds, which are responsible for the elasticity of that polymer. Prenylation reactions are not limited to elongations involving IPP; the same basic carbocationic mechanism permits the attachment of prenyl side chains to atoms of carbon, oxygen, nitrogen, or sulfur in a wide range of nonterpenoid compounds, including proteins.

The most extensively studied prenyltransferase, farnesyl diphosphate synthase, plays an important role in cholesterol biosynthesis in humans. Farnesyl diphosphate synthases from microbes, plants, and animals exhibit high sequence conservation. The first enzyme of the terpenoid pathway to be structurally defined is recombinant avian farnesyl diphosphate synthase, the crystal structure of which has been determined.

24.3.2 The enzyme limonene synthase is a model for monoterpene synthase action.

The families of enzymes responsible for the formation of terpenoids from GPP, FPP, and GGPP are known as monoterpene, sesquiterpene, and diterpene synthases, respectively. These synthases use the corresponding prenyl diphosphates as substrates to form the enormous diversity of carbon skeletons characteristic of terpenoids. Most terpenoids are cyclic, and many contain multiple ring systems, the basic structures of which are determined by the highly specific synthases. Terpenoid synthases that produce cyclic products are also referred to as "cyclases," although examples of synthases producing acyclic products are also known.

A diverse array of monoterpene synthases has been isolated from essential oil-producing angiosperm species and resin-producing gymnosperms. These enzymes use a common mechanism in which ionization

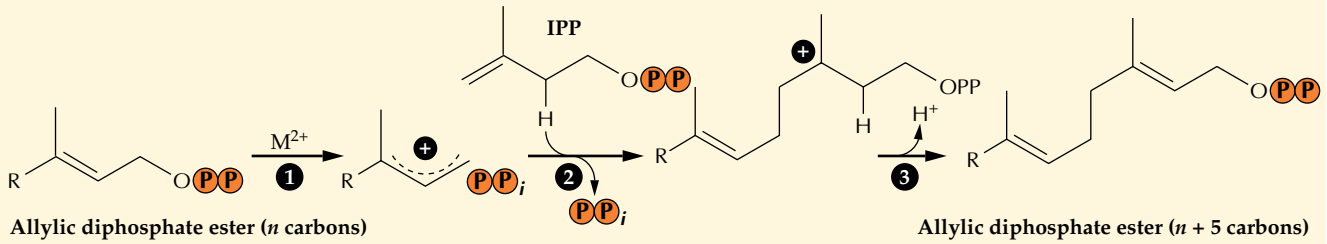
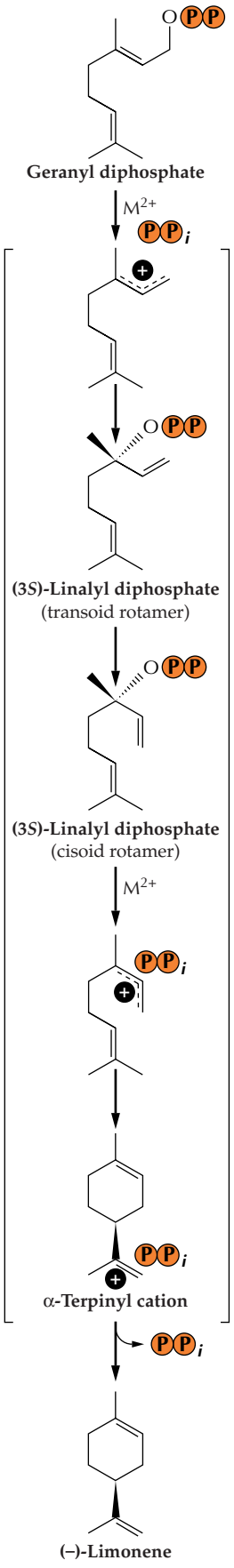


Figure 24.8
The prenyltransferase reaction.



of GPP leads initially to the tertiary allylic isomer linalyl diphosphate (LPP; Fig. 24.9). This isomerization step is required because GPP cannot cyclize directly, given the presence of the trans-double bond. Ionization of the enzyme-bound LPP intermediate promotes cyclization to a six-membered ring carbocation (the α-terpinyl cation), which may undergo additional electrophilic cyclizations, hydride shifts, or other rearrangements before the reaction is terminated by deprotonation of the carbocation or capture by a nucleophile (e.g., water). Variations on this simple mechanistic scheme, involving subsequent reactions of the α-terpinyl carbocation, are responsible for the enzymatic formation of most monoterpene skeletons (see Box 24.1).

The simplest monoterpene synthase reaction is catalyzed by limonene synthase, a useful model for all terpenoid cyclizations (Fig. 24.9). The electrophilic mechanism of action used by limonene synthase can be viewed as an intramolecular equivalent of the prenyltransferase reaction (see Fig. 24.8). Synthases that produce acyclic olefin products (e.g., myrcene) and bicyclic products (α- and β-pinene) from GPP are also known, as are enzymes that transform GPP to oxygenated derivatives such as 1,8-cineole and bornyl diphosphate (Fig. 24.10), the precursor of camphor (see Box 24.1).

An interesting feature of the monoterpene synthases is the ability of these enzymes to produce more than one product; for example, pinene synthase from several plant sources produces both α- and β-pinene. The pinenes are among the most common monoterpenes produced by plants and are principal components of turpentine of the pines, spruces, and firs. The compounds are toxic to bark beetles and their pathogenic fungal symbionts, which cause serious damage to conifer species worldwide. Many conifers respond to bark beetle infestation by up-regulating synthesis of monoterpenes, a process analogous to the production of antimicrobial phytoalexins, when under pathogen attack (Fig. 24.11). Other monoterpenes have quite different functions. Thus, linalool (see Fig. 24.10) and 1,8-cineole emitted by flowers serve as attractants for pollinators, including bees, moths, and bats. 1,8-Cineole and camphor act as foliar feeding deterrents to large herbivores such as hares and deer and also may provide a competitive advantage to several angiosperm species as allelopathic agents that inhibit germination of the seeds of other species.

Exceptions to the general pattern of head-to-tail joining of isoprene units seen in limonene, the pinenes, and most other monoterpenes derived from GPP are the "irregular" monoterpenes. An example of this type is the family of insecticidal monoterpene esters called pyrethrins, found in *Chrysanthemum* and *Tanacetum* species. These monoterpenoids, which exhibit a head-to-middle joining of C_5 units, have gained wide use as commercial insecticides because of their negligible toxicity to mammals and their limited persistence in the environment (see Fig. 24.10).

Figure 24.9
(–)-Limonene synthase catalyzes the simplest of all terpenoid cyclizations and serves as a model for this reaction type. Ionization of GPP, assisted by divalent metal ions, provides the delocalized carbocation–diphosphate anion pair, which collapses to form the enzyme-bound tertiary allylic intermediate linalyl diphosphate. This required isomerization step, followed by rotation about the C-2–C-3 single bond, overcomes the original stereochemical impediment to direct cyclization of the geranyl precursor. A subsequent assisted ionization of the linalyl diphosphate ester promotes an anti-endo-cyclization to the α-terpinyl cation, which undergoes deprotonation to form limonene, a compound now thought to be an important cancer preventive in humans.

24.3.3 Sesquiterpene synthases generate several compounds that function in plant defense.

The electrophilic mechanisms for the formation of the C_{15} sesquiterpenes from FPP closely resemble those used by monoterpene synthases, although the increased flexibility of the 15-carbon farnesyl chain eliminates the need for the preliminary isomerization step except in forming cyclohexanoid-type compounds. The additional C_5 unit and double bond of FPP also permit formation of a greater number of skeletal structures than in the monoterpene series. The best known sesquiterpene synthase of plant origin is *epi*-aristolochene synthase from tobacco, the crystal structure of which has been determined (Fig. 24.12). This enzyme cyclizes FPP

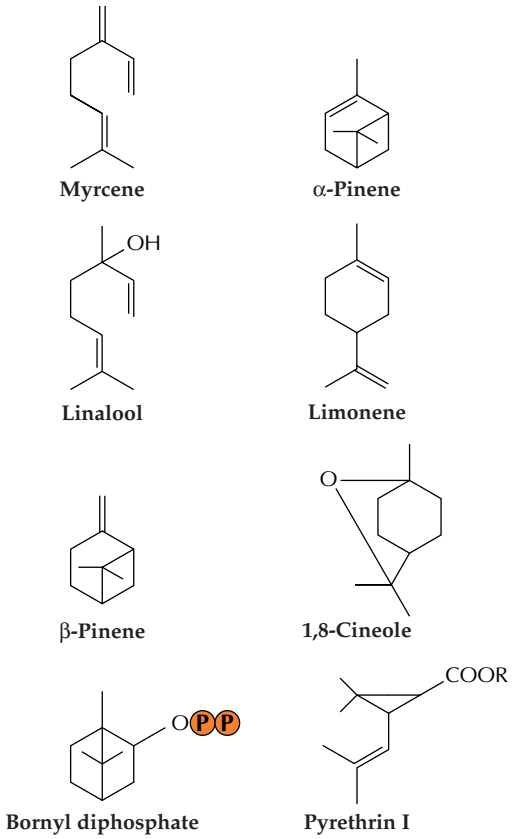


Figure 24.10
Structures of monoterpenes, including insecticidal compounds (α- and β-pinene, pyrethrin), pollinator attractants (linalool and 1,8-cineole), and antiherbivory agents (1,8-cineole).

Figure 24.11
Mass attack by mountain pine beetles on a lodgepole pine (*Pinus contorta*) bole. Each white spot on the trunk represents a beetle entry point at which resin has been secreted. This tree has survived the attack because turpentine production was sufficient to kill all of the bark beetles, which have been "pitched out" by resin outflow. On evaporation of the turpentine and exposure to air, the diterpenoid resin acids form a solid plug that seals the wound.

and catalyzes a methyl migration to yield the olefin precursor of the phytoalexin capsidiol, which is elicited by pathogen attack. Vetispiradiene synthase from potato provides the olefin precursor of the phytoalexin lubimin in this species, whereas δ-cadinene synthase from cotton yields the olefin precursor of the important defense compound gossypol, the latter being currently studied as a possible male contraceptive (Fig. 24.13). Some sesquiterpene synthases involved in the production of conifer resin are capable of individually producing more than 25 different olefins.

24.3.4 Diterpene synthases catalyze two distinct types of cyclization reactions.

Two fundamentally different types of enzymatic cyclization reactions occur in the transformation of GGPP to diterpenes (Fig. 24.14). The first resembles the reactions catalyzed by monoterpene and sesquiterpene synthases, in which the cyclization involves ionization of the diphosphate ester and attack of the resulting carbocation on an interior double bond of the geranylgeranyl substrate. An example of this type is casbene synthase, which is responsible for production of the phytoalexin casbene in castor bean. Taxadiene synthase from yew species uses a mechanistically similar, but more complex, cyclization to produce the tricyclic olefin precursor of taxol.

Abietadiene synthase from grand fir exemplifies the second type of cyclization, in which protonation of the terminal double bond to generate a carbonium ion initiates the first cyclization to a bicyclic intermediate (labdadienyl diphosphate, also known as copalyl diphosphate). Ionization of the diphosphate ester promotes the second cyclization step to give the tricyclic olefin product, abietadiene; a single enzyme catalyzes both

cyclization steps. Oxidation of a methyl group yields abietic acid (see Fig. 24.1), one of the most common diterpenoid resin acids of conifers and important for wound sealing in these species. Fossilization of this resin produces amber.

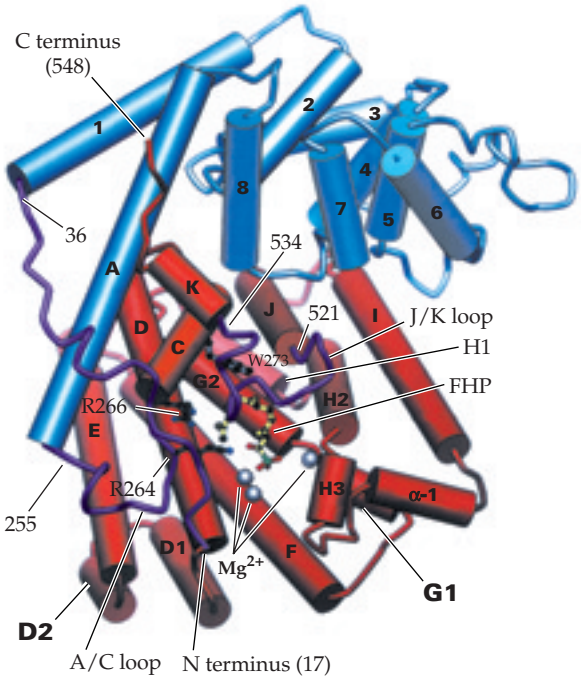


Figure 24.12
Schematic view of *epi*-aristolochene synthase complexed with the substrate analog farnesyl hydroxyphosphonate (FHP). Blue rods represent α-helices in the N-terminal domain; red rods represent α-helices in the C-terminal domain. Loop regions shown in purple are disordered in the native enzyme. Three Mg^{2+} ions and arginines 264 and 266 are involved in the initial steps of the reaction and are labeled near the entrance to the active site. Tryptophan 273, which serves as the general base in the final deprotonation step, is shown within the hydrophobic active-site pocket. The substrate analog FHP is shown in ball-and-stick representation, highlighted with yellow carbon–carbon bonds. Naming of helices in the C-terminal domain corresponds to the convention used for FPP synthase.

24.3.5 Triterpene synthesis proceeds from squalene, tetraterpene synthesis from phytoene.

Before cyclization can occur in the triterpene (C_{30}) series, two molecules of FPP (C_{15}) are first joined in a head-to-head condensation to produce squalene (see Fig. 24.7). The catalyst, squalene synthase, is a prenyltransferase that catalyzes a complex series of cationic rearrangements to accomplish the chemically difficult chore of joining the C-1 carbons of two farnesyl residues. Squalene is usually oxidized to form the 2,3-epoxide, oxidosqualene, and then cyclized in a protonation-initiated reaction to produce, for example,

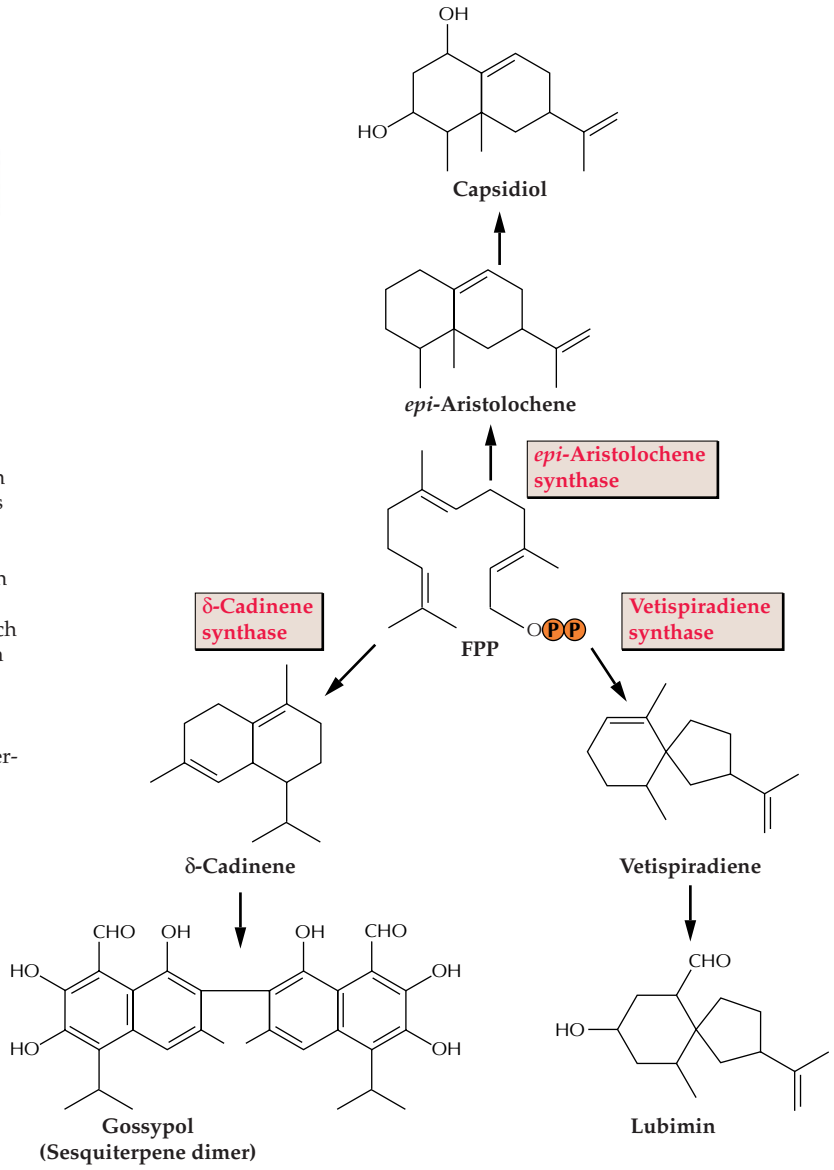


Figure 24.13
Structures of sesquiterpenes biosynthetically derived from FPP. The end products function in plant defense.

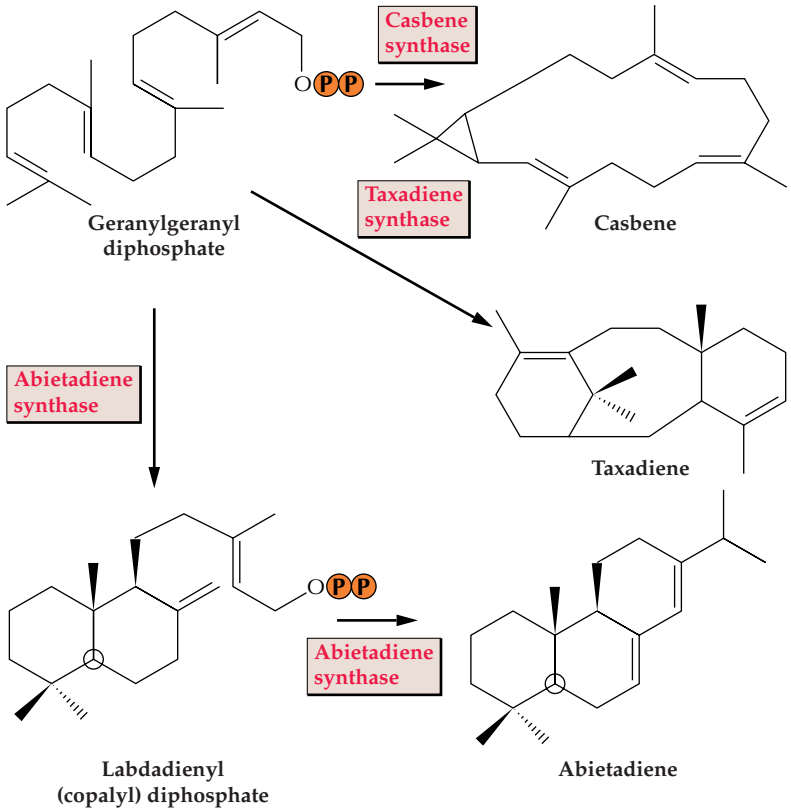


Figure 24.14
Cyclization of GGPP to form the diterpenes casbene, taxadiene, and abietadiene. Cyclization can proceed by one of two distinct mechanims, only one of which yields the intermediate labdadienyl (copalyl) diphosphate.

the common sterol cycloartenol (Fig. 24.15), a precursor of many other phytosterols and brassinosteroids (see Chapter 17). Several alternative modes of cyclization in the triterpene series are also known, such as that leading to the pentacyclic compound β-amyrin, the precursor of oleanolic acid found in the surface wax of several fruits (Fig. 24.15). Preliminary evidence suggests that sesquiterpene biosynthesis and triterpene biosynthesis (both of which utilize cytosolic FPP as a precursor) are reciprocally regulated during the induced defense responses, such that production of C_{15} defensive compounds is enhanced and C_{30} synthesis is repressed.

The tetraterpenes (C_{40}) are produced by joining two molecules of GGPP in head-to-head fashion to produce phytoene, in a manner analogous to the formation of squalene (see Fig. 24.7). The reaction is catalyzed by phytoene synthase, which deploys a mechanism very similar to that of squalene synthase. A series of desaturation steps precedes cyclization in the tetraterpene (carotenoid) series, usually involving formation of six-membered (ionone) rings at the chain termini to produce, for example, β-carotene from lycopene (see Chapter 12, Fig. 12.7).

24.4 Modification of terpenoid skeletons

Subsequent modifications of the basic parent skeletons produced by the terpenoid synthases are responsible for generating the myriad different terpenoids produced by plants. These secondary transformations most commonly involve oxidation, reduction, isomerization, and conjugation reactions, which impart functional properties to the terpenoid molecules. Several oxygenated derivatives of parent terpenoids have already been described in this chapter, including capsidiol, lubimin, gossypol, abietic acid, and oleanolic acid.

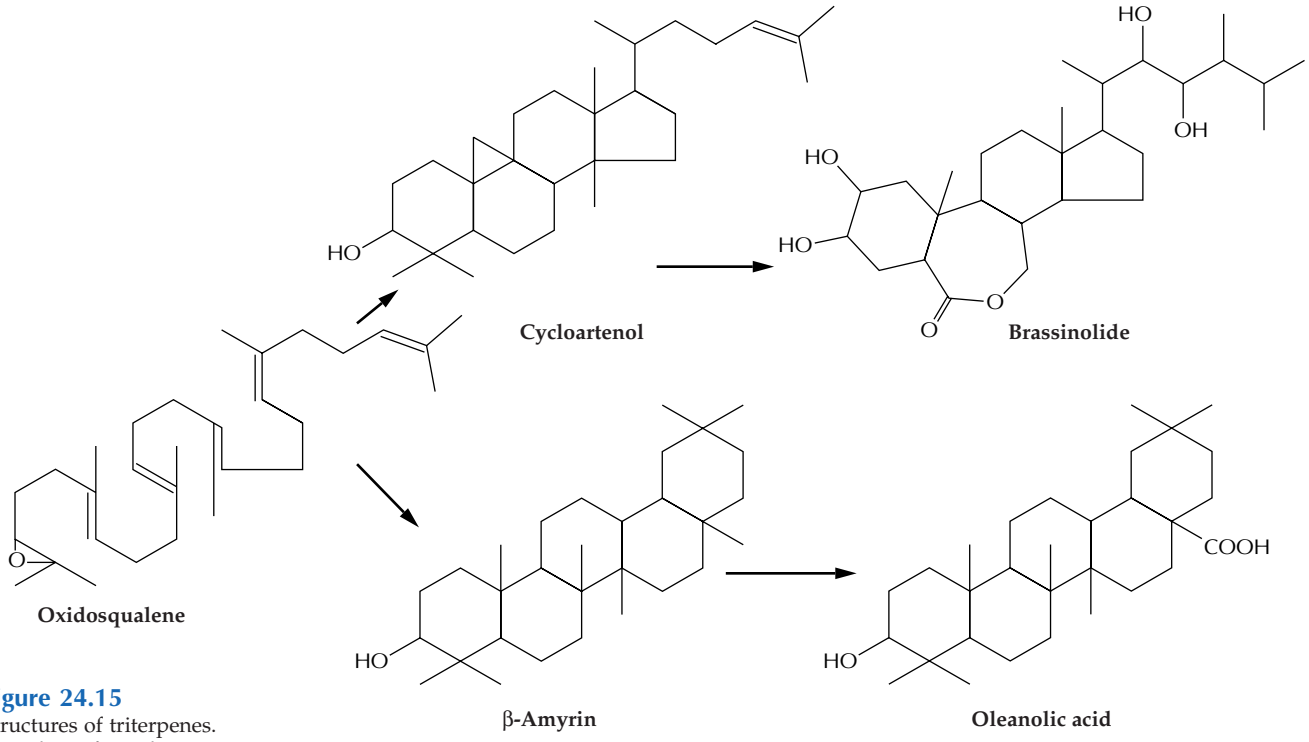


Figure 24.15
Structures of triterpenes. This class of squalene-derived products includes brassinosteroid regulators of plant growth and surface wax components.

Many of the hydroxylations or epoxidations involved in introducing oxygen atoms into the terpenoid skeletons are performed by cytochrome P450 mixed-function oxidases. Because these reactions are not unique to terpenoid biosynthesis, this section will not focus on specific enzyme types but rather on the general role of secondary transformations as the wellspring of diversity in terpenoid structure and function.

24.4.1 The conversion of (–)-limonene to (–)-menthol in peppermint and carvone in spearmint illustrates the biochemistry of terpenoid modification.

The principal and characteristic essential oil components of peppermint (*Mentha piperita*) and spearmint (*M. spicata*) are produced by secondary enzymatic transformations of (–)-limonene (Fig. 24.16). In peppermint, a microsomal cytochrome P450 limonene 3-hydroxylase introduces an oxygen atom at an allylic position to produce (–)-*trans*-isopiperitenol. A soluble $NADP^+$-dependent dehydrogenase oxidizes the alcohol to a ketone, (–)-isopiperitenone, thereby activating the adjacent double bond for reduction by a soluble, NADPH-dependent, regiospecific reductase to produce (+)-*cis*-isopulegone. An isomerase next moves the remaining double bond into conjugation with the carbonyl group, yielding (+)-pulegone. One regiospecific, NADPH-dependent, stereoselective reductase converts (–)-pulegone to either (+)-isomenthone or the predominant species, (–)-menthone. Similar reductases produce the menthol isomers from these ketones. (–)-Menthol greatly predominates among the menthol isomers (constituting as much as 40% of the essential oil) and is the component primarily responsible for the characteristic flavor and cooling sensation of peppermint. The menthol isomers are often found as acetate esters, formed by the action of an acetyl CoA-dependent acetyltransferase. The menthol and menthyl acetate content of peppermint oil glands increases with leaf maturity. Environmental factors greatly influence oil composition. Water stress and warm night growth conditions both promote the accumulation of the more-oxidized pathway intermediates such as (+)-pulegone.

The pathway in spearmint is much shorter. In this instance, a cytochrome P450 limonene 6-hydroxylase specifically introduces oxygen at the alternative allylic position to produce (–)-*trans*-carveol, which is oxidized to (–)-carvone by the soluble

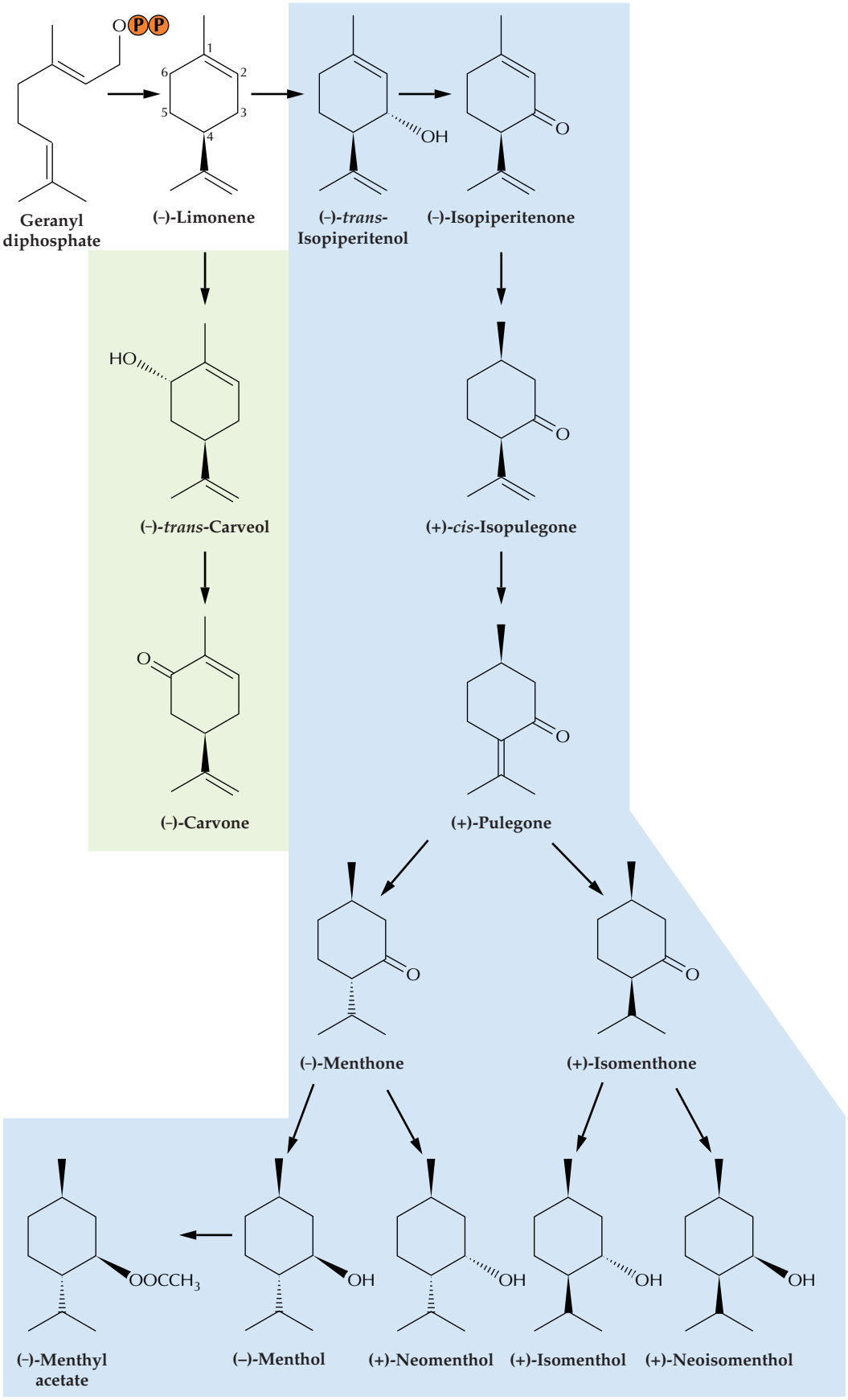


Figure 24.16
Essential oil synthesis in peppermint and spearmint. In peppermint, (–)-limonene is converted to (–)-isopiperitenone, which is modified to form (–)-menthol and related compounds. In spearmint, (–)-limonene is converted to (–)-carvone by a two-step pathway.

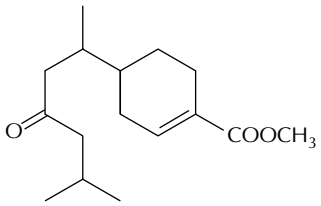
$NADP^+$-dependent dehydrogenase. Although most of the enzymatic machinery present in peppermint oil glands is also present in spearmint, the specificity of these enzymes is such that (–)-carvone is a very poor substrate. Consequently, carvone, the characteristic component of spearmint flavor, accumulates as the major essential oil component (about 70%). Similar reaction sequences initiated by allylic hydroxylations and subsequent redox metabolism and conjugations are very common in the monoterpene, sesquiterpene, and diterpene classes.

24.4.2 Some terpenoid skeletons are extensively decorated.

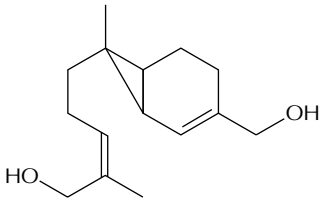
Reactions similar to those responsible for essential oil production in mints generate myriad terpenoid compounds of biological or pharmaceutical interest. Such reactions convert sesquiterpene olefin precursors to phytoalexins (see Fig. 24.13), allelopathic agents, and pollinator attractants. Additional sesquiterpenes generated by modifying olefin precursors include juvabione (Fig. 24.17), a compound from fir species that exhibits insect juvenile hormone activity; sirenin, a sperm attractant of the water mold allomyces; and artemisinin, a potent antimalarial drug from annual wormwood (*Artemisia annua,* also known as Qinghaosu, a plant used in traditional Chinese medicine since about 200 B.C.). A related enzymatic reaction sequence converts the parent diterpene olefin taxadiene to the anticancer drug taxol in yew species, in which the basic terpenoid nucleus is modified extensively by a complex pattern of hydroxylations and acylations. Esters of phorbol (another highly oxygenated diterpene) produced by species of the Euphorbiaceae are powerful irritants and cocarcinogens. After introduction of a hydroxyl group, subsequent oxidation can generate a carboxyl function such as that found in abietic acid (see Fig. 24.1) and oleanolic acid, and also provide the structural elements for lactone ring formation. Sesquiterpenes bearing such lactone rings, e.g., costunolide, are produced and accumulated in the glandular hairs on the leaf surfaces of members of the Asteraceae, where some of these compounds serve as feeding repellents to herbivorous insects and mammals. Monoterpene lactones include nepetalactone (the active principle of catnip as well as an aphid pheromone), a member of the iridoid family of monoterpenes, which are formed by a cyclization reaction quite different from that of other monoterpenes (Fig. 24.17).

The limonoids are a family of oxygenated nortriterpene antiherbivore compounds. Like the sesquiterpene lactones, these substances taste very bitter to humans and probably to other mammals as well. A powerful insect antifeedant compound is azadirachtin A, a highly modified limonoid from the neem tree (*Azadirachta indica*). Other oxygenated triterpenoid natural products with unusual biological properties include the phytoecdysones, a family of plant steroids that act as hormones and stimulate insect molting; the saponins, so named because of their soaplike, detergent properties; and the cardenolides, which, like the saponins, are glycosides, in that they bear one or more attached sugar residues. Ingestion of α-ecdysone by insects disrupts the molting cycle, usually with fatal consequences. The saponins and cardenolides are toxic to many vertebrate herbivores; this family of compounds includes well-known fish poisons and snail poisons of significance in the control of schistosomiasis. Many of these products are also cardioactive and anticholesterolemic agents of pharmacological significance. Digitoxin, the glycone (glycosylated form) of digitoxigenin (Fig. 24.17) extracted from foxglove (*Digitalis*), is used widely in carefully prescribed doses for treatment of congestive heart disease.

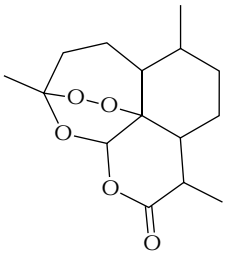
The broad range of insect and higher animal toxins and deterrents among the modified triterpenes leaves little doubt as to their role in plant defense. Interestingly, some herbivores have developed the means to circumvent the toxic effects of these terpenoids and adapt them to their own defense purposes. The classical example of this phenomenon is the monarch butterfly, a specialist feeder on milkweeds (*Asclepias*) which contain cardenolides that are toxic to most herbivores and are even associated with livestock poisoning. Monarch caterpillars, however, feed on milkweeds and accumulate the cardenolides without apparent ill effects. As a result, both caterpillars and the adult butterflies contain enough cardenolides to be toxic to their own predators such as birds.



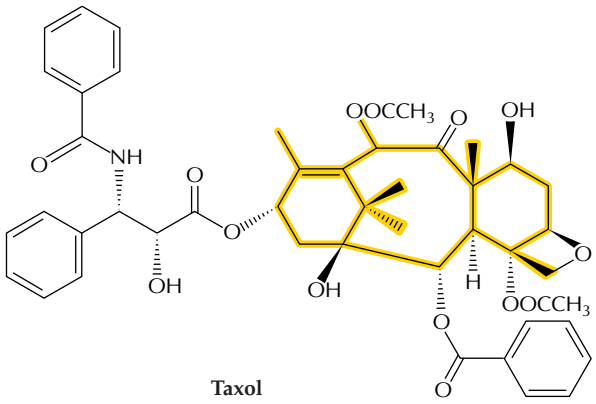
Juvabione
(insect juvenile hormone analog)



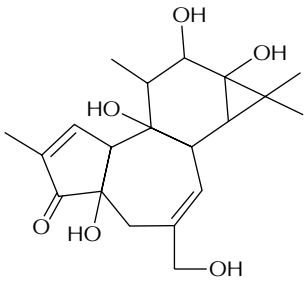
Sirenin
(sperm attractant in water molds)



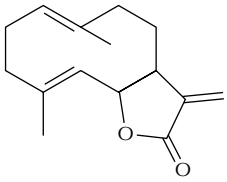
Artemisinin
(antimalarial drug)



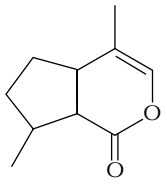
Taxol
(anticancer drug)



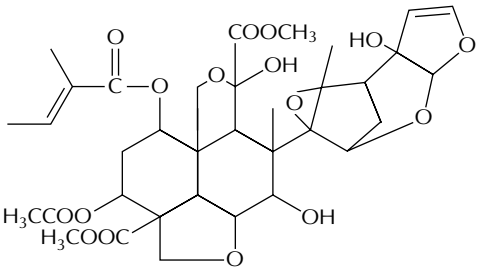
Phorbol
(irritant and cocarcinogen)



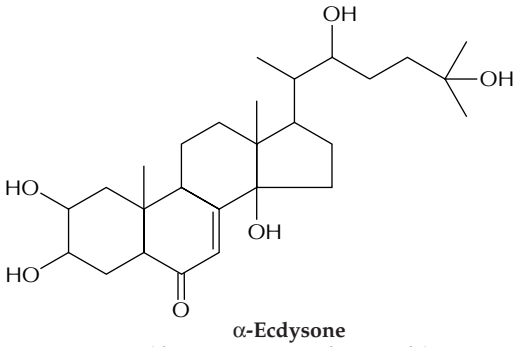
Costunolide
(insect repellent, mammal antifeedant)



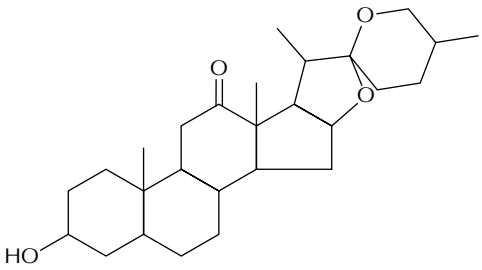
Nepetalactone
(active principle of catnip)



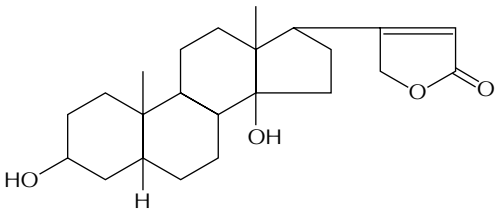
Azadirachtin A
(insect antifeedant)



α-Ecdysone
(disrupts insect molting cycle)



Hecogenin,
the aglycone of a saponin
(a detergent)



Digitoxigenin,
the aglycone of digitoxin, a cardenolide
(treatment of congestive heart disease)

Figure 24.17
Terpenoids formed by secondary transformations of parent cyclic compounds. The yellow highlighting delineates the terpenoid portion of the molecule taxol.

24.5 Toward transgenic terpenoid production

With recent success in the cloning of genes that encode enzymes of terpenoid synthesis, the transgenic manipulation of plant terpenoid metabolism may present a suitable avenue for achieving a number of goals. Several agriculturally important crop species have been bred selectively to produce relatively low amounts of unpalatable terpenoid defense compounds; in the process, these cultivars have lost not only defense capabilities but also, in some cases, quality attributes such as flavor and color. The selective reintroduction of terpenoid-based defense chemistry is certainly conceivable, as is the engineering of pathways into fruits and vegetables to impart desirable flavor properties. The aroma profiles of ornamental plant species might be modified by similar approaches. Likewise, transgene expression might accelerate the rate of slow biosynthetic steps and thereby increase the yields of essential oils used in flavors and perfumes, phytopharmaceuticals (e.g., artemisinin and taxol), insecticides (e.g., pyrethrins and azadirachtin), and a wide range of industrial intermediates that are economically inaccessible by traditional chemical synthesis.

The genetic engineering of terpenoid-based insect defenses is particularly appealing, given the array of available monoterpene, sesquiterpene, diterpene, and triterpene compounds that are toxic to insects not adapted to them. Attracting predators and parasitoids of the target insect or modifying host attractants, oviposition stimulators, and pheromone precursors offers even more sophisticated strategies for pest control. For effective transgenic manipulation of such terpenoid biosynthetic pathways, promoters for tissue-specific, developmentally controlled, and inducible expression are required, as are promoters for targeting production to secretory structures of essential oil plants and conifers. The latter are the most likely species for initial manipulation because they already are adapted for terpenoid accumulation, and the antecedent and subsequent metabolic steps are largely known.

The engineering of terpenoid biosynthetic pathways into plant species that do not ordinarily accumulate these natural products presents a greater opportunity but an even greater challenge, given that little metabolic context exists in these cases. In such species, issues of subcellular sites of synthesis, requirements for sufficiency of precursor flux, and the fate of the desired product might present additional difficulties. Clearly, targeting a terpenoid synthase to the cellular compartment containing the appropriate C_{10}, C_{15}, C_{20}, or C_{30} precursor will be an important consideration. Sufficient flux of IPP at the production site to drive the pathway also will be essential. Because constraints in precursor flow ultimately will limit the effectiveness of transgenes for subsequent pathway steps, information about the flux controls on IPP biosynthesis in both cytosol and plastid, and about the interactions of these controls, is sorely needed.

Very few published examples of the genetic engineering of terpenoid metabolism are currently available, although two notable successes have been achieved in the area of terpenoid vitamins. The ratio of beneficial tocopherol (vitamin E) isomers in oilseeds has been altered by this means, and an increased concentration of β-carotene (a vitamin A precursor) in both rice kernels and rapeseed has been obtained by manipulating the carotenoid pathway. In another, cautionary example, however, overexpression in a transgenic tomato of the enzyme that diverts GGPP to carotenoids resulted in a dwarf phenotype, an unintended consequence of depleting the precursor of the gibberellin plant hormones.

24.6 Alkaloids

24.6.1 Alkaloids have a 3000-year history of human use.

For much of human history, plant extracts have been used as ingredients in potions and poisons. In the eastern Mediterranean, use of the latex of the opium poppy (*Papaver somniferum;* Fig. 24.18) can be traced back at least to 1400 to 1200 B.C. The Sarpagandha root (*Rauwolfia serpentina*) has been used in India since approximately 1000 B.C. Ancient people used medicinal plant extracts as purgatives, antitussives, sedatives, and treatments for a wide range of ailments, including snakebite, fever, and insanity. As the use of medicinal plants spread westward across

Figure 24.18
(A) Maturing capsule of the opium poppy *Papaver somniferum.* When the capsule is wounded, a white, milky latex is exuded. Poppy latex contains morphine and related alkaloids such as codeine. When the exuded latex is allowed to dry, a hard, brown substance called opium is formed. (B) Statuette from Gazi of a goddess of sleep crowned with capsules of the opium poppy (1250–1200 B.C.).

Arabia and Europe, new infusions and decoctions played a role in famous events. During his execution in 399 B.C., the philosopher Socrates drank an extract of coniine-containing hemlock (*Conium maculatum;* Fig. 24.19). In the last century B.C., Queen Cleopatra used extracts of henbane (*Hyoscyamus*), which contains atropine (Fig. 24.20), to dilate her pupils and appear more alluring to her male political rivals.

Over the centuries, the king of all medicinals has been opium, which was widely consumed in the form of Theriak, a concoction consisting mainly of opium, dried snake meat, and wine (Box 24.2). Analysis of the individual components of opium led to the identification of morphine (Fig. 24.21A), named for Morpheus, the god of dreams in Greek mythology. The isolation of morphine in 1806 by German pharmacist Friedrich Sertürner gave rise to the study of **alkaloids.**

The term alkaloid, coined in 1819 in Halle, Germany, by another pharmacist, Carl Meissner, finds its origin in the Arabic name *al-qali,* the plant from which soda was first isolated. Alkaloids were originally defined as pharmacologically active, nitrogen-containing basic compounds of plant origin.

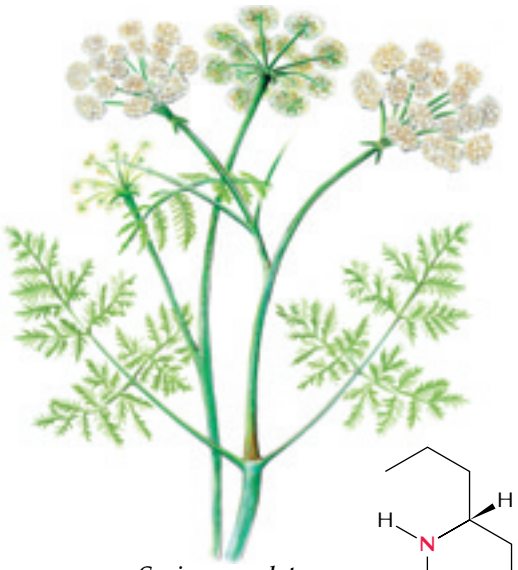
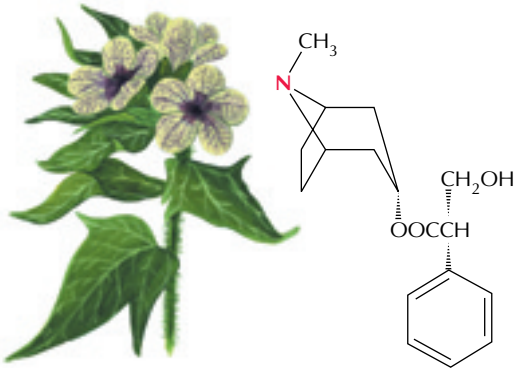


Figure 24.19
(A). The piperidine alkaloid coniine, the first alkaloid to be synthesized, is extremely toxic, causing paralysis of motor nerve endings. (B) In 399 B.C., the philosopher Socrates was executed by consuming an extract of coniine-containing poisonous hemlock. This depiction of the event, "The Death of Socrates," was painted by Jacques-Louis David in 1787.



Hyoscyamus niger **Atropine**

Figure 24.20
Stucture of the anticholinergic tropane alkaloid atropine from *Hyoscyamus niger.*

After 190 years of alkaloid research, this definition as such is no longer comprehensive enough to encompass the alkaloid field, but in many cases it is still appropriate. Alkaloids are not unique to plants. They have also been isolated from numerous animal sources (Fig. 24.21B and Box 24.3), although still to be determined is whether biosynthesis de novo occurs in each organism. Many of the alkaloids that have been discovered are not pharmacologically active in mammals and some are neutral rather than basic in character, despite the presence of a nitrogen atom in the molecule.

Alkaloid-containing plants were mankind's original "materia medica." Many are still in use today as prescription drugs (Table 24.1). One of the best-known prescription alkaloids is the antitussive and analgesic codeine from the opium poppy (Fig. 24.21A). Plant alkaloids have also served as models for modern synthetic drugs, such as the tropane alkaloid atropine for tropicamide used to dilate the pupil during eye examinations and the indole-derived antimalarial alkaloid quinine for chloroquine (Fig. 24.22).

In addition to having a major impact on modern medicine, alkaloids have also influenced world geopolitics. Notorious examples include the Opium Wars between China and Britain (1839–1859) and the efforts currently underway in various countries to eradicate

Box 24.2 Theriak, an ancient antipoisoning nostrum containing opium, wine, and snake meat, is still used today in rare instances.

One of the oldest and most long-lived medications in the history of mankind is Theriak. Originating in Greco-Roman culture, Theriak consists of mainly opium and wine with a variety of plant, animal, and mineral constituents. Panel A of the figure shows a recipe for Theriak from the French Pharmacopée Royale in 1676.

Theriak was developed as an antidote against poisoning, snake bites, spider bites, and scorpion stings. History has it that the Roman Emperor Nero contracted the Greek physician Andromachus to discover a medicine that was effective against all diseases and poisons. Andromachus improved the then-existing recipe to include, in addition to opium, five other plant poisons and 64 plant drugs. Another crucial component was dried snake meat, believed to act against snake bites by neutralizing the venom.

Today, Theriak is still prescribed in rare cases in Europe for pain and other ailments. Panel B shows a valuable Theriak-holding vessel made of Nymphenburg porcelain (in about 1820), which is on display in the Residenz Pharmacy in Munich, Germany.

(A)

(B)

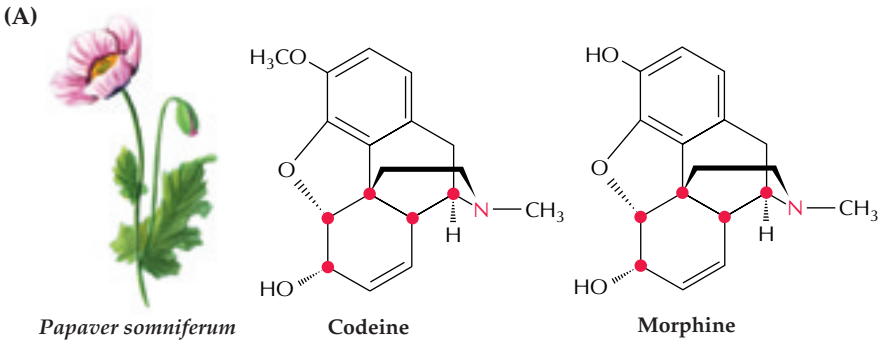


Figure 24.21
(A) Structures of the alkaloids codeine and morphine from the opium poppy *Papaver somniferum*. Asymmetric (chiral) carbons are highlighted with red dots. (B) The frog *Bufo marinus* accumulates a considerable amount of morphine in its skin.

illicit production of heroin, a semisynthetic compound derived by acetylation of morphine (Fig. 24.23), and cocaine, a naturally occurring alkaloid of the coca plant (Fig. 24.24). Because of their various pharmacological activities, alkaloids have influenced

Box 24.3 Some butterflies and moths use alkaloids for sexual signaling or for protection against predators.

Alkaloid-bearing species have been found in nearly all classes of organisms, including frogs, ants, butterflies, bacteria, sponges, fungi, spiders, beetles, and mammals. Alkaloids of various structures have been isolated from a variety of marine creatures. Some animals, such as amphibians, produce an array of either toxic or noxious alkaloids in the skin or the secretory glands. Others, such as the insects described below, use plant alkaloids as a source of attractants, pheromones, and defense substances.

Some butterflies gather alkaloidal precursors from plants that are not their food sources and convert these compounds into pheromones and defense compounds. Larvae of the cinnabar moth, *Tyria jacobaea,* continuously graze their plant host *Senecio jacobaea* until the plant is completely defoliated (see panel A). The alkaloids thus obtained by the larvae are retained throughout metamorphosis. Male Asian and American arctiid moths incorporate pyrrolizidine alkaloids into their reproductive biology by sequestering these alkaloids in abdominal scent organs called coremata, which are everted in the final stages of their courtship to release the pheromones necessary to gain acceptance by a female. The coremata of a male Asian arctiid moth (*Creatonotos transiens*) are directly proportional to the pyrrolizidine alkaloid content of its diet during the larval stage (see panel B). The courtship success of these male butterflies therefore depends on their ingesting alkaloids from higher plants.

The larvae of a second insect group, the Ithomiine butterflies, feed on solanaceous plants and sequester the plant toxins, including tropane alkaloids and steroidal glycoalkaloids. However, the adult Ithomiinae do not contain these Solanaceae alkaloids but prefer to ingest plants that produce pyrrolizidine alkaloids, sequestering these bitter substances as N-oxides and monoesters. The pyrrolizidine alkaloid derivatives protect Ithomiinae butterflies from an abundant predator, the giant tropical orb spider. The spider will release a field-caught butterfly from its web but will readily eat a freshly emerged adult that has not yet had an opportunity to feed on the preferred host plant. When palatable butterflies were painted externally with a solution of pyrrolizidine alkaloids, the spider released them from its web. In contrast, palatable butterflies treated the same way with Solanaceae alkaloids were devoured. In general, mostly male butterflies are found feeding on the pyrrolizidine alkaloid-accumulating plants; however, as much as 50% of the pyrrolizidine alkaloids present in these males is sequestered in the spermatophores and transferred to females at mating. In some butterfly species, the protective alkaloids are then transferred to the eggs.

Table 24.1 Physiologically active alkaloids used in modern medicine

Alkaloid	Plant source	Use
Ajmaline	*Rauwolfia serpentina*	Antiarrythmic that functions by inhibiting glucose uptake by heart tissue mitochondria
Atropine, -(±)-hyoscyamine	*Hyoscyamus niger*	Anticholinergic, antidote to nerve gas poisoning
Caffeine	*Coffea arabica*	Widely used central nervous system stimulant
Camptothecin	*Camptotheca acuminata*	Potent anticancer agent
Cocaine	*Erythroxylon coca*	Topical anaesthetic, potent central nervous system stimulant, and adrenergic blocking agent; drug of abuse
Codeine	*Papaver somniferum*	Relatively nonaddictive analgesic and antitussive
Coniine	*Conium maculatum*	First alkaloid to be synthesized; extremely toxic, causes paralysis of motor nerve endings, used in homeopathy in small doses
Emetine	*Uragoga ipecacuanha*	Orally active emetic, amoebicide
Morphine	*P. somniferum*	Powerful narcotic analgesic, addictive drug of abuse
Nicotine	*Nicotiana tabacum*	Highly toxic, causes respiratory paralysis, horticultural insecticide; drug of abuse
Pilocarpine	*Pilocarpus jaborandi*	Peripheral stimulant of the parasympathetic system, used to treat glaucoma
Quinine	*Cinchona officinalis*	Traditional antimalarial, important in treating *Plasmodium falciparum* strains that are resistant to other antimalarials
Sanguinarine	*Eschscholzia californica*	Antibacterial showing antiplaque activity, used in toothpastes and oral rinses
Scopolamine	*H. niger*	Powerful narcotic, used as a sedative for motion sickness
Strychnine	*Strychnos nux-vomica*	Violent tetanic poison, rat poison, used in homeopathy
(+)-Tubocurarine	*Chondrodendron tomentosm*	Nondepolarizing muscle relaxant producing paralysis, used as an adjuvant to anaesthesia
Vinblastine	*Catharanthus roseus*	Antineoplastic used to treat Hodgkin's disease and other lymphomas.

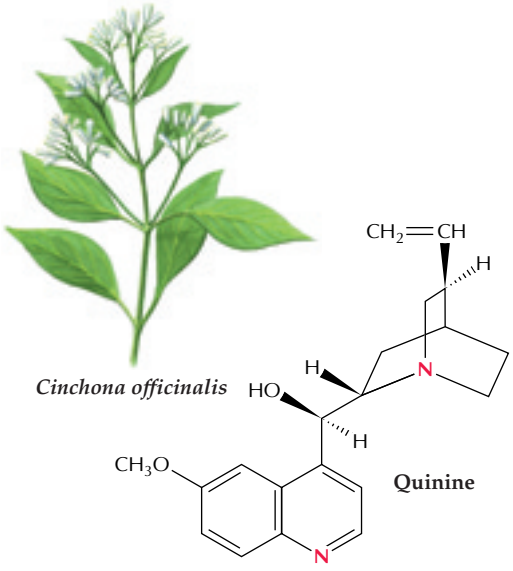


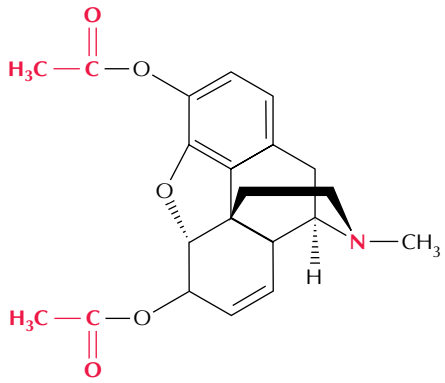
Figure 24.22
Structure of the monoterpenoid indole alkaloid-derived quinine from *Cinchona officinalis*. An antimalarial quinine-containing tonic prepared from the bark of *C. officinalis* greatly facilitated European exploration and inhabitation of the tropics during the past two centuries.

human history profoundly, both for good and ill. Of interest to plant biologists, however, is the evolutionary selection process in plants that has caused alkaloids to evolve into such a large number of complex structures and to remain effective over the millennia.

24.6.2 Physiologically active alkaloids participate in plant chemical defenses.

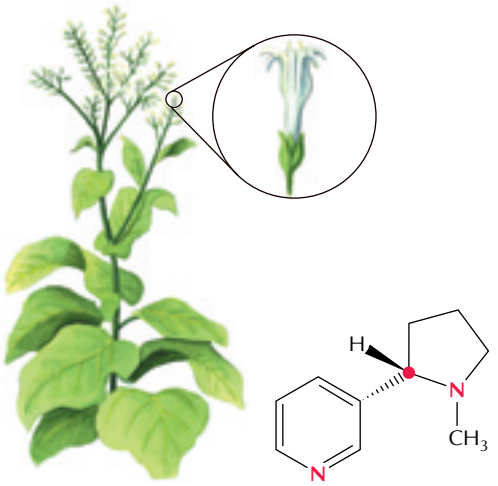
More than 12,000 alkaloids have been isolated since the discovery of morphine. About 20% of the species of flowering plants produce alkaloids, and each of these species accumulates the alkaloids in a unique, defined pattern. Some plants, such as the periwinkle (*Catharanthus roseus*) contain more than 100 different monoterpenoid indole alkaloids. Why should a plant invest so much nitrogen into synthesizing such a large number of alkaloids of such diverse structure? The role of alkaloids in plants has been a longstanding question, but a picture has begun to emerge that supports an ecochemical function for these compounds.

The role of chemical defense for alkaloids in plants is supported by their wide range of physiological effects on animals and by the antibiotic activities many alkaloids possess. Various alkaloids also are toxic to insects or function as feeding deterrents. For example, nicotine, found in tobacco, was one of the first insecticides used by humans and remains one of the most effective (Fig. 24.25). Herbivory has been found to stimulate nicotine biosynthesis in wild tobacco plants. Another effective insect toxin is caffeine, found in seeds and leaves of cocoa,



Heroin

Figure 24.23
Structure of diacetyl morphine, commonly known as heroin.

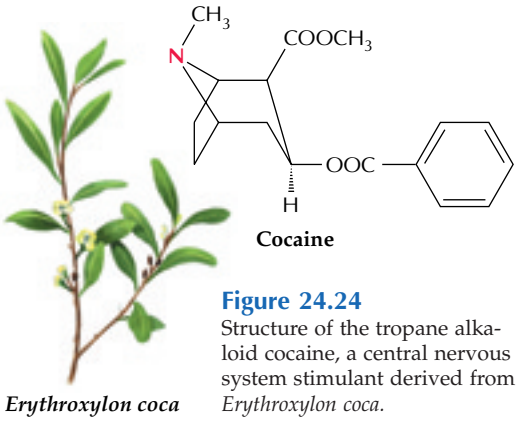


Nicotiana tabacum **Nicotine**

Figure 24.25
Structure of nicotine from *Nicotiana tabacum*. The asymmetric chiralcarbon is highlighted with a red dot.

coffee, cola, maté, and tea (Fig. 24.26). At a dietary concentration well below that found in fresh coffee beans or tea leaves, caffeine kills nearly all larvae of the tobacco hornworm (*Manduca sexta*) within 24 hours—primarily by inhibiting the phosphodiesterase that hydrolyzes cAMP. The steroid alkaloid α-solanine, a cholinesterase inhibitor found in potato tuber (Fig. 24.27), is the trace toxic constituent thought to be responsible for the teratogenicity of sprouting potatoes.

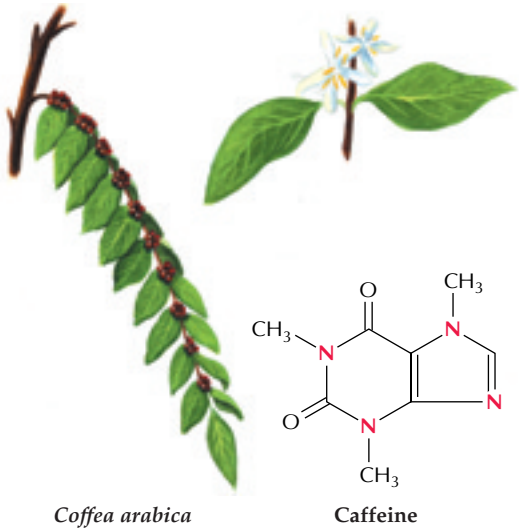
Two groups of alkaloids that have been well studied with respect to ecochemical function are the pyrrolizidine and quinolizidine alkaloids. The pyrrolizidine alkaloids, frequently found in members of the tribe Senecioneae (Asteraceae) and in the Boraginaceae, render most of these plants toxic to mammals. In *Senecio* species (Fig. 24.28), senecionine *N*-oxide is synthesized in the roots and translocated throughout the plant. In species such as *Senecio vulgaris* and *S. vernalis*, 60% to 80% of the pyrrolizidine alkaloids is found to accumulate in the inflorescences. Members of the *Senecio* genus are responsible for livestock poisonings and also represent a potential health hazard for humans. Naturally occurring pyrrolizidine alkaloids are harmless but become highly toxic when transformed by cytochrome P450 monooxygenases in the liver. On the other hand, several insect species have adapted to the pyrrolizidine alkaloids that accumulate in plants and have evolved mechanisms for using these alkaloids to their own benefit. Some insects can feed on pyrrolizidine alkaloid-producing plants and effectively and



Cocaine

Erythroxylon coca

Figure 24.24
Structure of the tropane alkaloid cocaine, a central nervous system stimulant derived from *Erythroxylon coca*.



Coffea arabica **Caffeine**

Figure 24.26
Structure of the purine alkaloid caffeine from *Coffea arabica*.

Solanum tuberosum

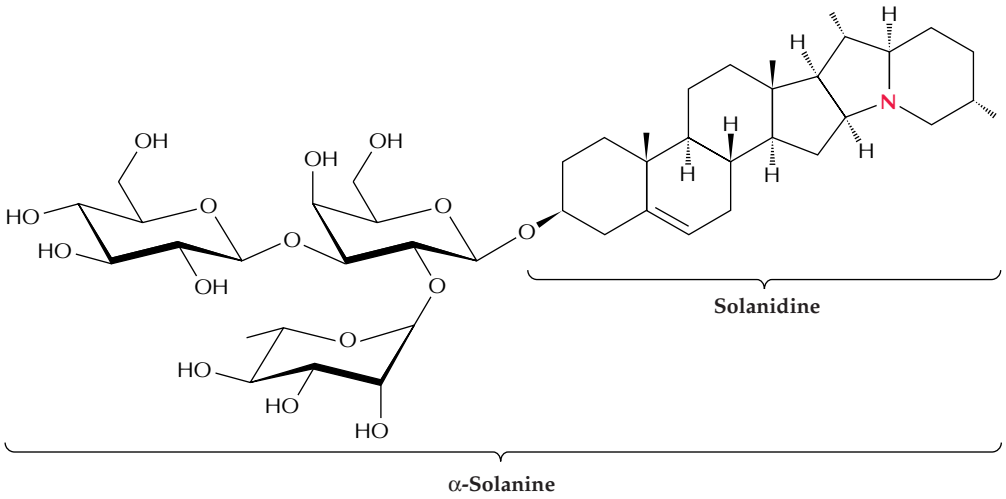
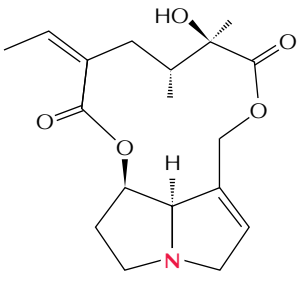


Figure 24.27
Structure of the steroid alkaloid glycoside α-solanine from *Solanum tuberosum* (potato). The aglycone solanidine is derived from cholesterol.

efficiently eliminate the alkaloids after enzymatic modification, such as formation of N-oxide derivatives. Other insects not only feed on these plants, but also store the pyrrolizidine alkaloids for their own defense or convert the ingested pyrrolizidine alkaloids to pheromones that attract prospective mates (Box 24.3).

The quinolizidine alkaloids occur primarily in the genus *Lupinus* and are frequently referred to as lupine alkaloids (Fig. 24.29); they are toxic to grazing animals, particularly to sheep. The highest incidence of livestock losses attributable to lupine alkaloid poisoning occurs in autumn during the seed-bearing stage of the plant life cycle—the seeds being the plant parts that accumulate the greatest quantities of these alkaloids. Because of their bitter taste, lupine alkaloids can also function as feeding deterrents. Given a mixed population of sweet and bitter lupines, rabbits and hares will readily eat the alkaloid-free sweet variety and avoid the lupine alkaloid-accumulating bitter variety, indicating that lupine alkaloids in plants serve to reduce herbivory by functioning both as bitter-tasting deterrents and toxins. Given this collection of examples, alkaloids can be viewed as a part of the chemical defense system of the plant that evolved under the selection pressure of predation.

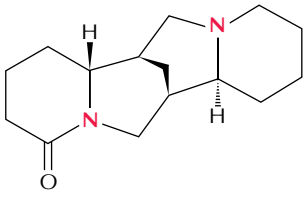
Senecio jacobaea



Senecionine

Figure 24.28
Structure of the pyrrolizidine alkaloid senecionine from ragwort (*Senecio jacobaea*).

Lupinus polyphyllus



Lupanine

Figure 24.29
Structure of the quinolizidine alkaloid lupanine from the bitter lupine *Lupinus polyphyllus*. Lupanine is a bitter compound that functions as a feeding deterrent.

24.6.3 Alkaloid biosynthesis research has been greatly aided by the development of techniques for culturing plant cells.

Many alkaloids have complex chemical structures and contain multiple asymmetric centers, complicating structure elucidation and making study of the biosynthesis of alkaloids quite difficult until relatively recently. For example, although nicotine (one asymmetric center; see Fig. 24.25) was discovered in 1828, its structure was not known until it was synthesized in 1904, and the structure of morphine (five asymmetric centers; see Fig. 24.21) was not unequivocally elucidated until 1952, almost 150 years after its isolation. Almost all of the enzymes involved in the biosynthesis of these two alkaloids have been identified, but 190 years after morphine was first isolated, its biosynthetic pathway remains incomplete.

Why has it been so difficult to elucidate alkaloid biosynthetic pathways? Plants synthesize natural products at a relatively sluggish rate, so steady-state concentrations of the alkaloid biosynthetic enzymes are low. In addition, the large amounts of tannins and other phenolics that accumulate in plants interfere with the extraction of active enzymes. Even when plants are treated with radiolabeled precursors and the resulting radioactive alkaloids are chemically degraded to identify the position of the label, the low rate of natural product metabolism can prevent the high rates of incorporation that yield clearly interpretable results. The use of polyvinylpyrrolidone and Dowex-1 in preparing protein extracts from plant tissues has helped overcome the enzyme inactivation by phenolic compounds, but isolation of the enzymes involved in natural product synthesis has had only limited success because of their very low concentrations in the plant.

Not until the 1970s were suspension cultures of plant cells established that were capable of producing high concentrations of alkaloids (Fig. 24.30). As an experimental system, cell culture provides several advantages over whole-plant studies, including the year-round availability of plant material; the undifferentiated, relatively uniform state of development of the cells; the absence of interfering microorganisms; and most importantly, the compressed vegetative cycle. Plant cell cultures can synthesize large amounts of secondary products within a two-week cultivation period. This is very favorable in comparison with in planta production, for which the time frame for alkaloid accumulation may vary from one season for annual plants to several years for some perennial species. In plant cell culture, the rate of alkaloid biosynthesis can be increased, greatly facilitating its study (Table 24.2). Moreover, the greater metabolic rates associated with cell cultures promote the incorporation of labeled precursors during alkaloid biosynthesis. Hormones regulate the accumulation of alkaloids in culture, and in many cases, alkaloid biosynthesis can be induced by the addition of abiotic and biotic elicitor substances to the culture. These advances have provided a powerful system with which to analyze the regulation of alkaloid biosynthesis. Since the advent of alkaloid production in culture, more than 80 new enzymes that catalyze steps in the biosynthesis of indole, isoquinoline, tropane, pyrrolizidine, acridone, and purine classes of alkaloids have been discovered and partially characterized.

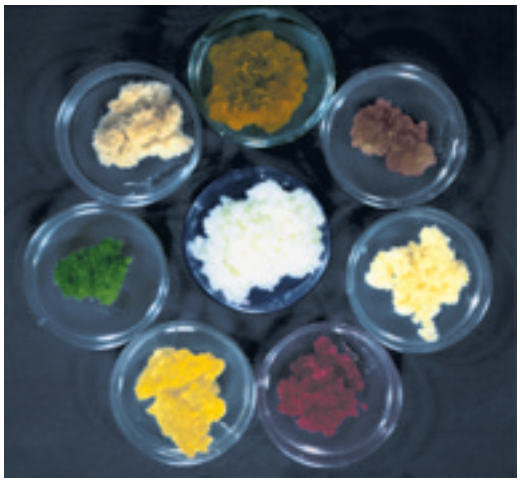
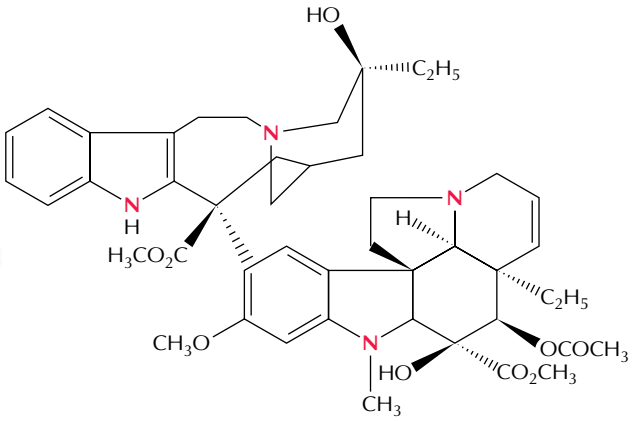


Figure 24.30
Callus cultures established from plants can be optimized to produce high concentrations of a wide variety of natural products. In some of the examples shown, metabolite pigments give the calli distinctive colors.

Table 24.2 Production of selected alkaloids in plant cell culture

Secondary metabolite	Species	Yield (g/l)	% Dry weight
Berberine	*Coptis japonica*	7.0	12
Jatrorrhizine	*Berberis wilsoniae*	3.0	12
Raucaffricine	*Rauwolfia serpentina*	1.6	3

Catharanthus roseus

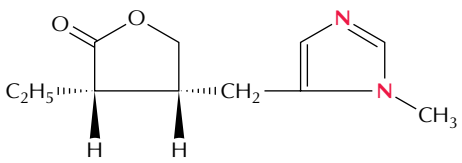


Vinblastine

Figure 24.31
Structure of the monoterpenoid indole alkaloid vinblastine from *Catharanthus roseus.*

At one time, plant cell suspension cultures were considered an alternative source of industrially significant secondary metabolites, particularly alkaloids of pharmaceutical importance. However, many important compounds such as vincristine, vinblastine (Fig. 24.31), pilocarpine (Fig. 24.32), morphine, and codeine, among many others, are not synthesized to any appreciable extent in cell culture. The reason for this is thought to be tissue-specific expression of alkaloid biosynthesis genes, because in some cases plants regenerated from nonproducing callus cells contained the same alkaloid profile as the parent plant. Although not currently used for commercial alkaloid production, plant cell culture continues to provide biochemists with a rich source of certain alkaloid biosynthesis enzymes and a convenient system with which to study enzyme regulation.

Pilocarpus jaborandi



Pilocarpine

Figure 24.32
The imidazole alkaloid pilocarpine from *Pilocarpus jaborandi.*

24.6.4 Although typically considered constitutive defense compounds, some alkaloids are synthesized in response to plant tissue damage.

Alkaloids are thought to be part of the constitutive chemical defense system of many plants. The ultimate test of this hypothesis may be future research into molecular genetic suppression of alkaloid biosynthesis. The phenotypes of mutants lacking specific gene products in an alkaloid biosynthesis pathway may provide a direct demonstration of the role of noninducible alkaloids produced constitutively in plants. Near-isogenic species of alkaloid-producing and nonproducing plants might then be subjected to experimental conditions to test their relative resistance.

In a few cases, such as that of nicotine in tobacco, convincing evidence has been presented that an alkaloid is involved in induced chemical defense. Wild species of tobacco have been found to be highly toxic to the hornworm, a tobacco-adapted species that is insensitive to nicotine but susceptible to N-acyl nicotines found in the tobacco leaf. The N-acyl derivatives are not found in unwounded *Nicotiana repanda* but their formation is induced by methyl jasmonate treatment. In response to leaf wounding, tobacco plants increase the alkaloid content of leaves that have not been subjected to wounding. *N*-Acetylnicotine accumulates very rapidly (within 10 hours). The alkaloid content increases and then returns to basal concentrations over a 14-day period. Recent isotope labeling experiments indicate that this

derivative is formed from a preexisting pool of nicotine. De novo nicotine biosynthesis occurs in roots, followed by transport to leaves, but only after 36 hours. The increase in nicotine biosynthesis results in a 10-fold increase of the alkaloid in the xylem fluid.

Freshly hatched hornworm larvae fed wounded leaves achieve only half the weight gain obtained by counterparts fed leaf material from unwounded plants. Recent studies demonstrate that, given the choice, hornworms will abandon a wounded plant. Hornworms not permitted to leave a wounded plant exhibit much higher mortality rates and much lower growth rates than those fed on unwounded plants.

Inducible synthesis of nicotine and other alkaloids appears to involve methyl jasmonate, a volatile plant growth regulator (see Chapter 17). Endogenous jasmonate pools increase rapidly when plant cells are treated with an elicitor prepared from yeast cell walls. In turn, jasmonates are known to induce accumulation of secondary metabolites in cell culture. More than 140 different cultured plant species respond to the addition of methyl jasmonate by increasing their production of natural products. Although studies of this type with intact plants are not as extensive as with cell suspension cultures, clear examples have been demonstrated with tobacco plants, in which leaf wounding produces an increase in endogenous jasmonic acid pools in shoots and roots. Moreover, the application of methyl jasmonate to tobacco leaves increases both endogenous jasmonic acid in roots and de novo nicotine biosynthesis. These results imply that jasmonate may play a role in regulating the defense responses of alkaloid-producing plants.

24.7 Alkaloid biosynthesis

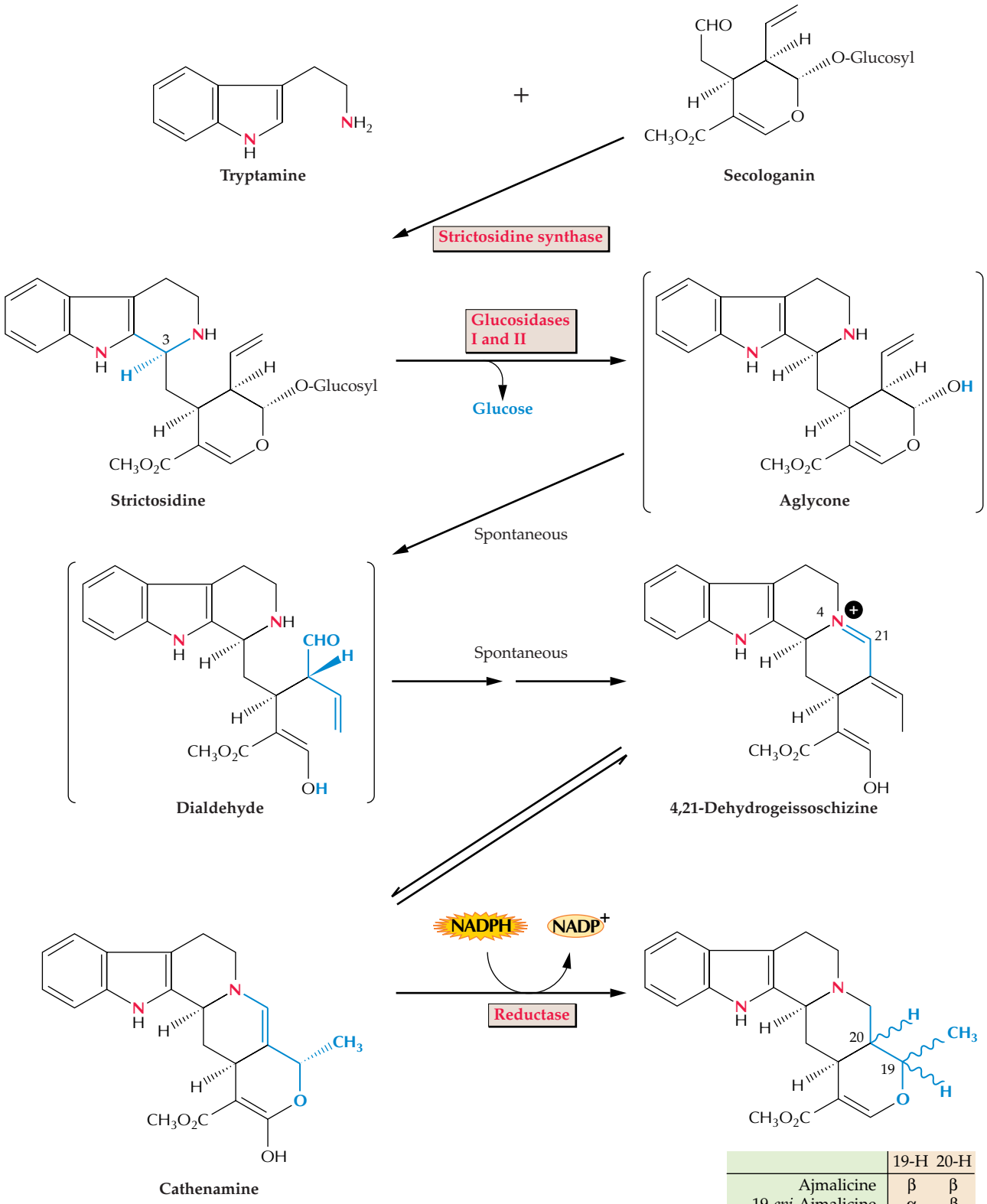
24.7.1 Plants biosynthesize alkaloids from simple precursors, using many unique enzymes.

Until the mid-20th century, our view of how alkaloids are synthesized in plants was based on biogenic hypotheses. Pathways suggested by illustrious natural product chemists such as Sir Robert Robinson, Clemens Schöpf, Ernst Winterstein, and Georg Trier were based on projections considered feasible within the realm of organic chemistry. In the 1950s, however, alkaloid biosynthesis became an experimental science, as radioactively labeled organic molecules became available for testing hypotheses. These early precursor-feeding experiments clearly established that alkaloids are in most cases formed from L-amino acids (e.g., tryptophan, tyrosine, phenylalanine, lysine, and arginine), either alone or in combination with a steroidal, secoiridoid (e.g., secologanin), or other terpenoid-type moiety. One or two transformations can convert these ubiquitous amino acids from primary metabolites to substrates for highly species-specific alkaloid metabolism. Although we do not thoroughly understand how most of the 12,000 known alkaloids are made by plants, several well-investigated systems can serve as examples of types of building blocks and enzymatic transformations that have evolved in alkaloid biosynthesis.

The L-tryptophan–derived monoterpenoid indole alkaloid ajmalicine was the first alkaloid for which biosynthesis was clarified at the enzyme level (Fig. 24.33); in that study plant cell suspension cultures of the Madagascar periwinkle *C. roseus* (see Fig. 24.31) were used. In plants, the biosynthesis of ajmalicine and more than 1800 other monoterpenoid indole alkaloids begins with the decarboxylation of the amino acid L-tryptophan by tryptophan decarboxylase to form tryptamine. Then tryptamine, by action of strictosidine synthase, is stereospecifically condensed with the secoiridoid secologanin (derived in multiple enzymatic steps from geraniol) to form 3α-strictosidine. Strictosidine can then be enzymatically permutated in a species-specific manner to form a multitude of diverse structures (Fig. 24.34). The elucidation of the enzymatic formation of ajmalicine by using plant cell cultures laid the groundwork for analysis of more-complex biosynthetic pathways, such as those leading to two other L-tryptophan–derived monoterpenoid indole alkaloids, ajmaline (Fig. 24.35) and vindoline.

24.7.2 The berberine synthesis pathway has been defined completely.

The first alkaloid for which each biosynthetic enzyme has been identified, isolated, and characterized from the primary metabolite



	19-H	20-H
Ajmalicine	β	β
19-*epi*-Ajmalicine	α	β
Tetrahydroalstonine	α	α

Figure 24.33
Biosynthesis of the monoterpenoid indole alkaloid ajmalicine and related compounds in *Catharanthus roseus*. Tryptamine is derived from L-tryptophan by decarboxylation through the action of tryptophan decarboxylase, and the secoiridoid secologanin is derived in multiple steps from the monoterpene geraniol.

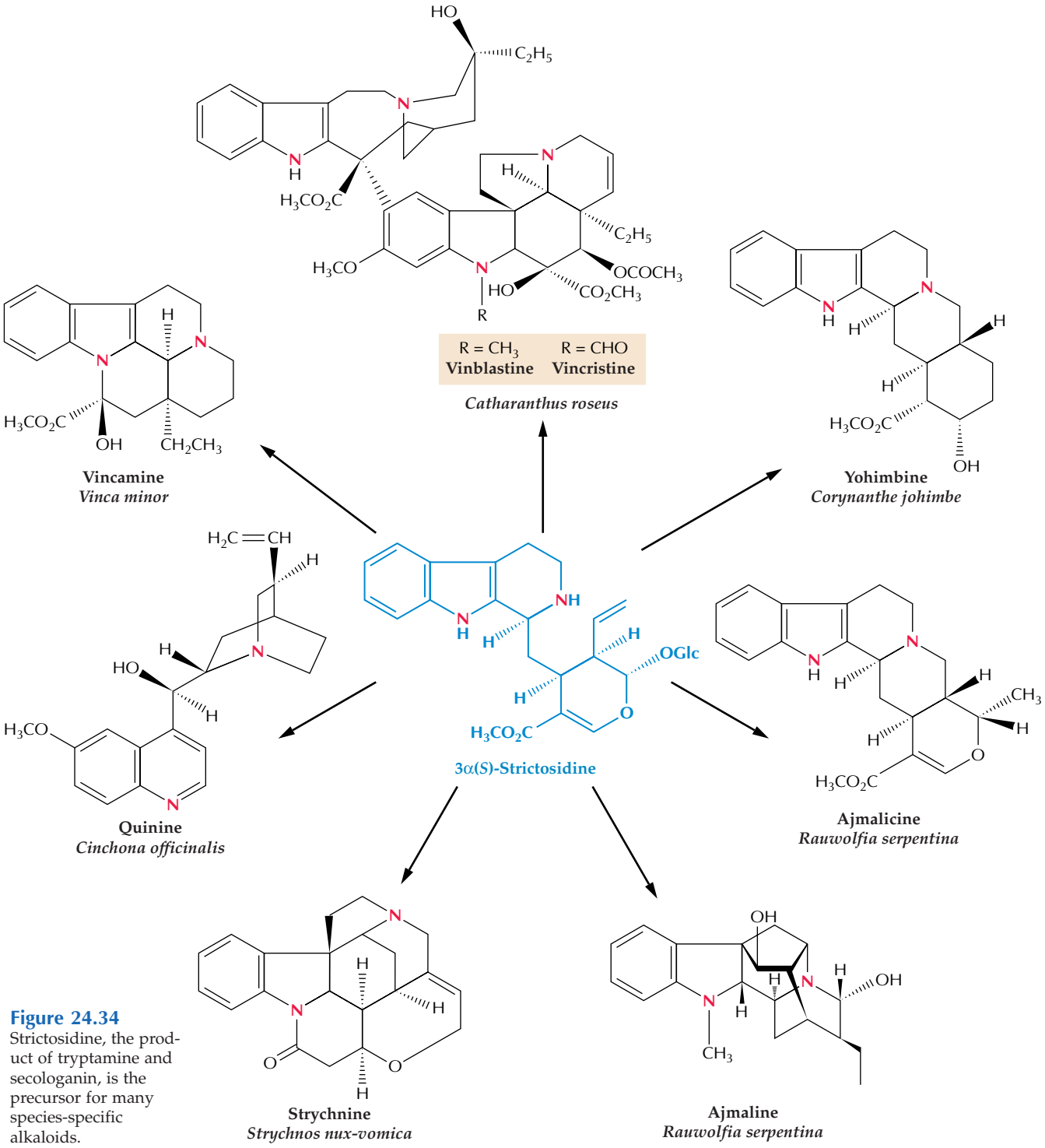
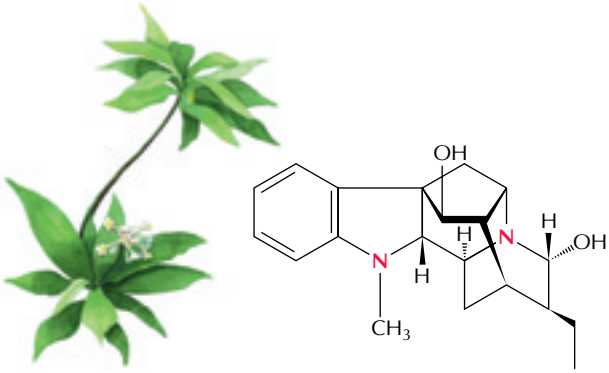


Figure 24.34
Strictosidine, the product of tryptamine and secologanin, is the precursor for many species-specific alkaloids.

precursor through to the end product alkaloid is the antimicrobial tetrahydrobenzylisoquinoline alkaloid, berberine, in *Berberis* (barberry) cell suspension cultures (Fig. 24.36). This pathway will be described in detail because it exemplifies the role of highly substrate-specific enzymes and of compartmentalization in alkaloid biosynthesis.

The biosynthesis of tetrahydrobenzylisoquinoline alkaloids in plants begins in the cytosol with a matrix of reactions that generates the first tetrahydrobenzylisoquinoline



Rauwolfia serpentina **Ajmaline**

Figure 24.35
Structure of the monoterpenoid indole alkaloid ajmaline from *Rauwolfia serpentina.*

alkaloid, (*S*)-norcoclaurine (Fig. 24.37). The pathway proceeds from two molecules of L-tyrosine. One is decarboxylated to form tyramine or is acted on by a phenol oxidase to form L-dopa. Dopamine can then be formed by decarboxylation of L-dopa or by the action of a phenol oxidase on tyramine. Determining which of these two pathways is predominant in a given plant is difficult because all of the enzyme activities are present in protein extracts. The benzyl moiety of (*S*)-norcoclaurine is formed by transamination of the second L-tyrosine molecule to form *p*-hydroxyphenylpyruvate, which is next decarboxylated to *p*-hydroxyphenylacetaldehyde. Dopamine and *p*-hydroxyphenylacetaldehyde are then stereoselectively condensed to form (*S*)-norcoclaurine. A series of methylation and oxidation reactions yield the branchpoint intermediate of benzylisoquinoline alkaloid biosynthesis, (*S*)-reticuline (Fig. 24.38).

In *Berberis,* the *N*-methyl group of (*S*)-reticuline is oxidized to the berberine bridge carbon C-8 of (*S*)-scoulerine (see Fig. 24.37). The specific pathway from (*S*)-scoulerine that leads to berberine proceeds with O-methylation to (*S*)-tetrahydrocolumbamine. The 3-*O*-methyl moiety of tetrahydrocolumbamine is converted to the methylenedioxy bridge of canadine by canadine synthase, a microsomal cytochrome P450–dependent oxidase. The final step in the biosynthesis of berberine is catalyzed by (*S*)-tetrahydroprotoberberine oxidase, an enzyme shown to contain a covalently bound flavin. The end product alkaloid berberine accumulates in the central vacuole of the *Berberis* cell.

The berberine bridge enzyme and (*S*)-tetrahydroprotoberberine oxidase are compartmentalized together in vesicles apparently derived from the smooth endoplasmic reticulum. Each of these enzymes consumes 1 mol of O_2 and produces 1 mol of H_2O_2 per mole of berberine formed. Overall, the course of reactions from 2 mol of L-tyrosine to 1 mol of berberine consumes 4 mol of *S*-adenosylmethionine and 2 mol of NADPH.

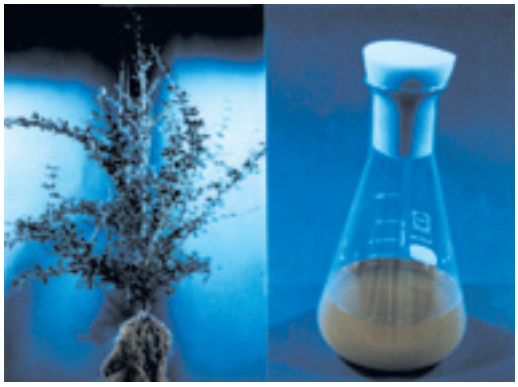


Figure 24.36
The *Berberis wilsoniae* plant (left) and cell suspension culture (right). The cell suspension culture derives its color from optimized production of the highly oxidized benzylisoquinoline alkaloid berberine. Plant cell cultures (like this one) that produce large quantities of alkaloids have led to the complete elucidation of several alkaloid biosynthetic pathways.

24.7.3 Elucidation of other alkaloid biosynthetic pathways is progressing.

The enzymes that catalyze the biosynthesis of the benzophenanthridine alkaloid macarpine in the California poppy *Eschscholzia californica* have also been identified, isolated, and characterized, as have nearly all of the enzymes of morphine biosynthesis in the opium poppy (Fig. 24.39). Good progress has been made toward understanding the enzymatic formation of the tropane alkaloid scopolamine in *Hyoscyamus niger* and of the acridone alkaloid furofoline-I in *Ruta graveolens.*

Studies have revealed that the chemical transformations required for alkaloid biosynthesis are catalyzed by highly stereo-, regio-, and substrate-specific enzymes that are present only in specific species. These enzymes

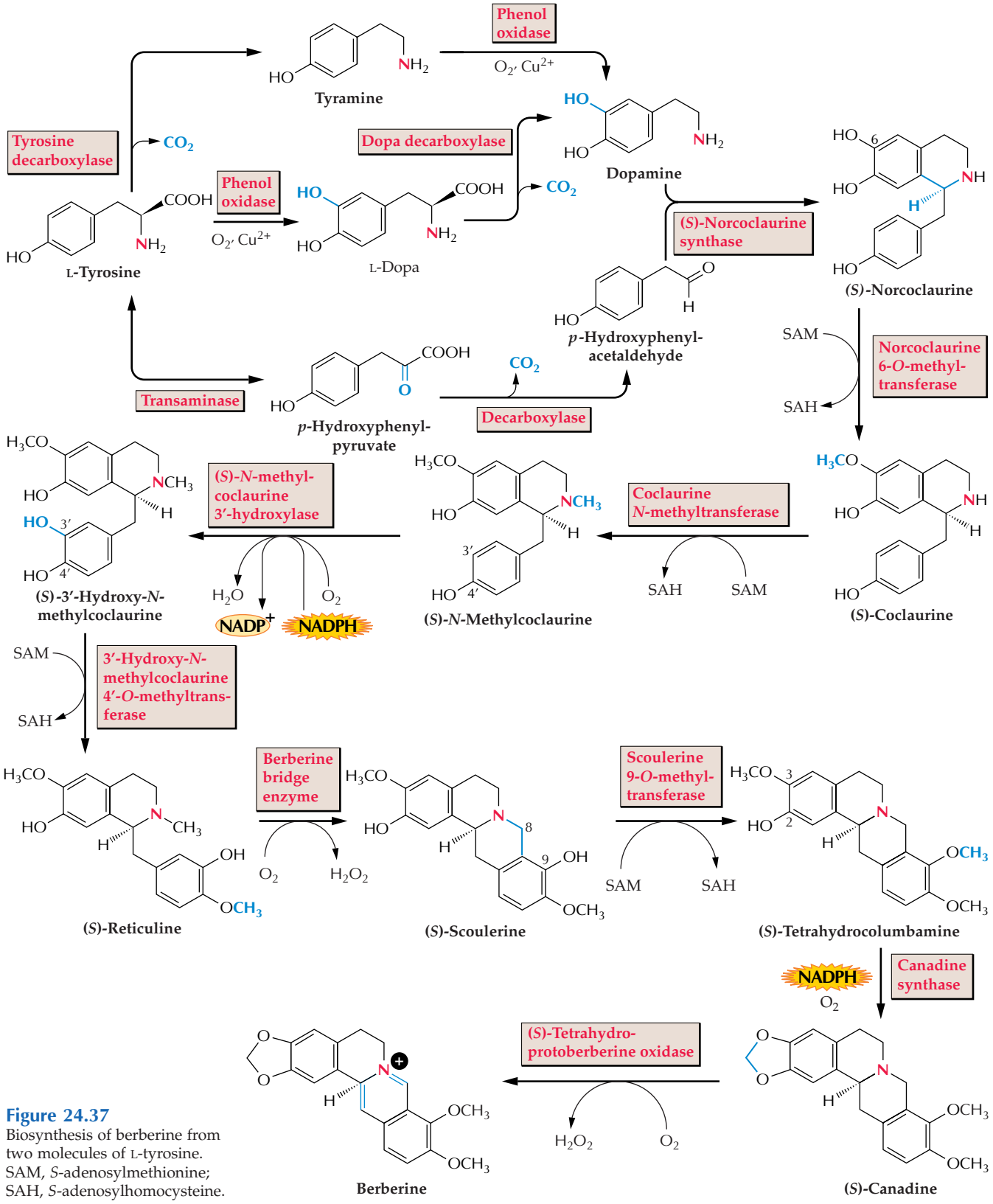


Figure 24.37
Biosynthesis of berberine from two molecules of L-tyrosine. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

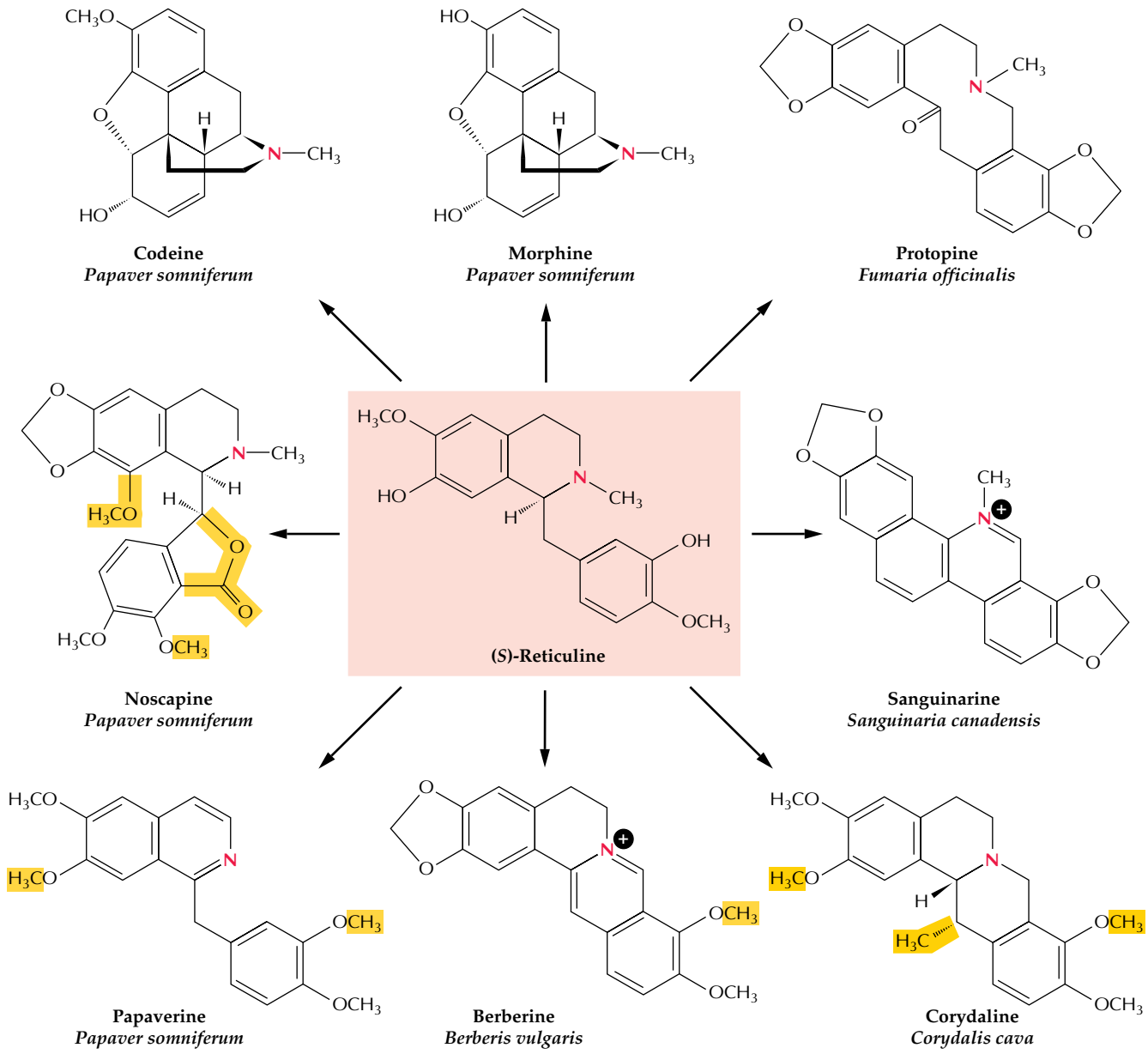


Figure 24.38
(*S*)-Reticuline has been called the chemical chameleon. Depending on how the molecule is twisted and turned before undergoing enzymatic oxidation, a vast array of tetrahydrobenzylisoquinoline-derived alkaloids of remarkably different structures can be formed.

do not appear to participate in primary metabolism. For example, the cytochrome P450–dependent monooxygenases and oxidases of alkaloid biosynthesis differ from the hepatic cytochrome P450–dependent monooxygenases and oxidases of mammals. Unlike the individual mammalian enzymes, which share a common catalytic mechanism and modify a broad range of substrates, the plant enzymes are highly substrate-specific and catalyze reactions previously unknown until discovered in the plant kingdom.

24.8 Biotechnological application of alkaloid biosynthesis research

24.8.1 Available techniques for biochemical and molecular genetic analysis facilitate identification, purification, and production of useful alkaloids.

The current status of the alkaloid branch of the field of natural products reflects the

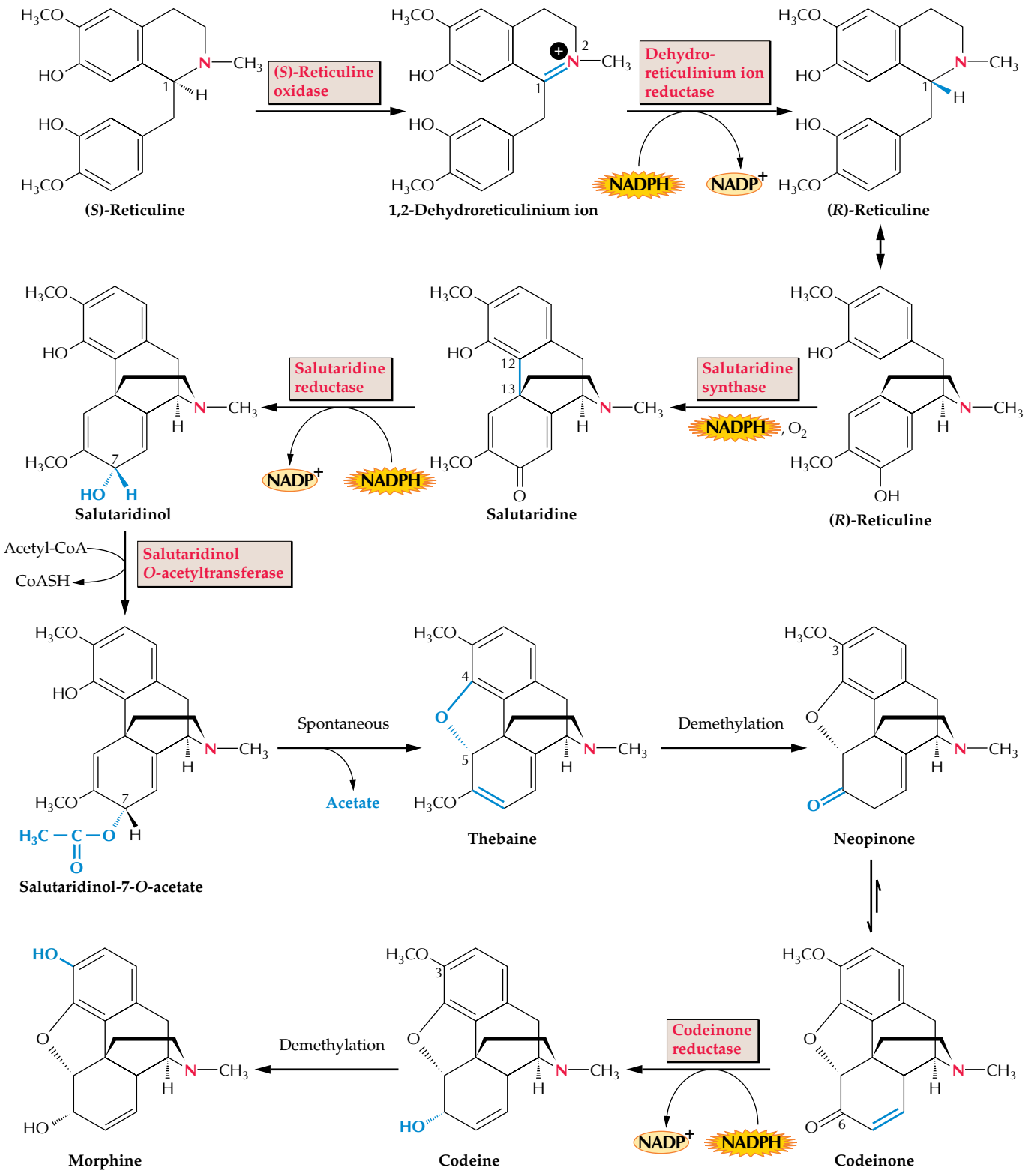


Figure 24.39
Isolation and characterization of all the enzymes of morphine biosynthesis in opium poppy are nearly complete 190 years after discovery of that alkaloid. Many of the equivalent morphine biosynthetic enzymes have been discovered in mammalian liver. Demonstration that the mammalian liver biosynthesizes morphine de novo would have tremendous implications concerning evolutionary development of the opiate receptor in humans.

many new advances in analytical chemistry, enzymology, and pharmacology. Only minimal quantities of a pure alkaloid are now necessary for a complete structure to be elucidated by mass and NMR spectroscopic analyses. Absolute stereochemistry can be unambiguously assigned by determining the crystal structure. The pharmacological activities of crude plant extracts or pure substances are determined by fully automated systems, such that millions of data points are collected each year in industrial screening programs. The factor that limits the number of biological activities for which we can test is the number of available target enzymes and receptors. As more of the underlying biochemical bases for diseases continue to be discovered, the number of test systems will increase.

What happens when a small quantity of an alkaloid of complex chemical structure from a rare plant is found to be physiologically active? The alkaloid must first pass animal and clinical trials; if these are successful, eventually enough material will be needed to satisfy market demand. Researchers can develop **biomimetic** syntheses, which duplicate at least part of the biosynthetic pathways of plants to yield synthetic compounds; alternatively, they can alter the metabolism of the plant to change the alkaloid profile (Fig. 24.40). The regulation of alkaloid biosynthesis in cell culture can also be influenced to produce a desired alkaloid. The following successful studies demonstrate the viability of these approaches.

24.8.2 Metabolic engineering of medicinal plants may be the pharmaceutical biotechnology of the future.

The tropane class of alkaloids, found mainly in the Solanaceae, contains the anticholinergic drugs hyoscyamine and scopolamine. Solanaceous plants have been used traditionally for their medicinal, hallucinogenic, and poisonous properties, which derive, in part, from tropane alkaloids. For obtaining improved sources of pharmaceuticals, metabolic engineering of the plants that serve as commercial sources of scopolamine could augment classical breeding in the effort to develop plants with an optimal alkaloid pattern. The current commercial source of scopolamine is *Duboisia*, which is cultivated on plantations in Australia, Indonesia, and Brazil. Certain other tropane alkaloid–producing species accumulate hyoscyamine instead of scopolamine as the major alkaloid. The question arises whether expression of a transgene in a medicinal plant would alter the alkaloid-producing pattern such that more of the pharmaceutically useful alkaloid, scopolamine, is obtained. To this end, a

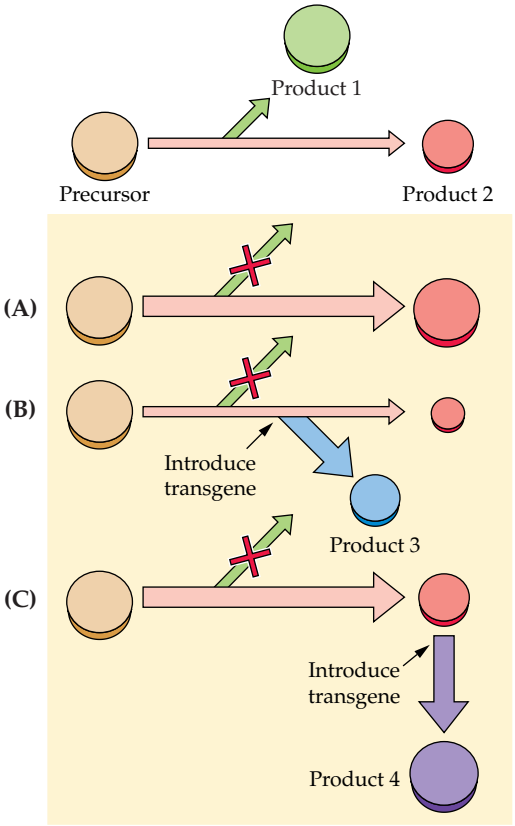


Figure 24.40
Using antisense/cosuppression technologies (see Chapter 7) or overexpression, medicinal plants can be tailored to produce pharmaceutically important alkaloids by eliminating interfering metabolic steps or by introducing desired metabolic steps. Expressing an entire alkaloid biosynthesis pathway of 20 to 30 enzymes in a single microorganism is currently beyond our technical capability. However, altering the pathway in a plant and producing the desired alkaloid either in culture or in the field may now be possible. For example, to accumulate more of the end product alkaloid, a side pathway that also uses the same precursor may have to be blocked (A). To accumulate an alkaloid not normally produced in a particular plant species, a transgene (from another plant or a microorganism) may be introduced (B). If the end product alkaloid would be more useful as a particular derivative, for example, as a more soluble glycoside, a gene that encodes a glycosyl transferase could be introduced (C).

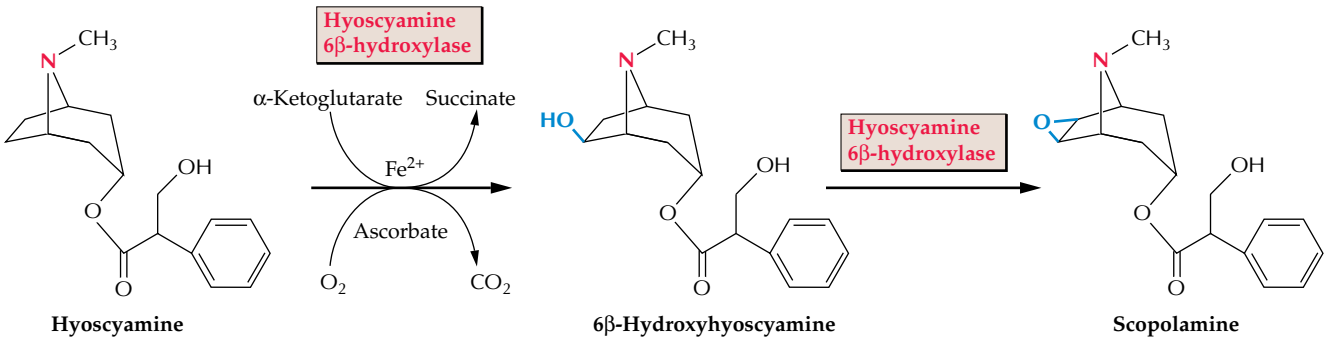


Figure 24.41
The reaction catalyzed by hyoscyamine 6β-hydroxylase along the biosynthetic pathway leading to the tropane alkaloid scopolamine in species of the genera *Hyoscyamus, Duboisia,* and *Datura.* This one enzyme catalyzes two consecutive steps, hydroxylation of hyoscyamine followed by epoxide formation to produce scopolamine.

cDNA encoding hyoscyamine 6β-hydroxylase from *H. niger* (black henbane) has been introduced into *Atropa belladonna* (deadly nightshade) by using *Agrobacterium tumefaciens*– and *A. rhizogenes*–mediated transformation (Fig. 24.41). The resulting transgenic plants and hairy roots each contained greater concentrations of scopolamine than did the wild-type plants. These transgenic *Atropa* plants provided the first example of how medicinal plants could be successfully altered by using molecular genetic techniques to produce increased quantities of a medicinally important alkaloid.

Designing meaningful transformation experiments requires a thorough knowledge of alkaloid biosynthetic pathways. Such studies are also limited by our ability to transform and regenerate medicinal plants. To date, expertise in this important area lags well behind that for tobacco, petunia, and cereal crops. For example, in the area of tropane alkaloids, transformation and regeneration of *Duboisia,* a plant for which plantation, harvesting, and purification techniques have already been established commercially, will have to be developed before any potential commercialization can be considered. Genetic manipulation of plant cell cultures may increase the concentrations of rate-limiting enzymes or may result in expression of gene products not normally induced in cultured cells. If so, alkaloid production in plant cell or tissue culture may become a viable industrial approach (Fig. 24.42).

Another successful example of how metabolic engineering can alter natural products synthesis has been provided by the

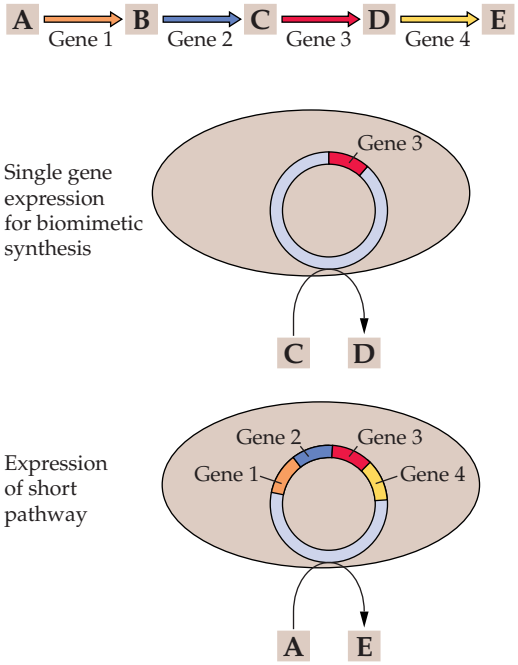


Figure 24.42
One alternative approach to the use of metabolically engineered plants is to use microbes to produce alkaloids. Short biosynthetic pathways can be expressed in either yeast or bacteria and used as a source of alkaloid. Plant alkaloid genes can be functionally expressed in microorganisms to produce either single biotransformation steps or short biosynthetic pathways. For example, a known plant biosynthetic pathway contains an enzyme encoded by gene 3, which catalyzes a transformation step that is difficult to achieve by chemical synthesis. After heterologous expression of gene 3 in a microorganism, the protein product can be used in a biomimetic synthesis of alkaloid D. Likewise, expression of the biosynthetic genes 1 through 4 in a microorganism might produce alkaloid E directly from precursor A.

transformation of *Brassica napus* (canola) with the cDNA encoding the *C. roseus* tryptophan decarboxylase used in biosynthesis of monoterpenoid indole alkaloids. Usefulness of seed from this oil-producing crop as animal feed has been limited in part by the presence of indole glucosinolates (see Chapters 8 and 16), sulfur-containing compounds that make the protein meal less palatable. The tryptophan decarboxylase transgene in canola redirects tryptophan pools away from indole glucosinolate biosynthesis and into tryptamine (Fig. 24.43). The mature seed of the transgenic canola plants contains less of the indole glucosinates and does not accumulate tryptamine, making it more suitable for use as animal feed and achieving a potentially economically useful product.

To date, the elucidation of enzymatic syntheses of at least eight alkaloids is either complete or nearly complete: ajmaline, vindoline, berberine, corydaline, macarpine, morphine, berbamunine, and scopolamine. Of these alkaloids, those in current industrial use, such as morphine and scoploamine, are still being isolated from the plants that produce them rather than synthesized. The future for research on these alkaloids lies in the development of alternative systems of production, such as plant cell or microbial cultures, and in the development of plants with an improved spectrum of alkaloids for a more efficient production of the pharmaceuticals currently isolated from field-grown plants. The design of these alternative systems and optimized plants requires molecular manipulation, which in turn requires knowledge of alkaloid biosynthetic pathways at the enzyme level. Much progress has been made with select alkaloids, but much remains to be discovered about the enzymatic synthesis of pharmaceutically important alkaloids such as camptothecin, quinine, and emetine, to name only a few examples. cDNAs have now been isolated for approximately 20 enzymes of alkaloid biosynthesis, and the rate at which new clones are identified is certain to increase in the coming years. As genes are isolated, we can anticipate that heterologous expression systems will be developed in bacterial, yeast, and insect cell culture systems to allow production of single enzymes, and perhaps even short pathways, for biomimetic syntheses of alkaloids. Our understanding of how the expression of alkaloid biosynthesis genes is regulated by elicitors or in specific tissues will also improve as the promoters of alkaloid biosynthetic genes are analyzed. The future will almost certainly bring genetically engineered microorganisms and eukaryotic cell cultures that produce alkaloids, metabolically engineered medicinal plants with tailored alkaloid spectra, pharmaceutically important alkaloids in plant cell culture, and even enzymatic synthesis of as yet unknown alkaloids through combinatorial biochemistry.

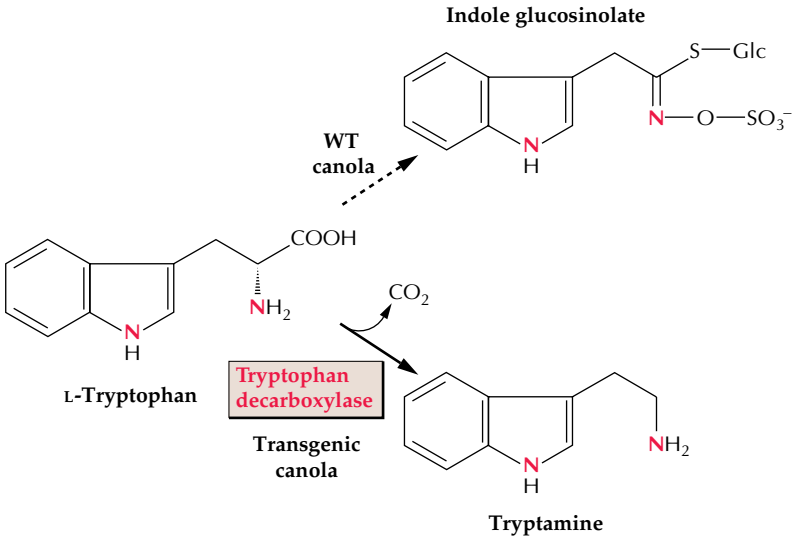


Figure 24.43
Metabolic engineering to improve the quality of canola oil. A canola cultivar is transformed with a gene from *Catharanthus roseus* that encodes tryptophan decarboxylase, an enzyme involved in biosynthesis of monoterpenoid indole alkaloids. The transgene effectively directs the L-tryptophan pool away from use in biosynthesis of the bitter indole glucosinolate and into the production of tryptamine. WT, wild type.

24.9 Phenylpropanoid and phenylpropanoid-acetate pathway metabolites

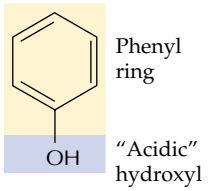
24.9.1 Plants contain a remarkably diverse array of phenolic compounds.

Plants originated in an aquatic environment. Their successful evolutionary adaptation to land was achieved largely by massive formation of "plant phenolic" compounds. Although the bulk of these substances assumed cell wall structural roles, a vast array of nonstructural constituents was

also formed, having such various roles as defending plants, determining certain distinguishing features of different woods and barks (e.g., durability), establishing flower color, and contributing substantially to certain flavors (tastes and odors). These functions and others performed by plant phenolics are essential for the continued survival of all types of vascular plants. Accounting for about 40% of organic carbon circulating in the biosphere, these phenolic compounds are primarily derived from **phenylpropanoid, phenylpropanoid-acetate,** and related biochemical pathways such as those leading to "hydrolyzable" tannins. Furthermore, it is their reassimilation back to carbon dioxide during biodegradation (mineralization) that presents the rate-limiting step in recycling biological carbon.

Plant phenolics are generally characterized as aromatic metabolites that possess, or formerly possessed, one or more "acidic" hydroxyl groups attached to the aromatic arene (phenyl) ring (Fig. 24.44). These compounds plagued plant scientists for years by interfering with experimental methods. For example, when exposed to air, plant phenolics readily oxidize and turn brown, generating products that form complexes with proteins and inhibit enzyme activity. Many protocols now used to isolate plant proteins and nucleic acids include special precautions designed to minimize interference by phenolic compounds. Cultured plant tissues can also release phenolics that inhibit growth of callus and regeneration of plantlets. At the same time, phenolic compounds are increasingly being recognized for their profound impact on plant growth, development, reproduction, and defense; indeed, scientists have come to appreciate their significance more fully, particularly over the past few decades.

The discussion of plant phenolic substances is a discussion of plant diversity itself. Characteristics unique to each of the roughly 250,000 species of vascular plants arise, at least in part, through differential deposition of highly specialized phenylpropanoid and phenylpropanoid-acetate derivatives. No single species can be used to illustrate the extraordinary diversity of "secondary" metabolites that exists within the plant kingdom, because many branches of the pathways are found or are amplified only in specific plant families. Placing undue emphasis on any single plant species can obscure the extremely broad variation in biosynthetic capabilities that has yielded this spectrum of different plant types.

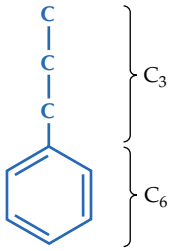


Phenol

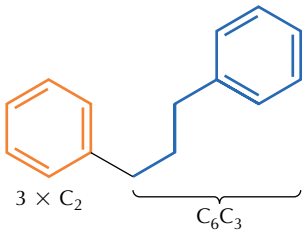
Figure 24.44
Structure of phenol.

24.9.2 Most, but not all, plant phenolic compounds are products of phenylpropanoid metabolism.

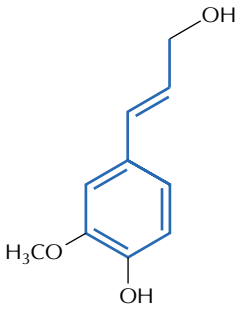
Most plant phenolics are derived from the phenylpropanoid and phenylpropanoid-acetate pathways (Fig. 24.45) and fulfill a very broad range of physiological roles in planta. In ferns, fern allies, and seed plants, polymeric **lignins** reinforce specialized cell walls, enabling them to support their massive weights on land and to transport water and minerals from roots to leaves. Closely related to lignins, the **lignans** can vary from dimers to higher oligomers. Widespread throughout the plant kingdom, lignans can, for example, either help defend



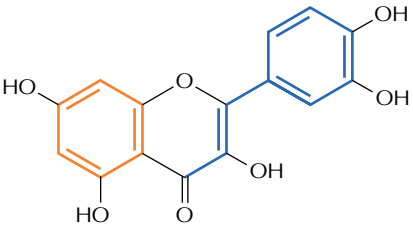
Phenylpropanoid skeleton (C_6C_3)



Phenylpropanoid–acetate skeleton (C_6C_3–C_6), with phenylpropanoid-derived (C_6C_3) and acetate-derived (3 × C_2) rings



Coniferyl alcohol, a component of lignins and many lignans



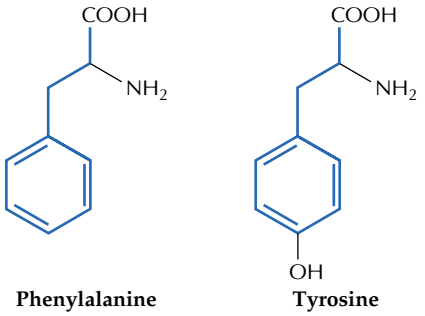
Quercetin, a flavonoid (C_6C_3–C_6)

Figure 24.45
Phenylpropanoid and phenylpropanoid-acetate skeletons and representative plant compounds based on those structures.

Phenylpropanoid skeleton
Acetate-derived rings

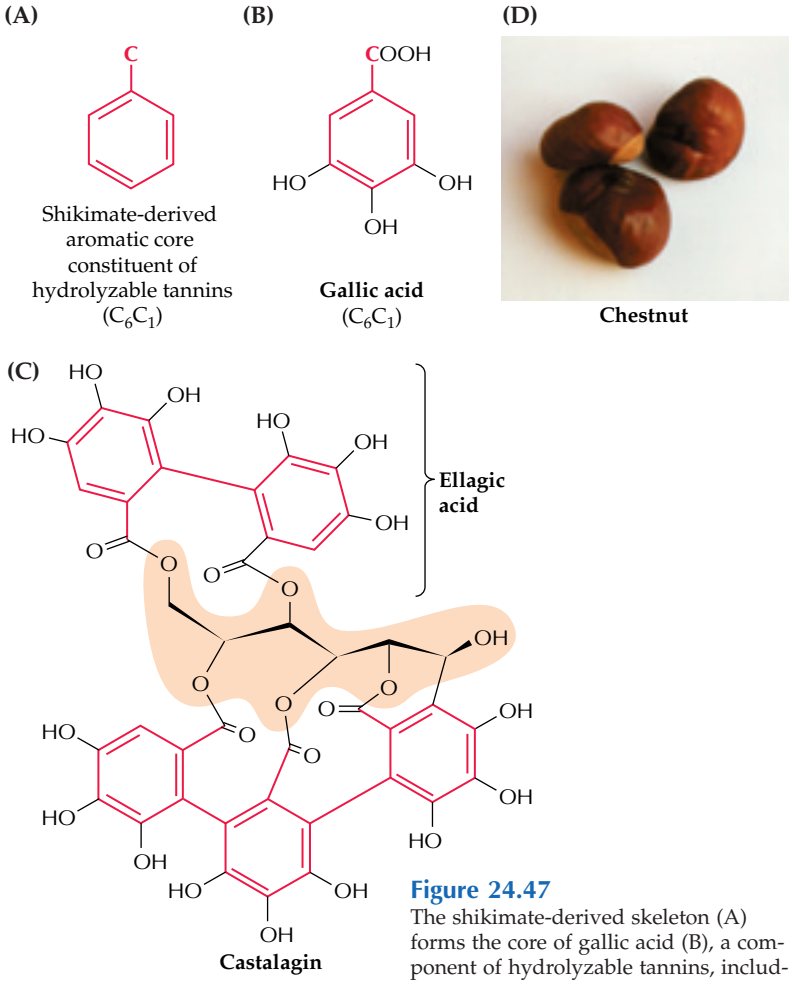
against various pathogens or act as antioxidants in flowers, seeds, seed coats, stems, nuts, bark, leaves, and roots. **Suberized** tissues contain alternating layers of hydrophobic (aliphatic) and hydrophilic (phenolic) structural substances. Present in cork, bark, roots, and certain periderm tissues (e.g., potato skin), suberized tissues function, for example, by providing a protective barrier, thereby limiting the effects of desiccation from the atmosphere and pathogen attack. The **flavonoids** comprise an astonishingly diverse group of more than 4500 compounds. Among their subclasses are the anthocyanins (pigments), proanthocyanidins or condensed tannins (feeding deterrents and wood protectants), and isoflavonoids (defensive products and signaling molecules). The **coumarins, furanocoumarins,** and **stilbenes** protect against bacterial and fungal pathogens, discourage herbivory, and inhibit seed germination. Numerous miscellaneous phenolics also play defensive roles or impart characteristic tastes and odors to plant material.

Although most plant phenolics are products of phenylpropanoid metabolism, with the phenylpropanoids, in turn, being derived from phenylalanine and tyrosine (Fig. 24.46), some phenolic compounds are generated through alternative pathways. For example, hydrolyzable tannins, a group of mostly polymeric substances that appear to act in plant defense, are typically copolymers of carbohydrates and the shikimate-derived gallic and ellagic acids (Fig. 24.47). Found in the leaves, fruits, pods, and galls of some woody dicots, hydrolyzable tannins have not yet been identified in monocots. "Condensed tannins," on the other hand, are widespread and occur in practically all trees and shrubs. Known as proanthocyanidins, these compounds are synthesized by the phenylpropanoid-acetate pathway. Some others, such as the phenolic psychoactive compounds of cannabis, the tetrahydrocannabinoids, are polyketide (acetate) or terpenoid derivatives (Fig. 24.48).



Phenylalanine **Tyrosine**

Figure 24.46
The aromatic amino acids phenylalanine and tyrosine are derivatives of the shikimic–chorismic acid pathway (see Chapter 8).



Shikimate-derived aromatic core constituent of hydrolyzable tannins (C_6C_1)

Gallic acid (C_6C_1)

Chestnut

Castalagin

Figure 24.47
The shikimate-derived skeleton (A) forms the core of gallic acid (B), a component of hydrolyzable tannins, including castalagin (C) from chestnut (D).

24.10 Phenylpropanoid and phenylpropanoid-acetate biosynthesis

24.10.1 Phenylalanine (tyrosine) ammonia-lyase is a central enzyme in phenylpropanoid metabolism.

One enzyme directs carbon from aromatic amino acids to the synthesis of phenylpropanoid metabolites. This enzyme converts phenylalanine (PAL) to cinnamic acid and tyrosine (TAL) to *p*-coumaric acid (Fig. 24.49, reactions 1 and 2). Interestingly, in

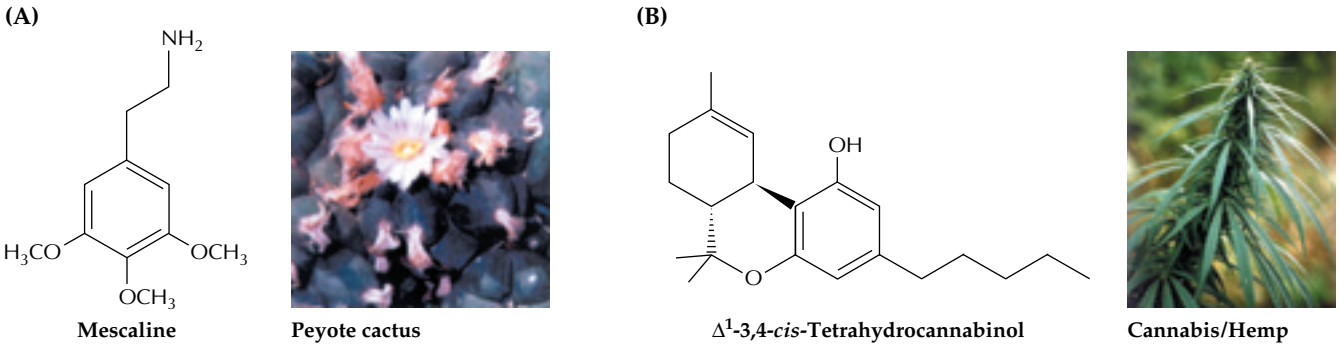


Figure 24.48
Not all plant phenolic compounds are derived from phenylpropanoid substrates. Whereas mescaline, the psychoactive component of peyote, is a phenylpropanoid derivative (A), the phenolic compound Δ^1-3,4-*cis*-tetrahydrocannabinol (B), a psychoactive component of cannabis, is a product of polyketide synthesis, the repeated condensation of acetyl-CoA units derived from malonyl-CoA.

most vascular plants, Phe is the highly preferred substrate, but the monocot enzyme can utilize both Phe and Tyr. PAL has been detected in a few aquatic plants, where it probably functions in formation of simple flavonoids, such as the C-glucosyl–linked lucenin and vicenin of *Nitella* species (Fig. 24.50). Lignins, however, are not present in aquatic plants. Thus, this PAL (TAL) enzymatic step and the products of the various phenylpropanoid and phenylpropanoid-acetate pathways appear to have been key to the plant colonization of land.

PAL is the most extensively studied enzyme in the phenylpropanoid pathway, perhaps in all secondary metabolism. In some plants, PAL appears to be encoded by a single gene, whereas in others it is the product of a multigene family. The enzyme requires no cofactor for activity. The ammonium ion liberated by the PAL reaction is recycled by way of glutamine synthetase and glutamate synthetase (GS-GOGAT; see Chapter 8). Once assimilated into glutamate, the amino group can be donated to prephenate, forming arogenate, a precursor of both phenylalanine and tyrosine (Fig. 24.51). This nitrogen-cycling process ensures a steady supply of the aromatic amino acids from which plant phenolics are derived.

24.10.2 Biochemical pathways to distinct phenolic classes share many common features.

During the 1960s and early 1970s, impressive progress was made in defining many of the salient features of the pathway that converts cinnamic acid to the monolignols (see Fig. 24.49). This pathway essentially comprises four types of enzymatic reactions: aromatic hydroxylations, O-methylations, CoA ligations, and NADPH-dependent reductions. More recently, the precise enzymology involved in earlier parts of the pathway has come under renewed attention, focusing particularly on aromatic hydroxylations and on whether the O-methylation steps utilize the free acids or CoA esters.

Aromatic ring hydroxylation involves three distinct hydroxylation conversions, all of which are believed to be microsomal. The best studied of these enzymes, cinnamate-4-hydroxylase, is an oxygen-requiring, NADPH-dependent, cytochrome P450 enzyme that catalyzes the regiospecific hydroxylation at the *para*-position of cinnamic acid to give *p*-coumaric acid (see Fig. 24.49, reaction 3). The other two hydroxylases originally were thought to introduce hydroxyl groups into the free acids *p*-coumarate or ferulate (or their CoA ester forms), yielding the diphenol (catechol) products caffeic acid or 5-hydroxyferulic acid (or their CoA derivatives), respectively (see Fig. 24.49, reaction 4). At this time, however, substantial confusion remains as to how the caffeoyl moiety of caffeic acid or caffeoyl-CoA is formed. Whether this biosynthesis involves a nonspecific phenolase-catalyzed conversion or whether some other enzymatic step (e.g., one involving an NADPH-dependent cytochrome P450) is used is still not known. Additionally, although ferulate-5-hydroxylase has been established as an NADPH-dependent cytochrome P450 enzyme, there

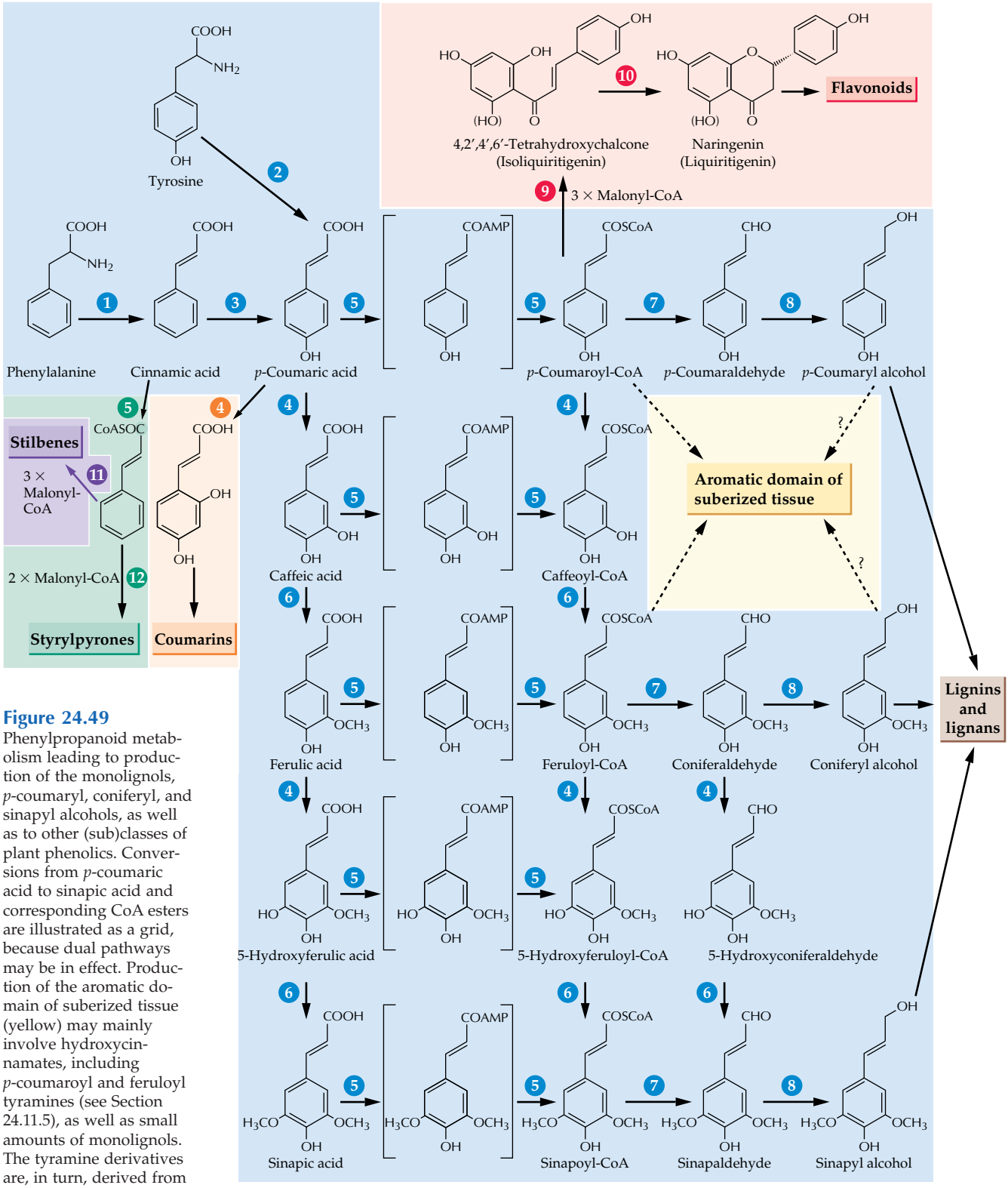


Figure 24.49
Phenylpropanoid metabolism leading to production of the monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols, as well as to other (sub)classes of plant phenolics. Conversions from *p*-coumaric acid to sinapic acid and corresponding CoA esters are illustrated as a grid, because dual pathways may be in effect. Production of the aromatic domain of suberized tissue (yellow) may mainly involve hydroxycinnamates, including *p*-coumaroyl and feruloyl tyramines (see Section 24.11.5), as well as small amounts of monolignols. The tyramine derivatives are, in turn, derived from *p*-coumaroyl-CoA and feruloyl-CoA. Enzymes (and their cofactors) are as follows: 1. PAL; 2. PAL (or TAL), found mainly in grasses; 3. cinnamate-4-hydroxylase (O_2, cytochrome P450, NADPH); 4. hydroxylases (O_2, cyt. P450, NADPH); 5. CoA ligases that participate in ligation of AMP and CoA (CoASH, ATP); 6. *O*-methyltransferases (SAM); 7. cinnamoyl-CoA:NADPH oxidoreductases (NADPH); 8. cinnamoyl alcohol dehydrogenases (NADPH); 9. chalcone synthase; 10. chalcone isomerase; 11. stilbene synthase; 12. styrylpyrone synthase. Products in parentheses refer to less common pathways. [Note: Sequence of intermediates in the pathways leading to sinapyl alcohol awaits experimental confirmation at the time of writing. The reader is encouraged to read the pertinent literature on developments in this area.]

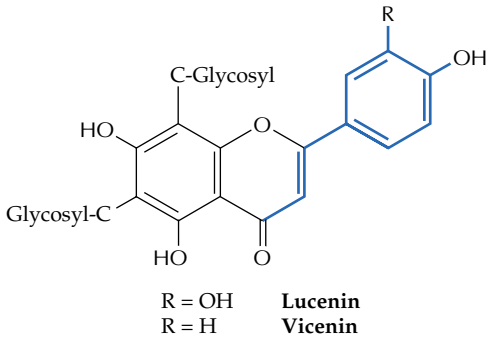


Figure 24.50
C-Glycosyl flavonoid types reported to be present in a green alga, *Nitella hookeri* (Charophyceae).

is still some uncertainty as to whether it is ferulic acid, feruloyl-CoA, coniferaldehyde, or coniferyl alcohol that serves as the physiological substrate.

Researchers have not yet determined whether in some instances the O-methylation steps precede CoA-ligation, or whether both routes are possible (see Fig. 24.49, reaction 6). In any case, O-methyltransferases, whether acting on free acids or CoA esters, introduce methyl groups in a highly regiospecific manner, methylating the *meta*-hydroxyl group but not the group at the *para*-position. The enzyme catalyzing this transformation uses *S*-adenosylmethionine (SAM) as a cofactor, whereas CoA ligation requires ATP and CoASH. This two-step ligation first generates the AMP derivative, then converts it into the corresponding CoA ester.

After the CoA ester is formed, two sequential NADPH-dependent reductions produce the monolignols, completing the general phenylpropanoid pathway (see Fig. 24.49, reactions 7 and 8). The first of these enzymes, cinnamoyl-CoA reductase, catalyzes formation of *p*-coumaraldehyde (*p*-hydroxycinnamaldehyde), coniferaldehyde, and possibly sinapaldehyde. This type B reductase

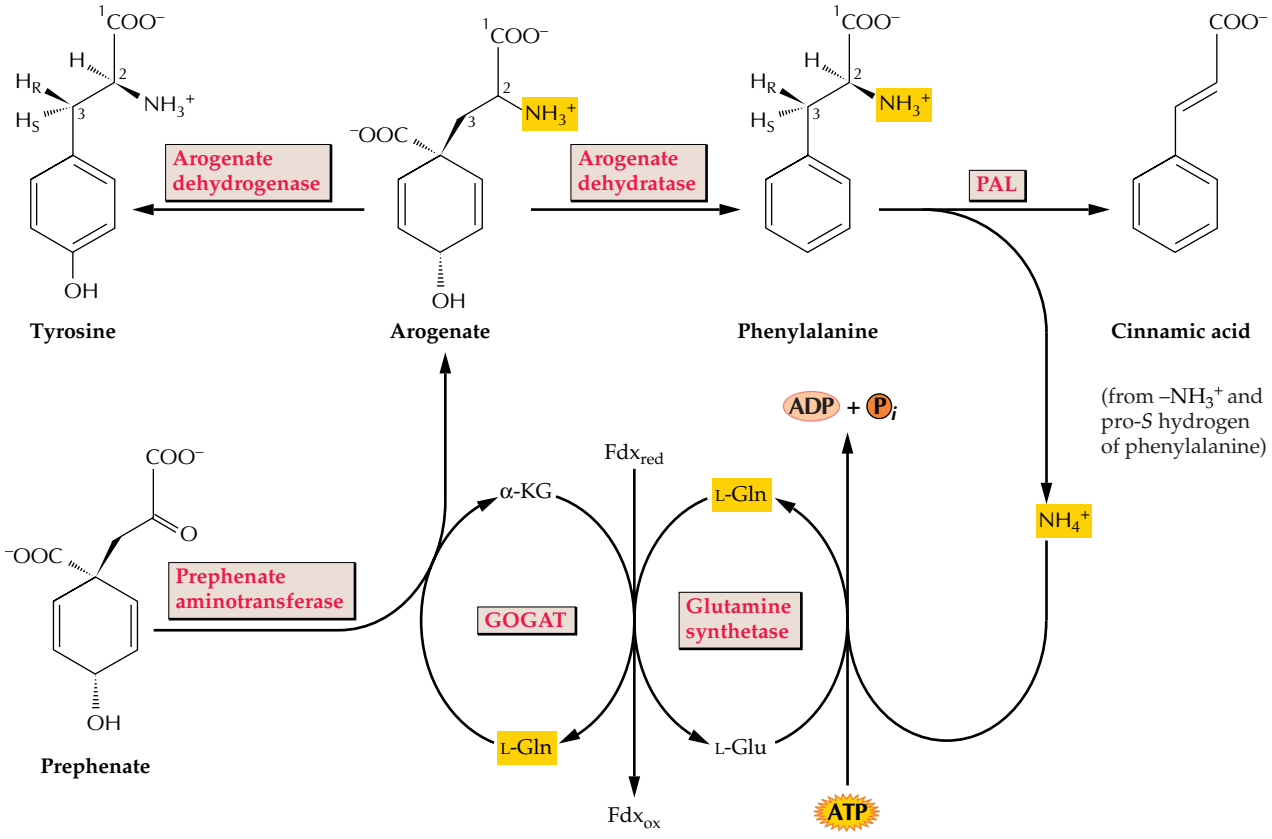


Figure 24.51
During active phenylpropanoid metabolism, nitrogen from phenylalanine is recycled. Although TAL activity has been reported in certain plant species, no report has yet established a comparable nitrogen-recycling system for tyrosine. GOGAT, glutamine: α-ketoglutarate aminotransferase; L-Gln, glutamine; L-Glu, glutamate; α-KG, α-ketoglutarate; Fdx_{red}, reduced ferredoxin; Fdx_{ox}, oxidized ferredoxin.

abstracts the pro-*S* hydride (Fig. 24.52) from behind the nicotinamide plane of NADPH during reduction. The second enzyme, cinnamyl alcohol dehydrogenase, is a type A reductase that abstracts the pro-*R* hydride from in front of the nicotinamide plane to yield the monolignols *p*-coumaryl, coniferyl, and sinapyl alcohols (Fig. 24.52).

The above description is a brief account of the overall biochemical steps that culminate in monolignol formation. However, the pathway shown in Figure 24.49 is deceptive. Not all cells, tissues, or species of plants utilize the entire pathway. In many instances, plants utilize only a small pathway segment that directs substrates to one or more of the main metabolic branchpoints; moreover, they may express that truncated pathway only in specific tissues. Researchers do not yet fully understand metabolic flux and compartmentalization in the phenylpropanoid pathway. Elucidation of these processes will be a necessary step toward defining or identifying the control points in the pathway.

24.11 Biosynthesis of lignans, lignins, and suberization

The monolignols are primarily converted into two distinct classes of plant metabolites: the lignans and the lignins. Most metabolic flux through the phenylpropanoid biosynthetic pathway is directed to the production of the lignins, which are structural components of cell walls. Free radicals participate in the reactions that produce both dimeric/oligomeric lignans and lignins as well as related complex plant polymers such as those in suberized tissue.

24.11.1 Dimeric and oligomeric lignans are formed primarily from coniferyl alcohol.

The term lignan was initially coined by Robert Downs Haworth in 1936 to describe a class of dimeric phenylpropanoid (C_6C_3) metabolites linked by way of their 8–8′ bonds. More recently, another term, **neolignan,** was used to define all of the other types of linkages (e.g., 8–1′-linked dimers), but has since been modified to encompass substances derived from allylphenol compounds, such as isoeugenol (Fig. 24.53). In this chapter, however, we have chosen to use the more convenient name lignan to describe all possible phenylpropanoid (C_6C_3) coupling products, so long as the coupling mode (e.g., 8–8′, 8–5′) is specified (Fig. 24.54). Interestingly, although several thousand lignans are now known in nature, relatively few coupling modes have been encountered.

Lignan dimers are found in ferns, gymnosperms, and angiosperms, but higher

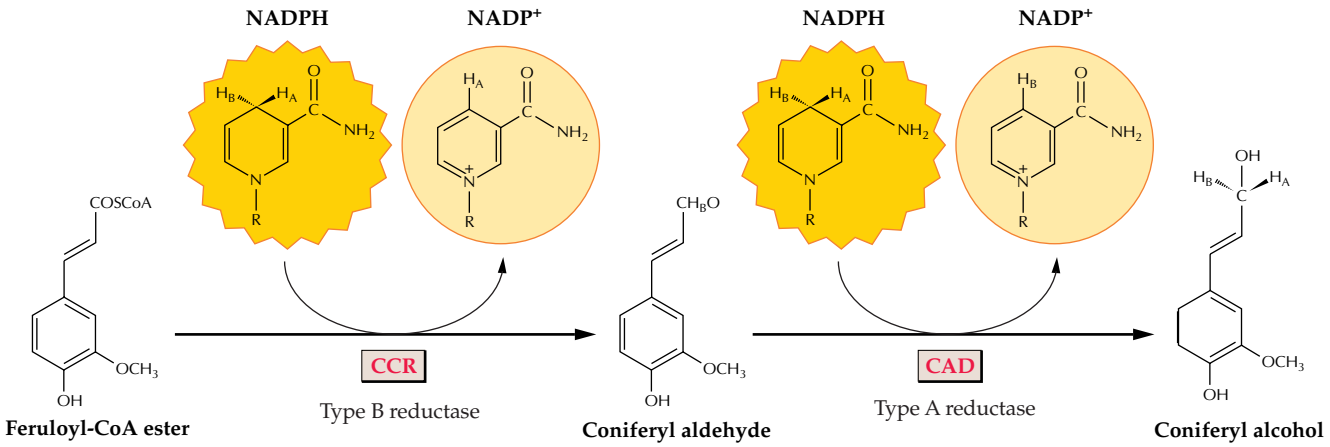


Figure 24.52
The stereospecificity of a type B reductase, NADPH-dependent cinnamoyl-CoA reductase (CCR), and a type A reductase, cinnamyl alcohol dehydrogenase (CAD). H_A, pro-*R* (the hydrogen projecting upward from the A-face of the nicotinamide ring, i.e., out from the plane of the page); H_B, pro-*S* (the hydrogen projecting upward from the B-face of the nicotinamide ring, i.e., behind the plane of the page). R, adenine nucleotide diphosphate.

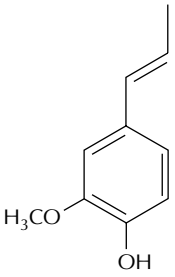


Figure 24.53
Isoeugenol, an allylphenol.

oligomeric forms also occur. Lignan formation utilizes coniferyl alcohol predominantly, along with other monolignols, allylphenols, and phenylpropanoid monomers to a lesser extent. Most lignans are optically active, although the particular antipode (enantiomer) can vary with the plant source.

The biochemistry of lignan formation has only very recently begun to be delineated. To date, work has focused mainly on generation of the most common 8–8′-linked lignans. This class of natural products is formed by a strict stereoselective coupling of two coniferyl alcohol molecules. The first demonstrated example of stereoselective control of phenolic coupling was the in vitro synthesis of (+)-pinoresinol (Fig. 24.55). This overall reaction, discovered in *Forsythia* species, is as follows: A laccase or laccase-like enzyme catalyzes a one-electron oxidation that forms the corresponding free radicals, and a **dirigent protein** (Latin: *dirigere,* to guide or align) orients the putative free radical substrates in such a way that random coupling cannot occur; only formation of the 8–8′-coupled intermediate, (+)-pinoresinol, is permitted. The particular antipode (optical form) of pinoresinol formed also varies with the plant species in question; for example, flax seeds accumulate (–)-pinoresinol. Once formed, pinoresinol can then undergo a variety of conversions, depending on the plant species.

The gene encoding the *Forsythia* dirigent protein has been cloned and the functional recombinant protein expressed. It is not homologous to any other protein. Given the existence of lignans linked by way of other distinct bonding modes and the increasing number of homologous genes and expressed sequence tags found in this and other species,

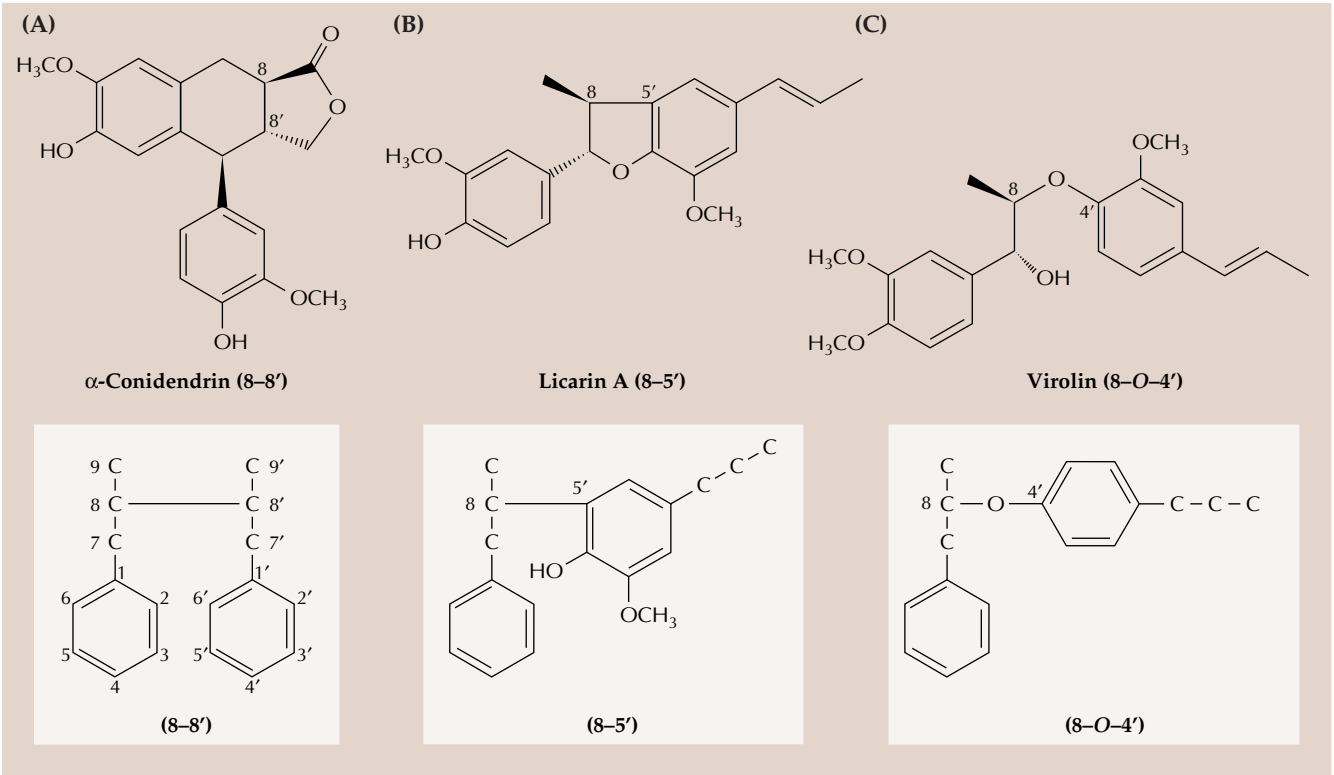


Figure 24.54
Examples of lignans derived by distinct coupling modes, e.g., 8–8′, 8–5′, and 8–*O*–4′.

Forsythia intermedia

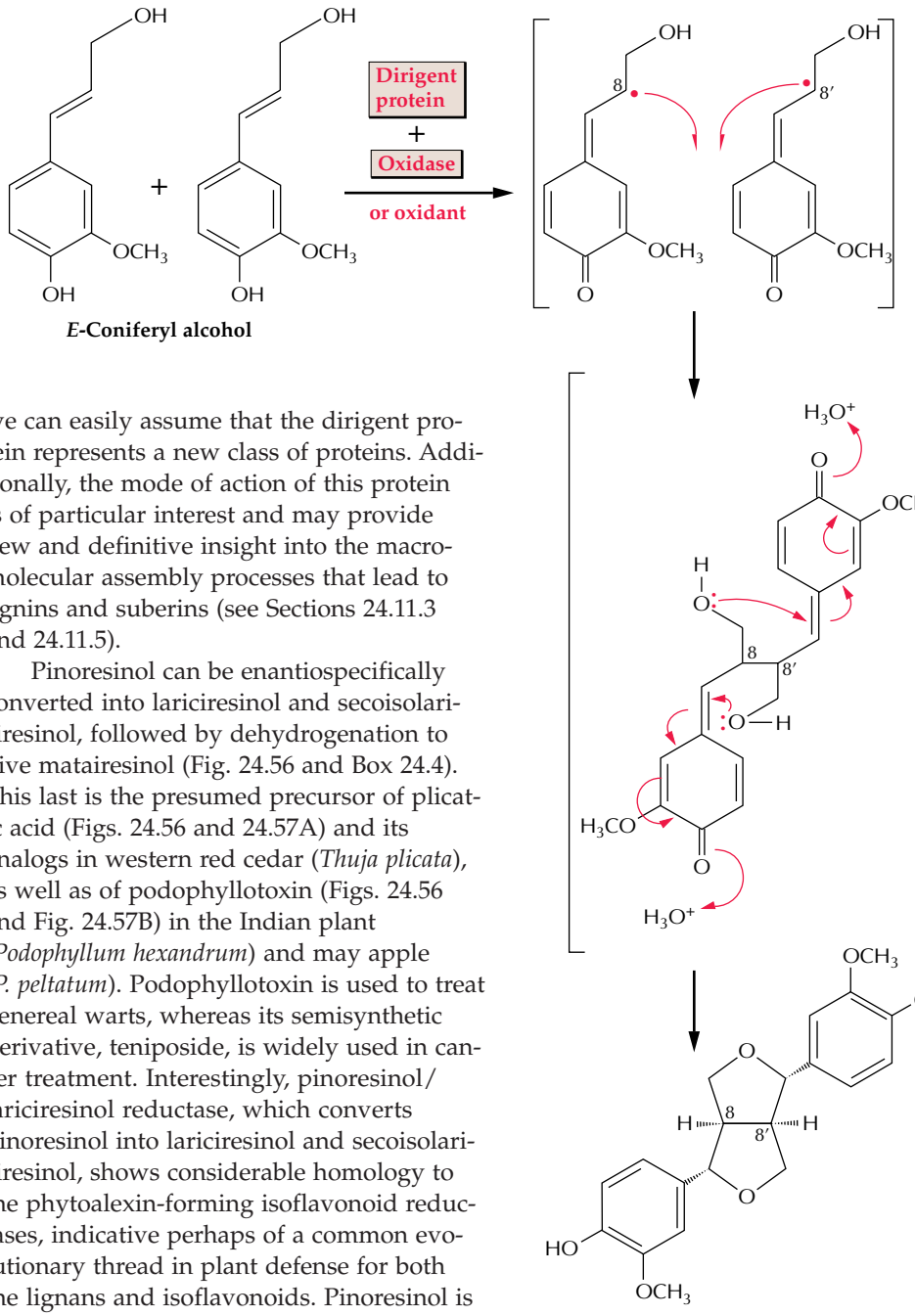


Figure 24.55
Proposed biochemical mechanism accounting for stereoselective control (regio- and stereochemistry) of *E*-coniferyl alcohol coupling in *Forsythia* species. The particular enantiomer of pinoresinol formed can vary with plant species.

we can easily assume that the dirigent protein represents a new class of proteins. Additionally, the mode of action of this protein is of particular interest and may provide new and definitive insight into the macromolecular assembly processes that lead to lignins and suberins (see Sections 24.11.3 and 24.11.5).

Pinoresinol can be enantiospecifically converted into lariciresinol and secoisolariciresinol, followed by dehydrogenation to give matairesinol (Fig. 24.56 and Box 24.4). This last is the presumed precursor of plicatic acid (Figs. 24.56 and 24.57A) and its analogs in western red cedar (*Thuja plicata*), as well as of podophyllotoxin (Figs. 24.56 and Fig. 24.57B) in the Indian plant (*Podophyllum hexandrum*) and may apple (*P. peltatum*). Podophyllotoxin is used to treat venereal warts, whereas its semisynthetic derivative, teniposide, is widely used in cancer treatment. Interestingly, pinoresinol/lariciresinol reductase, which converts pinoresinol into lariciresinol and secoisolariciresinol, shows considerable homology to the phytoalexin-forming isoflavonoid reductases, indicative perhaps of a common evolutionary thread in plant defense for both the lignans and isoflavonoids. Pinoresinol is also the precursor of the antioxidant sesamin (Fig. 24.57C) in the seeds of sesame (*Sesamum indicum*).

24.11.2 Lignin biosynthesis has been described as a largely nonenzymatic process, but differences between synthetic and biologically derived lignins cast doubt on this premise.

Derived from the Latin *lignum* (wood), the term lignin initially was coined to describe the noncellulosic encrusting substance present in woody tissue. After cellulose, lignins are the most abundant organic natural products known, accounting for as much as 20% to 30% of all vascular plant tissue. Deposition of lignins in plants results in the formation of woody secondary xylem tissues in trees, as well as reinforcement of vascular tissues in herbaceous plants and grasses. There are still no methods available for

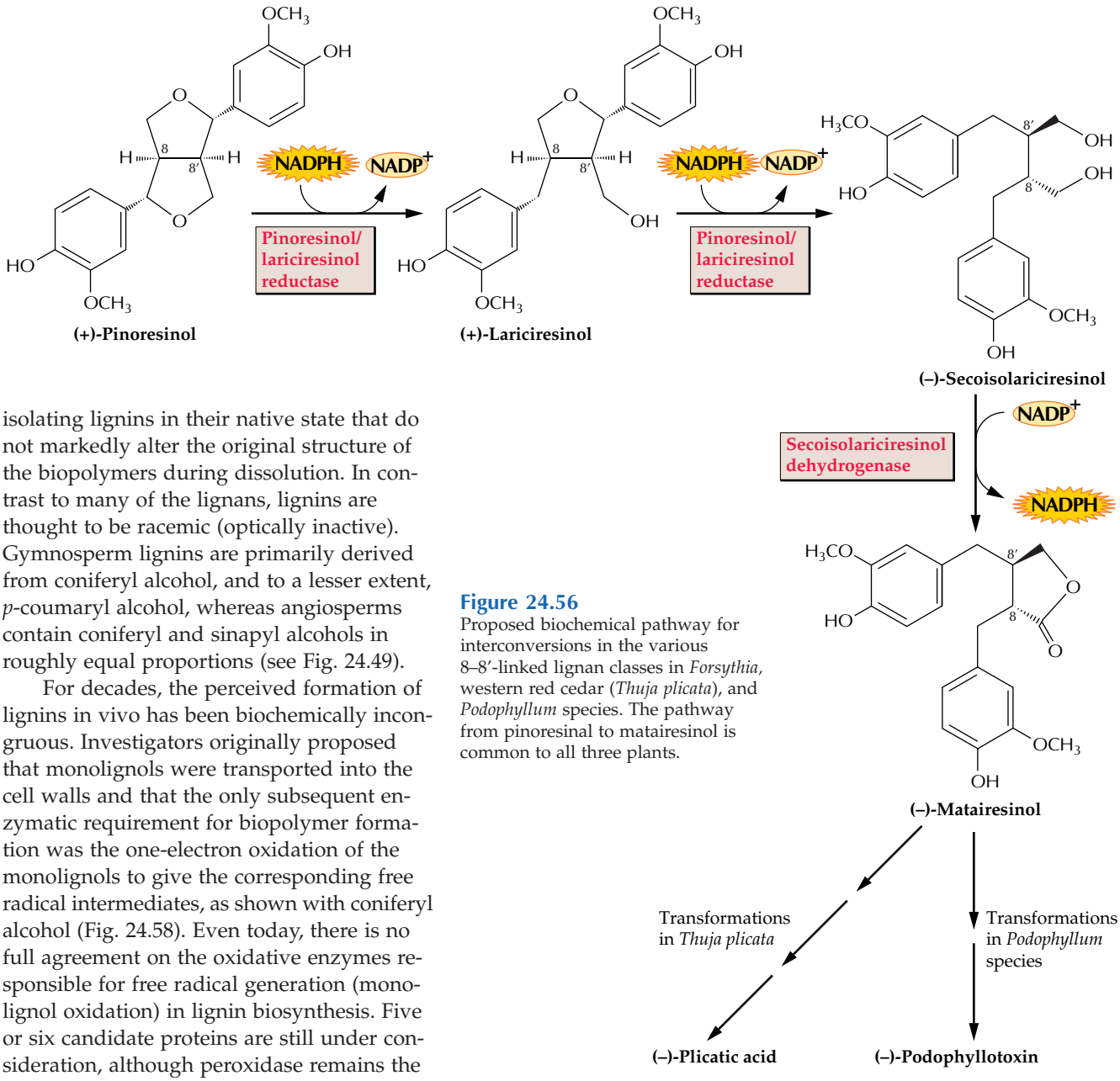


Figure 24.56
Proposed biochemical pathway for interconversions in the various 8–8′-linked lignan classes in *Forsythia*, western red cedar (*Thuja plicata*), and *Podophyllum* species. The pathway from pinoresinal to matairesinol is common to all three plants.

isolating lignins in their native state that do not markedly alter the original structure of the biopolymers during dissolution. In contrast to many of the lignans, lignins are thought to be racemic (optically inactive). Gymnosperm lignins are primarily derived from coniferyl alcohol, and to a lesser extent, *p*-coumaryl alcohol, whereas angiosperms contain coniferyl and sinapyl alcohols in roughly equal proportions (see Fig. 24.49).

For decades, the perceived formation of lignins in vivo has been biochemically incongruous. Investigators originally proposed that monolignols were transported into the cell walls and that the only subsequent enzymatic requirement for biopolymer formation was the one-electron oxidation of the monolignols to give the corresponding free radical intermediates, as shown with coniferyl alcohol (Fig. 24.58). Even today, there is no full agreement on the oxidative enzymes responsible for free radical generation (monolignol oxidation) in lignin biosynthesis. Five or six candidate proteins are still under consideration, although peroxidase remains the most favored.

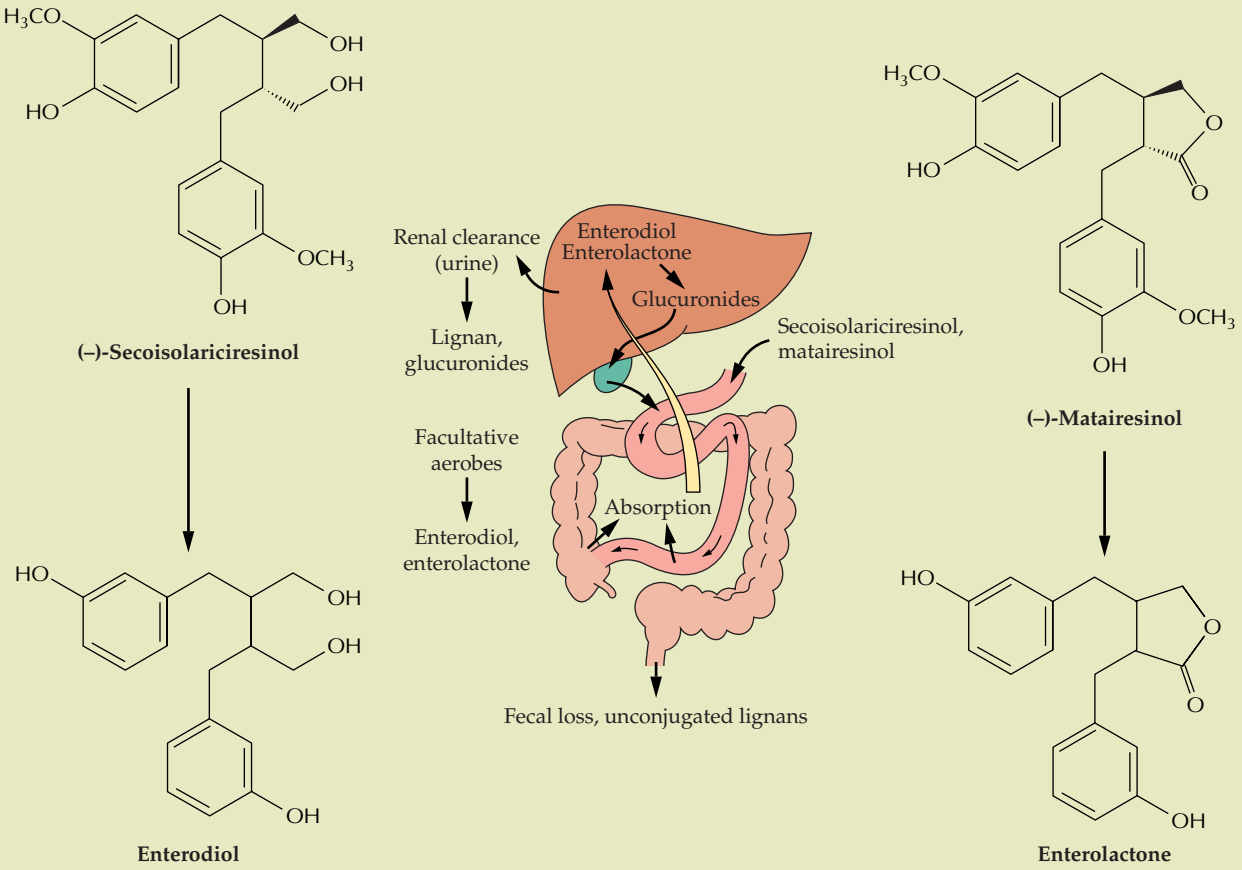
The free radical intermediates formed by oxidation were initially believed to couple together in a manner requiring no further enzymatic control or input. These nonenzymatic free radical coupling reactions were thought to generate dimeric lignan structures that underwent further reoxidation and coupling to yield the lignin biopolymer (Fig. 24.58). In other words, the random reactions of monolignol-derived free radical intermediates in a test tube were considered to give preparations identical to the lignins formed in vivo. According to this model, nature's second most abundant substance is the only natural product for which its formation is not under enzymatic control.

However, although it is rarely recognized, natural and synthetic lignins differ in terms of bonding frequency, bonding type, and macromolecular size. For example, for lignins in vivo, the 8–*O*–4′ interunit linkage predominates (more than 50%), with the 8–5′ substructure found in much lower amounts (about 9–12%) (Fig. 24.59). In contrast, in synthetic in vitro preparations, the 8–*O*–4′ substructure is present to only a very small extent, and the 8–5′ and 8–8′ linkages

Box 24.4 Dietary lignans have health-protecting functions.

Secoisolariciresinol and matairesinol are common constituents of various plants, including *Forsythia intermedia,* flax, and certain vegetables and grains (e.g., green beans and rye). These lignans have important nutritional functions in health protection. During digestion, intestinal bacteria convert secoisolariciresinol and matairesinol to enterodiol and enterolactone, respectively (see figure). These "mammalian" lignans undergo enterohepatic circulation, in which they are conjugated in the liver, excreted in the bile, deconjugated in the intestine by bacterial enzymes, absorbed across the intestinal mucosa, and returned to the liver in the portal circulation (see figure).

Enterodiol and enterolactone are believed to be responsible for preventing the onset of and substantially reducing the rate of incidence of prostate and breast cancers. The protection accrues to individuals on diets rich in grains, vegetables, and berries that contain high concentrations of secoisolariciresinol and matairesinol. In contrast, typical Western diets tend to be poor in these foods and do not afford comparable protection.



predominate. This disparity suggests that within woody tissues some mechanism in the lignifying cell wall regulates or mandates the interunit linkage pattern within the native biopolymer.

As is becoming increasingly clear, the lignification process in situ is under very tight biochemical control as part of a cell-specific programmed process. In the following section, we describe known elements that control lignification in vivo. Undoubtedly, more details will emerge as this important process is investigated systematically.

24.11.3 Lignin biosynthesis is controlled spatially and temporally and may involve a proteinaceous template.

Before lignin biosynthesis is initiated, the cells destined to form secondary xylem (i.e., wood; Fig. 24.60) undergo specific

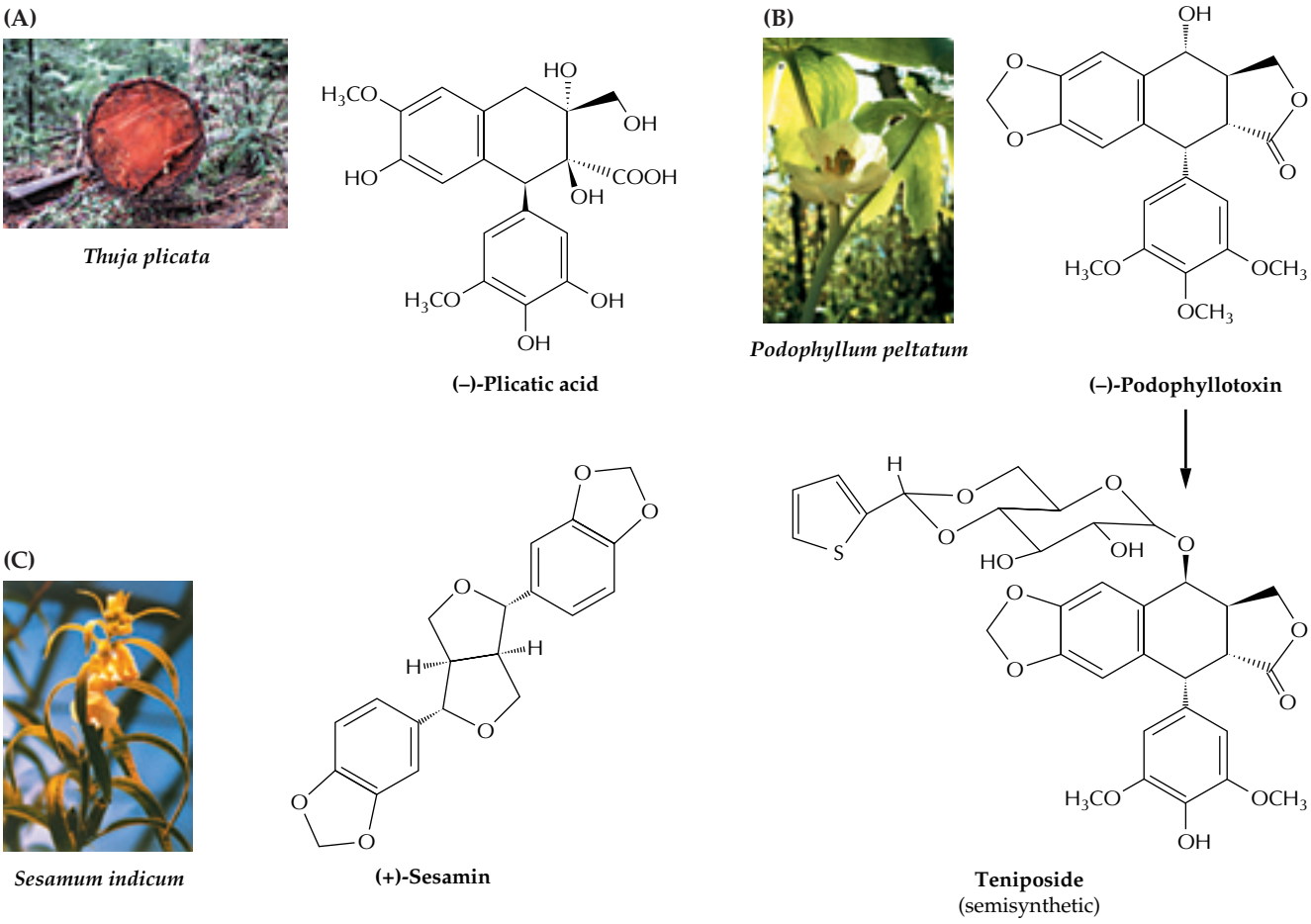


Figure 24.57
Examples of 8–8′-linked lignans. (A) Plicatic acid (and its oligomeric congeners, not shown) are deposited en masse during heartwood formation in western red cedar. The congeners contribute substantially to the color, quality, and durability of this heartwood and are major components of the biochemical protection that enables such species to survive for more than 3000 years. (B) Podophyllotoxin, from the may apple. The etoposide or teniposide derivatives of this compound are also used in treatment of certain cancers. (C) Sesamin, from the sesame seed, has in vitro antioxidant properties that stabilize sesame oil against turning rancid during commercial storage.

irreversible changes that ultimately lead to cell death and the formation of conducting elements (e.g., tracheids, vessels) and structural supporting tissues, such as fibers. These cells experience a programmed expansion of their primary walls, followed by so-called secondary thickenings, which involve ordered deposition of cellulose, hemicellulose, pectin, and structural proteins. Thus, the overall architecture of the plant cell wall is established largely before lignification takes place.

At the start of lignin biosynthesis, monolignols are transported from the cytosol into the cell wall during a specific stage of wall development. Electron microscopy investigations have shown that lignin biosynthesis is initiated at defined sites in the cell corners and middle lamella, i.e., at the locations farthest from the cytosol and plasma membrane. These loci in the cell walls then form distinctive domains that extend inward through the various cell wall layers, toward the plasma membrane. The domains ultimately coalesce.

UV-microscopy and radiochemical labeling indicate that individual monolignols are deposited differentially. For example, in conifers, *p*-coumaryl alcohol is primarily laid down at the early stages of lignin biosynthesis in the cell corners and middle lamella, whereas coniferyl alcohol is deposited predominantly in the secondary wall (Fig. 24.60A). This controlled deposition of specific

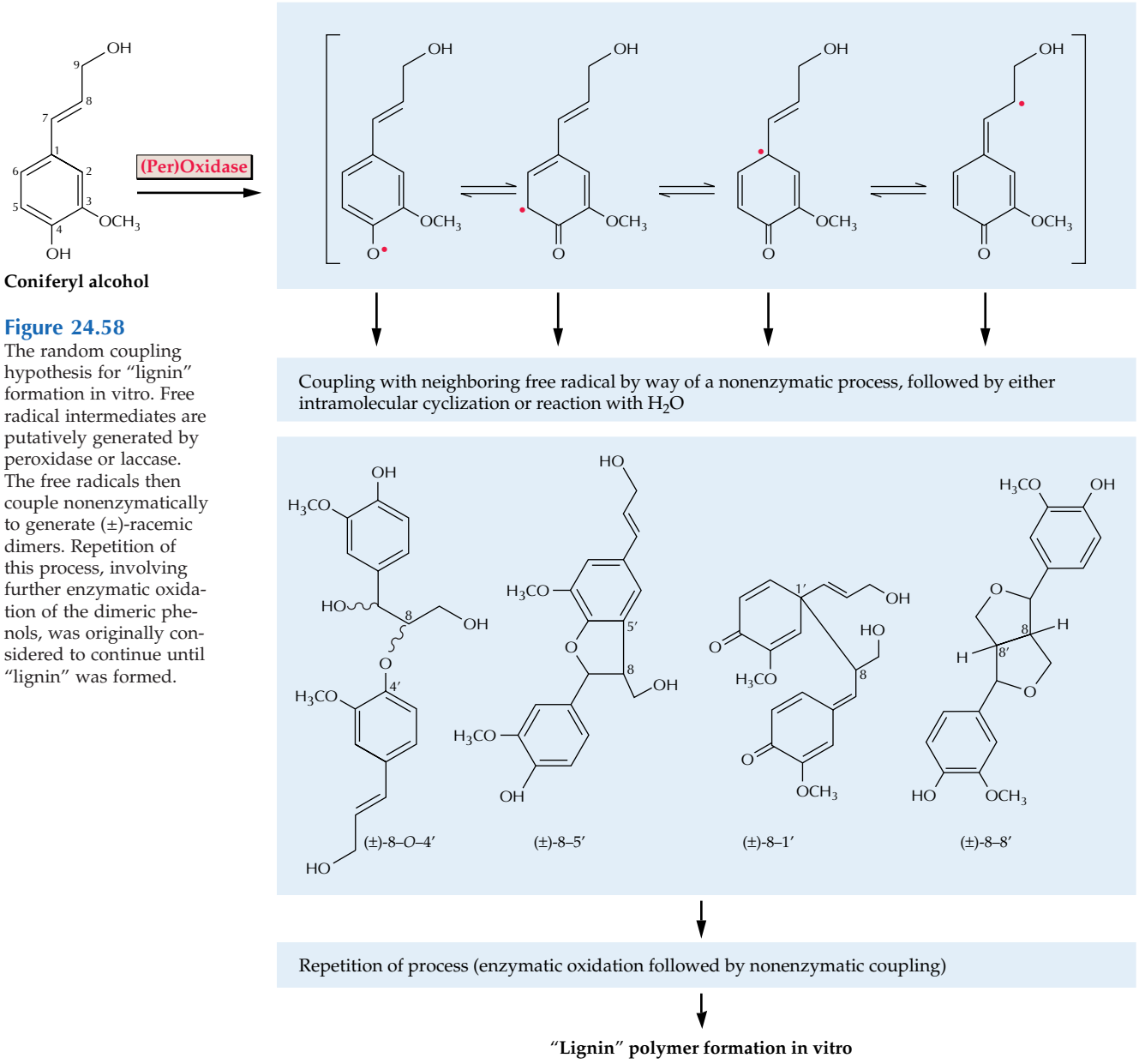


Figure 24.58
The random coupling hypothesis for "lignin" formation in vitro. Free radical intermediates are putatively generated by peroxidase or laccase. The free radicals then couple nonenzymatically to generate (±)-racemic dimers. Repetition of this process, involving further enzymatic oxidation of the dimeric phenols, was originally considered to continue until "lignin" was formed.

monolignols creates domains with distinct structural configurations.

Perhaps most interesting of all, immunochemical studies demonstrate that initiation of lignin biosynthesis is both temporally and spatially associated with the secretion of distinct proteins from the Golgi apparatus and their deposition into the cell wall, including some that are proline-rich. These or related polypeptides, including some proline-rich proteins, may participate in lignification and may be related to the dirigents identified in lignan biosynthesis. Indeed, dirigent sites have been detected in regions where lignification is initiated. Thus, lignin biopolymer assembly may be under the control of a proteinaceous template.

Taken together, this evidence suggests that lignin assembly in vivo is subject to biochemical regulation, whereby the appropriate monomers are linked in a specific manner to yield a limited number of coupling modes in characteristic proportions. This model assumes that elongation of the primary lignin chain occurs by end-wise polymerization and is guided by an array of proteinaceous sites that stipulate or control linkage type and configuration. Moreover, in this

way, the cytosol predetermines the outcome of phenoxy radical coupling. Lignin chain replication is thus envisaged to involve primitive self-replicating polymerization templates, and even the presumed lack of optical activity in lignins might result from, for example, the self-replication process involving generation of mirror-image polymeric assemblies. How lignification is ultimately achieved and what is the precise nature and mechanism of the putative proteinaceous templates now await full clarification at the biochemical level. As we establish the salient details of how lignin biopolymer assembly is controlled, plants are beginning to yield some of the long-hidden secrets involved in cell wall formation.

24.11.4 Variations on lignin deposition can be observed in the formation of reaction wood and in lignification in nonwoody plants.

A programmed plasticity of sorts is built into the overall macromolecular assembly of lignified cell walls. Perhaps the best example of this is seen in the formation of so-called **reaction wood.** When the woody stem becomes misaligned from its vertical axis, reaction wood forms to buttress the growing stem and gradually realign the photosynthetic canopy (Fig. 24.61). In this region, some of the cells originally fated to form ordinary xylem (see Fig. 24.60B) are reprogrammed to generate reaction wood instead. These cells then undergo massive changes in the macromolecular assembly of their cell walls (Fig. 24.61). In conifers, the cell walls of reaction wood, called **compression wood,** become thicker and rounder, the cellulose content is reduced relative to normal wood, and the cellulose microfibril angle is increased; the quantity of lignin also increases, primarily through an increase in the *p*-coumaryl alcohol content. In contrast, the reaction wood formed in angiosperms is known as **tension wood,** because the affected tissue is placed under tension rather than compression. Tension wood forms on the

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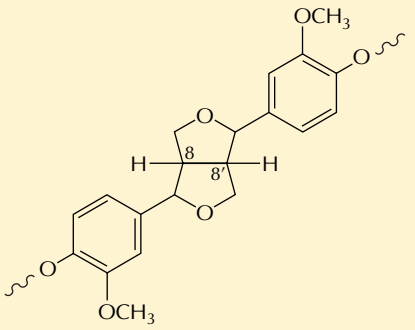
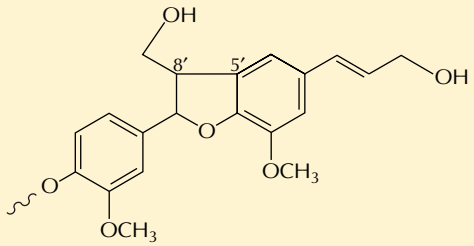
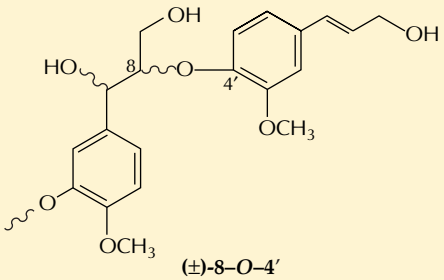
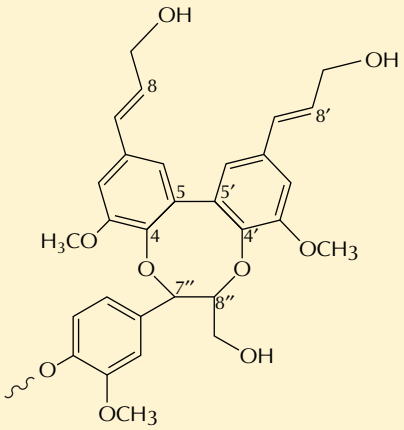
Traces	 (±)-8–8′ Pinoresinol
9–12%	 (±)-8–5′ Dehydrodiconiferyl alcohol
>50%	 (±)-8–*O*–4′ (*erythro*/*threo*) Guaiacylglycerol 8–*O*–4′-coniferyl alcohol ethers
18–20%	 (±)-5–5′–*O*–4′ Dibenzodioxocin

Figure 24.59
Prevalence of selected interunit linkages in native lignin biopolymers from the gymnosperm Norway spruce (*Picea abies*).

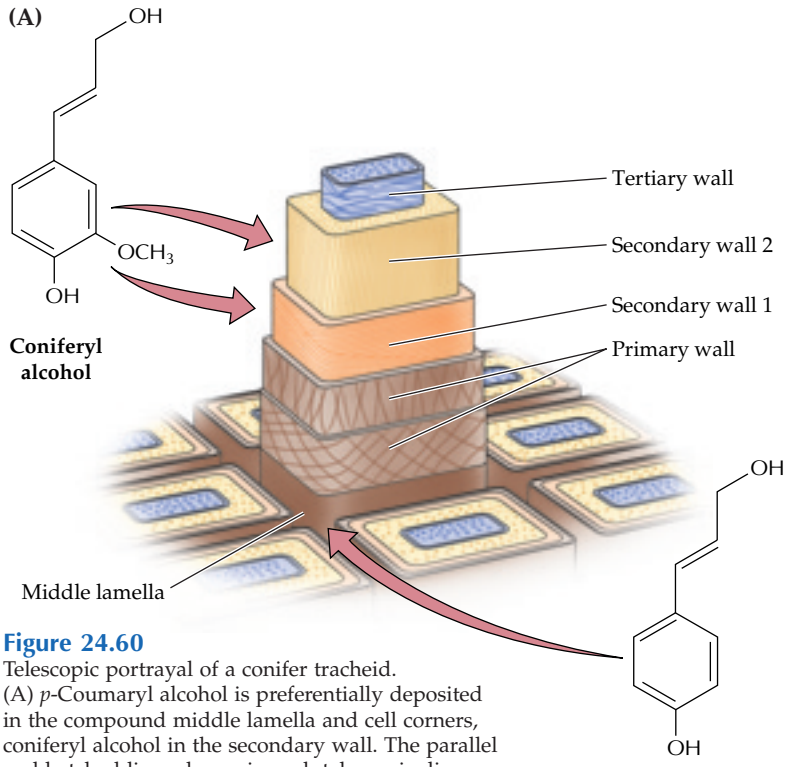


Figure 24.60
Telescopic portrayal of a conifer tracheid. (A) *p*-Coumaryl alcohol is preferentially deposited in the compound middle lamella and cell corners, coniferyl alcohol in the secondary wall. The parallel and hatched lines shown in such telescopic diagrams indicate the orientation of cellulose microfibrils. (B) Light micrograph cross-section of normal secondary xylem of Douglas fir (*Pseudotsuga menziesii*).

upperside of the stem, compression wood on the underside. Characteristics of tension wood include increased cellulose content and the presence of a carbohydrate-derived gelatinous layer. The amount of lignin present may decrease or remain the same, depending on the species. The underlying biochemical mechanisms that engender formation of both compression and tension wood are not known.

Lignification in nonwoody herbaceous plants and grasses differs to some extent from lignin biosynthesis during wood formation. Nonwoody plants contain lignins that appear to be formed from mixtures of monolignols and hydroxycinnamic acids. The lignin interunit linkages seem to follow those generally described for woody tissue lignin, except that hydroxycinnamic acids are also involved. To date, no extensive biochemical studies have focused on how the macromolecular assembly of lignin in nonwoody plants actually occurs, although the involvement of proline-rich polymers has been implicated here as well through the immunochemical studies discussed above.

24.11.5 Suberization protects tissues from water loss and pathogen invasion.

Suberized tissues are found in various underground organs (e.g., roots, stolons, tubers) as well as in periderm layers (e.g., cork, bark). They are also formed as part of the wound- and pathogen-induced defenses of specific organs and cell types, perhaps the most familiar example being the browning and subsequent encrustation of sliced potato tubers. Suberized tissues are formed as multilamellar domains consisting of alternating polyaliphatic and polyaromatic layers (Fig. 24.62), as shown in the wound-healing layers in potato. These layers contribute to cell wall strength and provide a means to limit uncontrolled water loss by the intact organism by forming impenetrable barriers. From an evolutionary perspective, suberization was of utmost importance in plant adaptation to living on land and may even have preceded lignification.

As with lignin, no methods are yet available to obtain either of the two domains of suberin in a native or unaltered condition. The aliphatic component is located between the primary wall and the plasmalemma. Suberin aliphatics are generally long-chain (more than 20 carbons) lipid substances; they also include α,ω-fatty dioic acids, such as C_{16}- or C_{18}-alkan-α,ω-dioic acids, which are considered diagnostic of suberized tissue (Fig. 24.63). Interestingly, the polyaromatic domain located in the cell wall is apparently formed before the aliphatics, primarily from distinctive monomeric building blocks that contain hydroxycinnamate-derived substances (Fig. 24.64). Thus, the formation of suberized tissue is very distinct from the lignification of secondary xylem (where deposition is the last biochemical act of the xylem-forming cells before cell death).

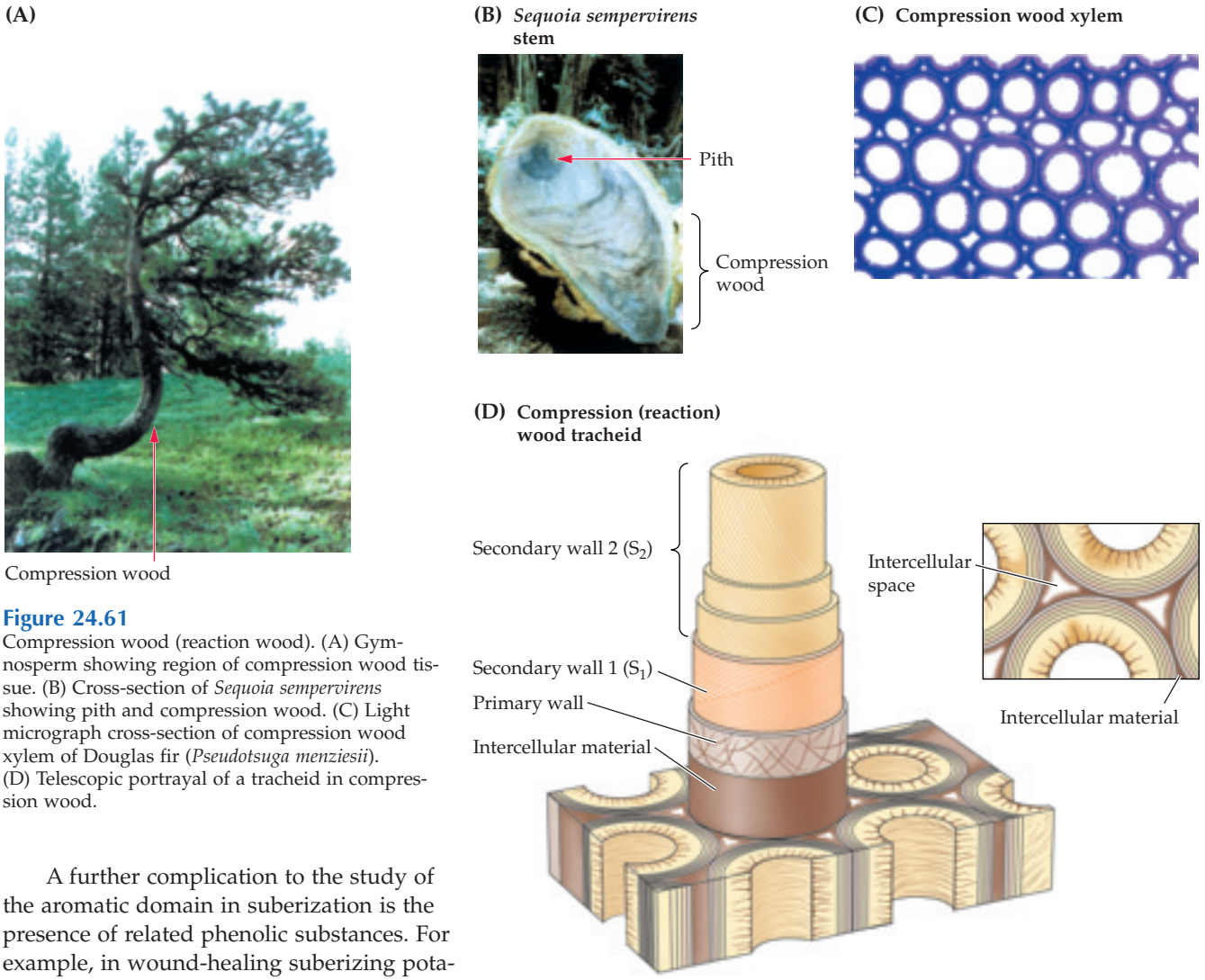


Figure 24.61
Compression wood (reaction wood). (A) Gymnosperm showing region of compression wood tissue. (B) Cross-section of *Sequoia sempervirens* showing pith and compression wood. (C) Light micrograph cross-section of compression wood xylem of Douglas fir (*Pseudotsuga menziesii*). (D) Telescopic portrayal of a tracheid in compression wood.

A further complication to the study of the aromatic domain in suberization is the presence of related phenolic substances. For example, in wound-healing suberizing potato periderm tissues, chlorogenic acid and miscellaneous other phenolics are also present (Fig. 24.65). These compounds do not appear to function in suberization per se but rather may provide a means for topical disinfection of the exposed cell surfaces, thereby preventing or limiting infection/contamination. Some evidence also suggests the presence of low amounts of monolignols in suberized tissues, but these may be from small amounts of lignin.

Although the polymeric suberin phenolic constituents are predominantly derived from hydroxycinnamate, how this aromatic domain is assembled is unknown. Recent studies demonstrated that potato tuber wound-healing suberizing tissues contain two hydroxycinnamoyl-CoA transferases, which catalyze formation of various alkyl ferulates and (*p*-coumaroyl) feruloyl tyramine derivatives, respectively. How, or if,

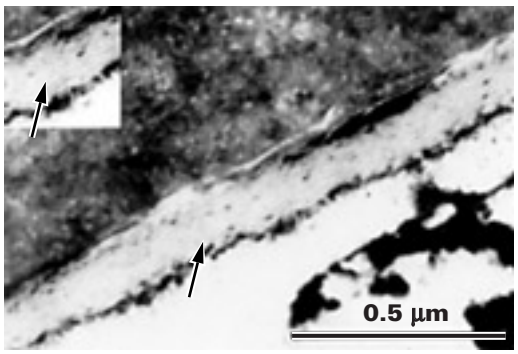


Figure 24.62
Suberized tissue consists of layered polyaliphatic and polyaromatic domains, as shown for wound-healing potato tuber slices exposed to air. A suberized layer (see arrows) forms five days after exposure of potato tuber slices to air.

Fatty acid

$H_3C(CH_2)_nCOOH$

n = 14, 16, 18, 20, 22

α,ω-Fatty dioic acids

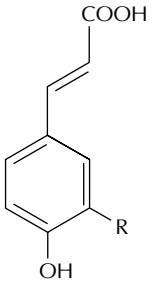
$HOOC(CH_2)_nCOOH$

n = 14, 16, 18, 20, 22

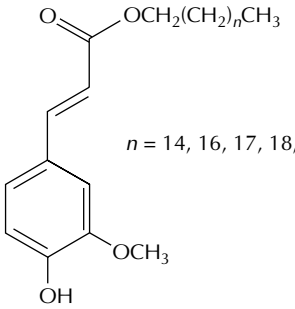
α,ω-Hydroxy fatty acids

$HO(CH_2)_nCOOH$

n = 15, 17, 19, 21, 23, 25

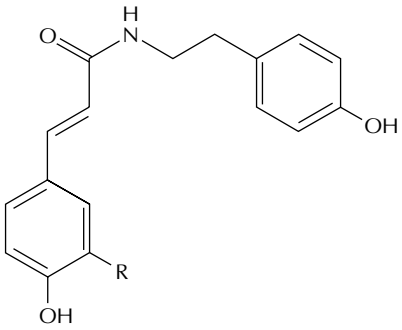


R = H **p-Coumaric acid**
R = OCH_3 **Ferulic acid**

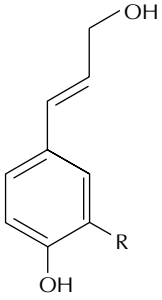


n = 14, 16, 17, 18, 19, 20, 22, 24, 26

Alkyl ferulates



R = H **p-Coumaroyl tyramine**
R = OCH_3 **Feruloyl tyramine**



R = H **p-Coumaryl alcohol**
R = OCH_3 **Coniferyl alcohol**

Figure 24.64
Aromatic components of suberized tissue, derived primarily from hydroxycinnamates, including alkyl ferulates and *p*-coumaroyl and feruloyl tyramines. Suberized tissue may also contain small amounts of monolignols.

Figure 24.63
Aliphatic components of suberized tissue. Found in combination, these compounds are considered diagnostic for suberin. An α,ω-dioic acid has carboxyl groups on both of the end carbons. An α,ω-hydroxy acid has a hydroxyl on one of the end carbons.

these are integrated into the aromatic domain of suberin of potato remains to be established, although an anionic peroxidase has been implicated in the polymerization process. Additionally, the finding that the appearance of proline-rich proteins seems to correlate temporally and spatially with deposition of the aromatic domain of suberized tissue may be important.

Much remains to be understood about formation of both the polyaromatic and the polyaliphatic domains of suberized tissue. In particular, we do not know yet which features are common to all plants and which are species-specific. For example, the suberized tissues seen in various root, periderm, and woody bark tissues are not identical to one another. This underscores the need to identify the basic biochemical requirements for suberization and to determine how these differ with regard to tissue-specific addition of particular phenolic substances.

24.12 Flavonoids

With more than 4500 different representatives known thus far, the flavonoids constitute an enormous class of phenolic natural products. Present in most plant tissues, often in vacuoles, flavonoids can occur as monomers, dimers, and higher oligomers. They are also found as mixtures of colored oligomeric/polymeric components in various heartwoods and barks.

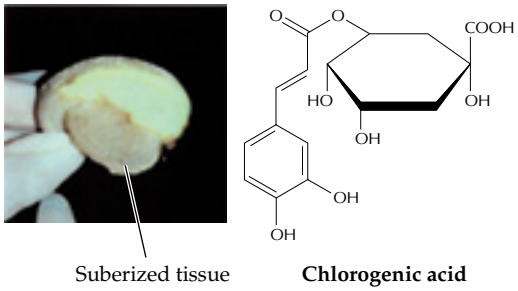


Figure 24.65
Suberin deposition has been studied in wounded potato tubers. In these tissues, suberin formation is accompanied by the production of an unrelated phenolic compound, chlorogenic acid.

24.12.1 Flavonoids comprise a diverse set of compounds and perform a wide range of functions.

Many plant–animal interactions are influenced by flavonoids. The colors of flowers and fruits, which often function to attract pollinators and seed dispersers, result primarily from vacuolar anthocyanins (Fig. 24.66) such as the pelargonidins (orange, salmon, pink, and red), the cyanidins (magenta and crimson), and the delphinidins (purple, mauve, and blue). Related flavonoids, such as flavonols, flavones, chalcones, and aurones, also contribute to color definition. Manipulating flower color by targeting various enzymatic steps and genes in flavonoid biosynthesis has been quite successful, particularly in petunia.

Specific flavonoids can also function to protect plants against UV-B irradiation, a role sometimes ascribed to kaempferol (Fig. 24.67). Others can act as insect feeding attractants, such as isoquercetin in mulberry, a factor involved in silkworm recognition of its host species. In contrast, condensed tannins such as the proanthocyanidins add a distinct bitterness or astringency to the taste of certain plant tissues and function as antifeedants (Fig. 24.68). The flavonoids apigenin and luteolin serve as signal molecules in legume–rhizobium bacteria interactions, facilitating nitrogen fixation (Fig. 24.69). In a related function, isoflavonoids are involved in inducible defense against fungal attack in alfalfa (e.g., medicarpin; Fig. 24.69) and other plant species. Perhaps the most poorly studied and least understood classes of the flavonoids are the oligomeric and polymeric substances associated with formation of certain heartwood and bark tissues. These

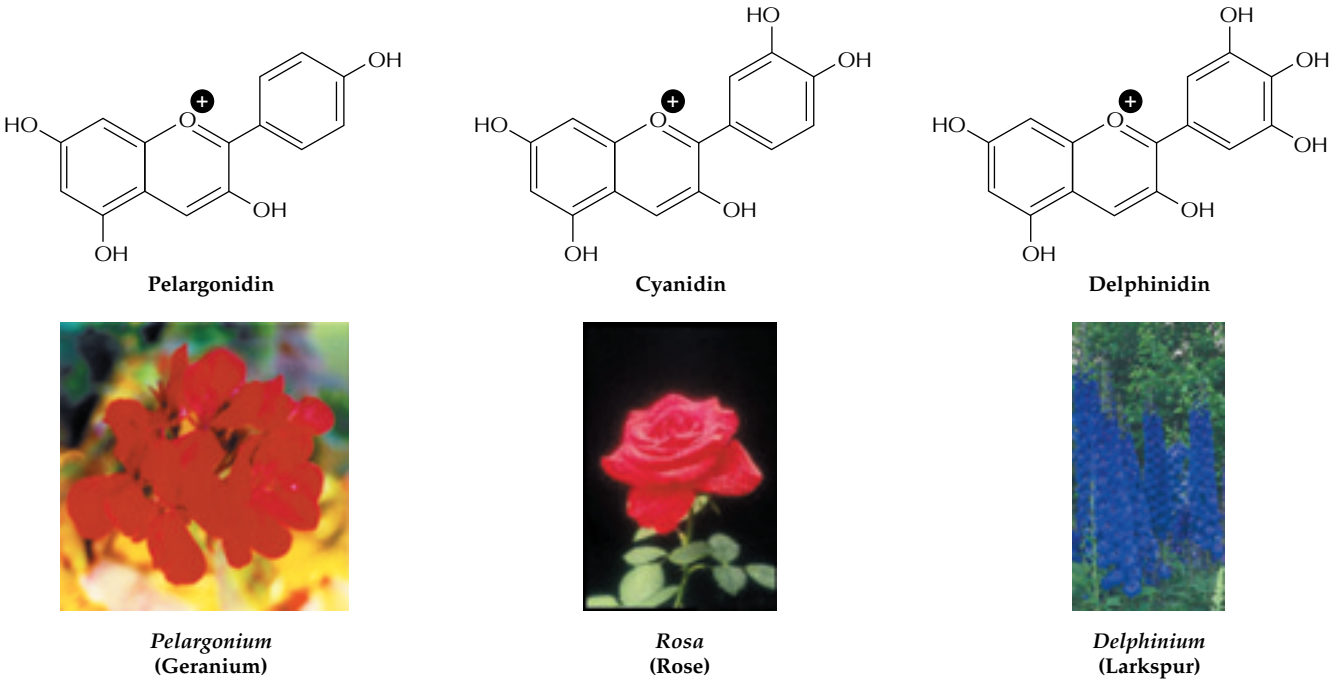
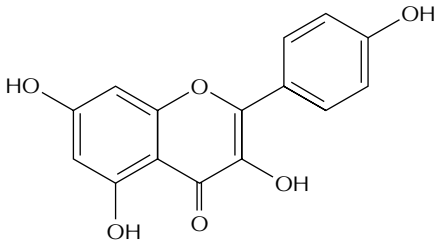


Figure 24.66
Selected anthocyanin pigments: pelargonidin, cyanidin, and delphinidin from geranium, rose, and larkspur, respectively.

Soybean

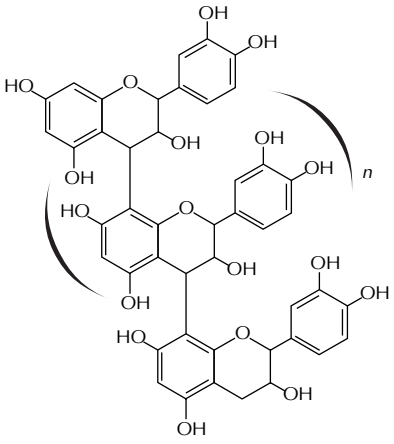


Kaempferol

Figure 24.67
Kaempferol, a UV-B protectant, is present in many plants such as soybean (*Glycine max*).

compounds include proanthocyanidins and their congeners in woody gymnosperms and isoflavonoids in woody legumes from the tropics. In both cases, their massive deposition during heartwood formation contributes significantly and characteristically to the overall color, quality, and rot resistance of wood. These metabolites can be misidentified as lignins because some constituents are not readily solubilized and are frequently dissolved only under the same conditions that effect lignin dissolution.

Red sorghum



Proanthocyanidin (n = 1–30)

Figure 24.68
Red sorghum produces proanthocyanidin antifeedant compounds—condensed tannins, which deter birds from feeding on the seed. White sorghum, which is deficient in these compounds, is rapidly consumed by birds. Similar compounds are present in the heartwood of Douglas fir (not shown).

Various flavonoids have also been studied extensively from the perspectives of health protection and pharmacological utility, for which mammalian enzyme systems have been used to assess flavonoid activity. Flavonoids have been analyzed as modulators of immune and inflammatory responses, for their impact on smooth muscle function, and as anticancer, antiviral, antitoxic, and hepatoprotective agents. There is considerable current interest in the use of isoflavonoids in cancer prevention. Dietary consumption of the isoflavonoids daidzein and genistein (Fig. 24.70), which are present in soybeans, is thought to reduce substantially the incidence of breast and prostate cancers in humans.

24.12.2 The flavonoid biosynthesis pathway has several important branchpoints.

The flavonoids consist of various groups of plant metabolites, which include chalcones, aurones, flavonones, isoflavonoids, flavones, flavonols, leucoanthocyanidins (flavan-3,4-diols), catechins, and anthocyanins (Figs. 24.70 and 24.71).

The first committed step of the flavonoid pathway is catalyzed by chalcone synthase (CHS; see Fig. 24.70). Three molecules of acetate-derived malonyl-CoA and one molecule of *p*-coumaryl-CoA are condensed to generate a tetrahydroxychalcone (see Fig. 24.49, reaction 9). CHS, a dimeric polyketide synthase with each subunit at about 42 kDa, has no cofactor requirements. In certain species, the coordinated action of CHS and an NADPH-dependent reductase generates a 6-deoxychalcone (isoliquiritigenin). Both chalcones can then be converted into aurones, a subclass of flavonoids found in certain plant species. Beyond CHS, the next step shared by most of the flavonoid biosynthesis pathways is catalyzed by chalcone isomerase (CHI), which catalyzes a stereospecific ring closure isomerization step to form the 2*S*-flavanones, naringenin, and (less commonly) liquiritigenin (see Fig. 24.70). The flavanones may represent the most important branching point in flavonoid metabolism, because isomerization of these compounds yields the phytoalexin isoflavonoids (Fig. 24.70), introduction of a C-2–C-3 double bond affords flavones and

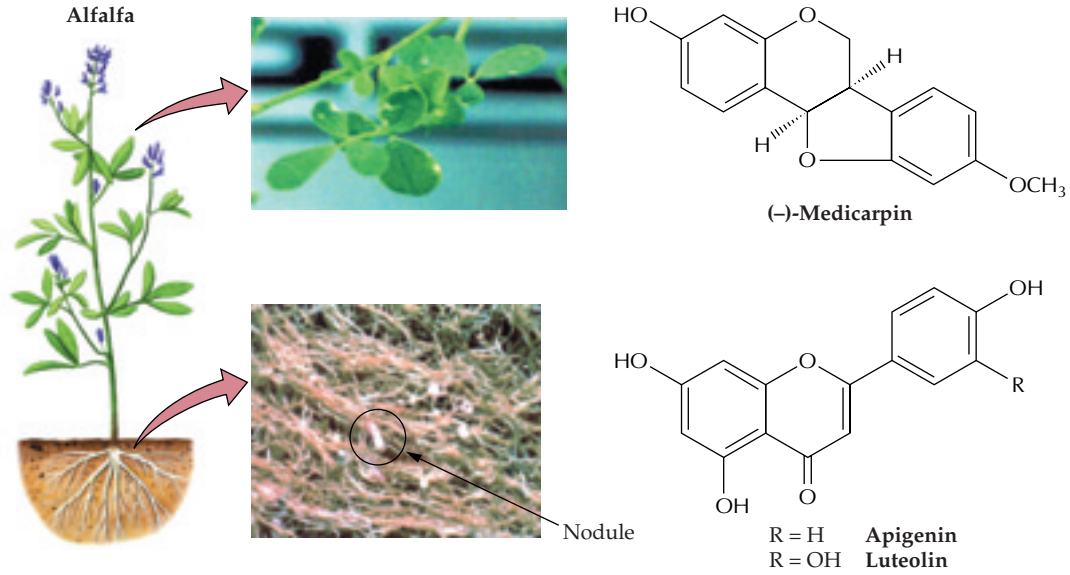


Figure 24.69
Flavonoids perform diverse functions in alfalfa (*Medicago sativa*). The flavonoids apigenin and luteolin function as signaling molecules that induce *Nod* gene expression in compatible *Rhizobium* bacteria, facilitating the development of nitrogen-fixing root nodules. The phytoalexin isoflavonoid medicarpin participates in inducible plant defense.

flavonols (Fig. 24.71), and hydroxylation of the 3-position generates dihydroflavonols (Fig. 24.71).

Entry into the isoflavonoid branchpoint occurs by way of two enzymes (see Fig. 24.70). The first, isoflavone synthase (IFS), catalyzes an unusual C-2 to C-3 aryl migration and hydroxylation to give the 2-hydroxyisoflavanones and has recently been shown to be an NADPH-dependent cytochrome P450 enzyme. Dehydration of the 2-hydroxyisoflavanones, catalyzed by 2-hydroxyisoflavanone dehydratase (IFD), forms the isoflavonoids genistein and daidzein. The isoflavonoids can be further metabolized, primarily in the Fabaceae, to yield phytoalexins (e.g., medicarpin in alfalfa; see Fig. 24.70) or to generate isoflavonoid-derived substances known as rotenoids in tropical legumes (e.g., 9-demethylmunduserone from *Amorpha fruticosa;* see Fig. 24.70). The rotenoids, which are isolated mainly from *Derris elliptica* and related species, are used extensively as insecticidal agents but have other applications as well. For example, rotenone is used as a rat poison and an inhibitor of NADH dehydrogenase. Interestingly, the NADPH-dependent isoflavone reductase (IFR) step involved in isoflavonoid formation shows considerable homology to pinoresinol/lariciresinol reductase (see Fig. 24.56 and Section 24.11.1), suggesting a phylogenetic link between both lignan and isoflavonoid pathways for plant defense.

The second branching point in general flavonoid metabolism involves that of dehydration of naringenin at the C-2/C-3 positions to give such abundant flavones as apigenin (Fig. 24.71). This conversion is catalyzed by flavone synthase (FNS), which varies in enzymatic type depending on the plant species. For example, in parsley cell cultures, flavone formation is catalyzed by an α-ketoglutarate–dependent dioxygenase (FNS I in Fig. 24.71), whereas an NADPH-dependent microsomal preparation engenders this reaction in *Antirrhinum* flowers (FNS II in Fig. 24.71).

The third major branchpoint in flavonoid metabolism is stereospecific 3-hydroxylation of naringenin (or its 3′-hydroxylated analog) to give dihydroflavonols (Fig. 24.71) such as dihydrokaempferol (or dihydroquercetin). The enzyme involved, flavanone 3-hydroxylase, is an Fe^{2+}-requiring, α-ketoglutarate–dependent dioxygenase. Specific hydroxylation involving an NADPH-dependent cytochrome P450 monooxygenase of naringenin can also directly give dihydroquercetin, which can be converted to quercetin (a flavanol) by

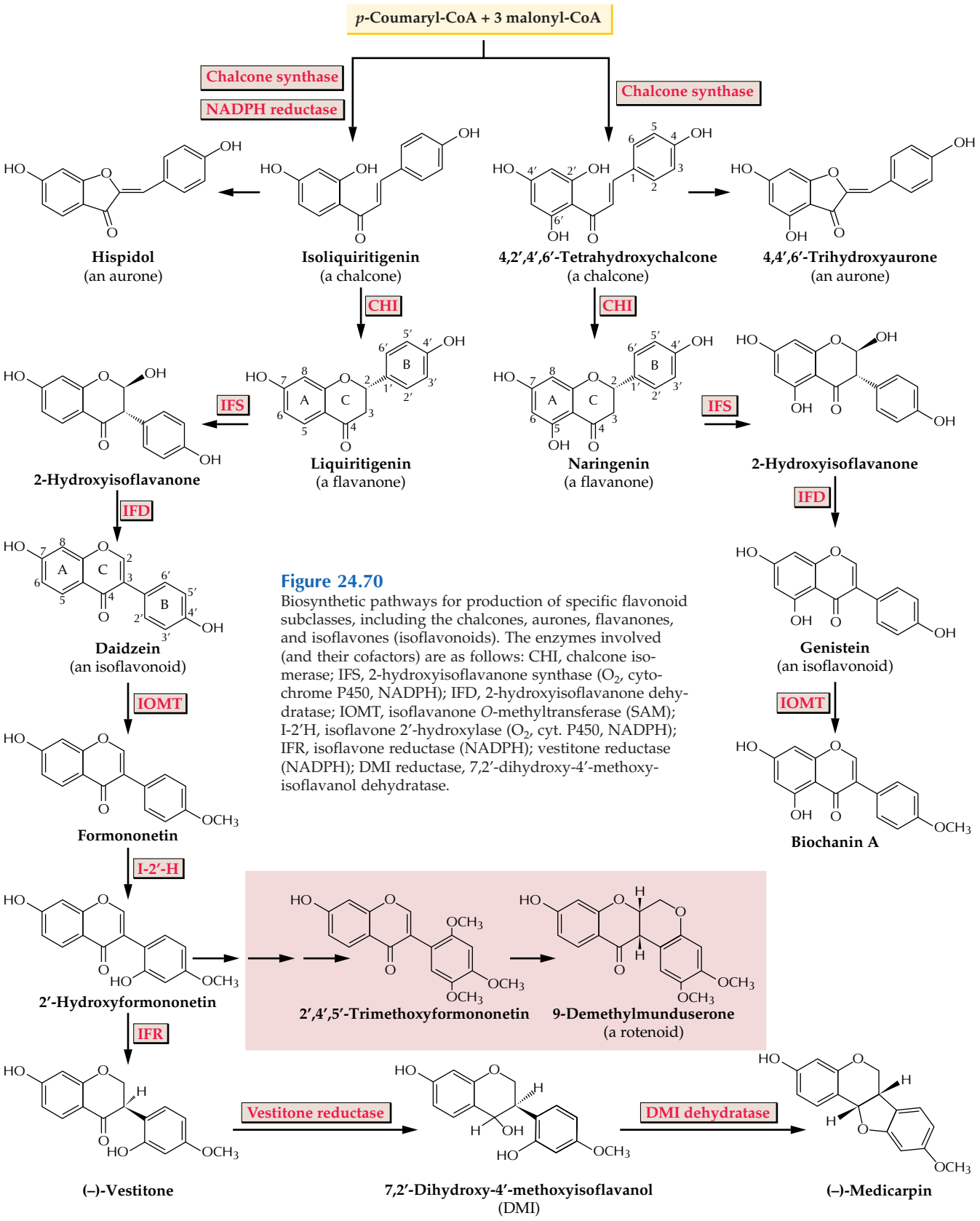


Figure 24.70
Biosynthetic pathways for production of specific flavonoid subclasses, including the chalcones, aurones, flavanones, and isoflavones (isoflavonoids). The enzymes involved (and their cofactors) are as follows: CHI, chalcone isomerase; IFS, 2-hydroxyisoflavanone synthase (O_2, cytochrome P450, NADPH); IFD, 2-hydroxyisoflavanone dehydratase; IOMT, isoflavanone *O*-methyltransferase (SAM); I-2′H, isoflavone 2′-hydroxylase (O_2, cyt. P450, NADPH); IFR, isoflavone reductase (NADPH); vestitone reductase (NADPH); DMI reductase, 7,2′-dihydroxy-4′-methoxyisoflavanol dehydratase.

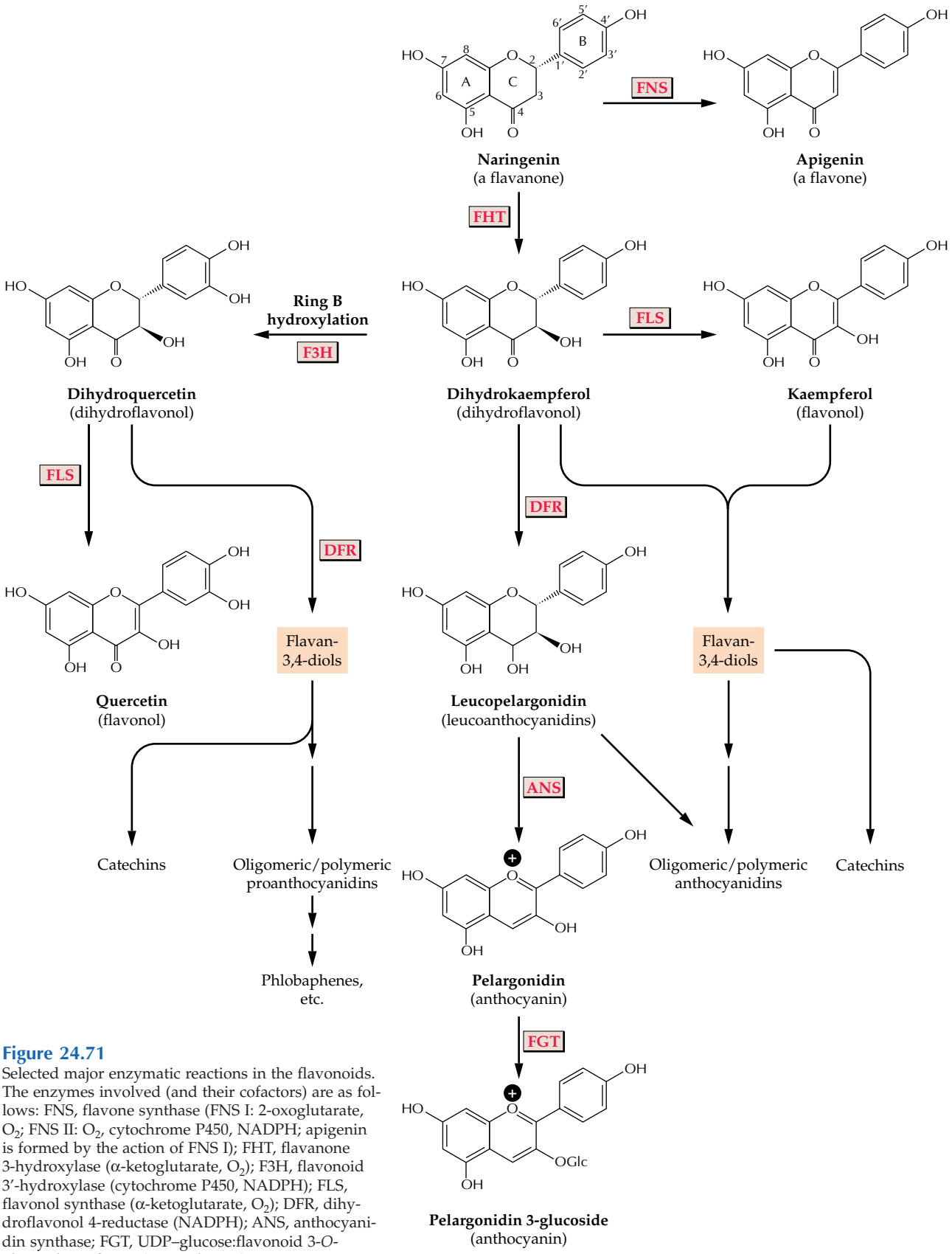


Figure 24.71
Selected major enzymatic reactions in the flavonoids. The enzymes involved (and their cofactors) are as follows: FNS, flavone synthase (FNS I: 2-oxoglutarate, O_2; FNS II: O_2, cytochrome P450, NADPH; apigenin is formed by the action of FNS I); FHT, flavanone 3-hydroxylase (α-ketoglutarate, O_2); F3H, flavonoid 3′-hydroxylase (cytochrome P450, NADPH); FLS, flavonol synthase (α-ketoglutarate, O_2); DFR, dihydroflavonol 4-reductase (NADPH); ANS, anthocyanidin synthase; FGT, UDP–glucose:flavonoid 3-*O*-glucosyltransferase (UDP–glucose).

flavonol synthase (FLS)–catalyzed C-2–C-3 double bond formation; FLS is an α-ketoglutarate–dependent dioxygenase. Alternatively, dihydroquercetin can be reduced by an NADPH-dependent dihydroflavonol reductase (DFR) to give the corresponding flavan-3,4-diols (Fig. 24.71).

Subsequent species- and tissue-specific enzymatic conversions can create vast arrays of structurally diverse groups of flavonoids. For example, in flower petals, the leucoanthocyanidins (e.g., leucopelargonidin) can be converted to the colored anthocyanins (e.g., pelargonidin) through the action of a dehydratase, anthocyanidin synthase (ANS), which is thought to be an α-ketoglutarate–dependent dioxygenase (Fig. 24.71). Leucoanthocyanidins can also serve as precursors of the (*epi*-)catechins and condensed tannins. The enzymology associated with those coupling processes, chain extension mechanisms, and oxidative modifications, however, is not yet established.

24.13 Coumarins, stilbenes, styrylpyrones, and arylpyrones

24.13.1 Some coumarins, a class of plant defense compounds, can cause internal bleeding or dermatitis.

Coumarins (e.g., coumarin; Fig. 24.72A) belong to a widespread family of plant metabolites called the benzopyranones, with more than 1500 representatives in more than 800 species. In plants, these compounds can occur in seed coats, fruits, flowers, roots, leaves, and stems, although in general the greatest concentrations are found in fruits and flowers. Their roles in plants appear to be mainly defense-related, given their antimicrobial, antifeedant, UV-screening, and germination inhibitor properties.

The best known properties of coumarins indirectly highlight their roles in plant defense. Ingesting coumarins from plants such as clover can cause massive internal bleeding in mammals. This discovery ultimately led to the development of the rodenticide Warfarin (Fig. 24.72B) and to the use of related compounds to treat and prevent stroke. Likewise, the photosensitizing compound 8-methoxypsoralen, present in leaf tissue of *Heracleum mantegazzianum* (giant hogweed), can cause photophytodermititis on skin contact and subsequent exposure to UV-A radiation (Fig. 24.73). A comparable form of coumarin-induced dermatitis can also occur during celery handling. Psoralen (Fig. 24.74), however, is now successfully used to treat various skin disorders (eczema, psoriasis) by means of a combination of oral ingestion and UV-A treatment.

The structure of a representative simple coumarin, 7-hydroxycoumarin, is shown in Figure 24.75. Additional families of plant coumarins (see Fig. 24.74) include linear furanocoumarins (e.g., psoralen), angular furanocoumarins (e.g., angelicin), pyranocoumarins (e.g., seselin), and pyrone-substituted coumarins (e.g., 4-hydroxycoumarin).

24.13.2 Coumarin biosynthesis pathways have not yet been fully elucidated.

The biosynthetic pathways to the coumarins are only partially determined at this point; they mainly involve aromatic hydroxylations and additional reactions catalyzed by *trans/cis*-hydroxycinnamic acid isomerases, dimethylallyltransferases, various P450/NADPH/O_2–dependent synthases and *O*-methyltransferases (Fig. 24.75). The simplest

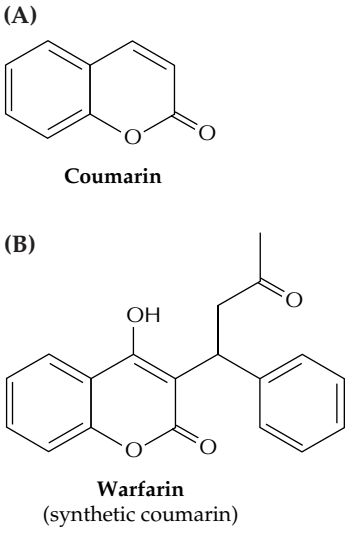
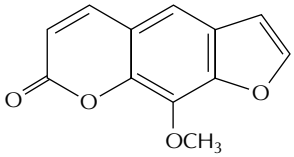


Figure 24.72
Structures of (A) coumarin (from clover), and (B) a synthetic coumarin, the rodenticide Warfarin.

Heracleum



8-Methoxypsoralen
(a furanocoumarin)

Figure 24.73
A linear furanocoumarin, 8-methoxypsoralen, sensitizes human skin to UV-A light. This compound, present in external tissues of *Heracleum* species, causes severe blistering on skin contact followed by exposure to UV-irradiation.

examples, coumarin and 7-hydroxycoumarin (umbelliferone), are believed to be formed by O-hydroxylation of cinnamic and *p*-coumaric acids, respectively, followed by trans/cis-isomerization and ring closure. However, neither the enzymes nor their encoding genes have yet been obtained.

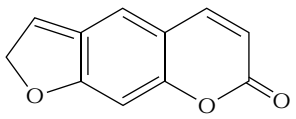
On the other hand, much more is known about the biosynthesis of both linear and angular furanocoumarins. These involve regiospecific prenylation through the action of the corresponding tranferases to yield demethylsuberosin and osthenol, respectively. The subsequent transformations are believed to involve various NADPH-dependent, cytochrome P450 oxidase–catalyzed conversions and O-methylations (Fig. 24.75).

Various fungi and yeasts also biosynthesize coumarins, e.g., the toxic aflatoxins. However, these metabolites are polyketide derivatives and hence are biochemically distinct from their plant analogs.

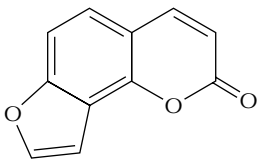
24.13.3 Stilbenes, styrylpyrones, and arylpyrones constitute another class of chemical defense compounds.

In addition to the products of the flavonoid pathway, cinnamoyl-CoA and malonyl-CoA (acetate-derived) pathways can in certain plant species also undergo condensation reactions to yield the corresponding stilbenes, styrylpyrones, and arylpyrones (Fig. 24.76). Comparison of gene sequences for each entry-point enzyme (CHS and stilbene synthase) reveals significant homology, as would be expected for similar enzymatic systems. Beyond the initial synthases, however, little has yet been described about subsequent transformations.

Stilbenes are present in bryophytes, pteridophytes, gymnosperms, and angiosperms, with more than 300 different stilbenoids known today. The stilbenes play important roles in plants, particularly in heartwood protection, and also have significance in pharmacology and human health. In plants, they can function as both constitutive and inducible defense mechanisms. Stilbenes display weak antibacterial properties

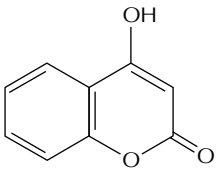


Psoralen
(a linear furanocoumarin)



Angelicin
(an angular furanocoumarin)

Seselin
(a pyranocoumarin)



4-Hydroxycoumarin
(a pyrone-substituted coumarin)

Figure 24.74
Structures of the linear furanocoumarin psoralen, the angular furanocoumarin angelicin, the pyranocoumarin seselin, and the pyrone-substituted coumarin 4-hydroxycoumarin.

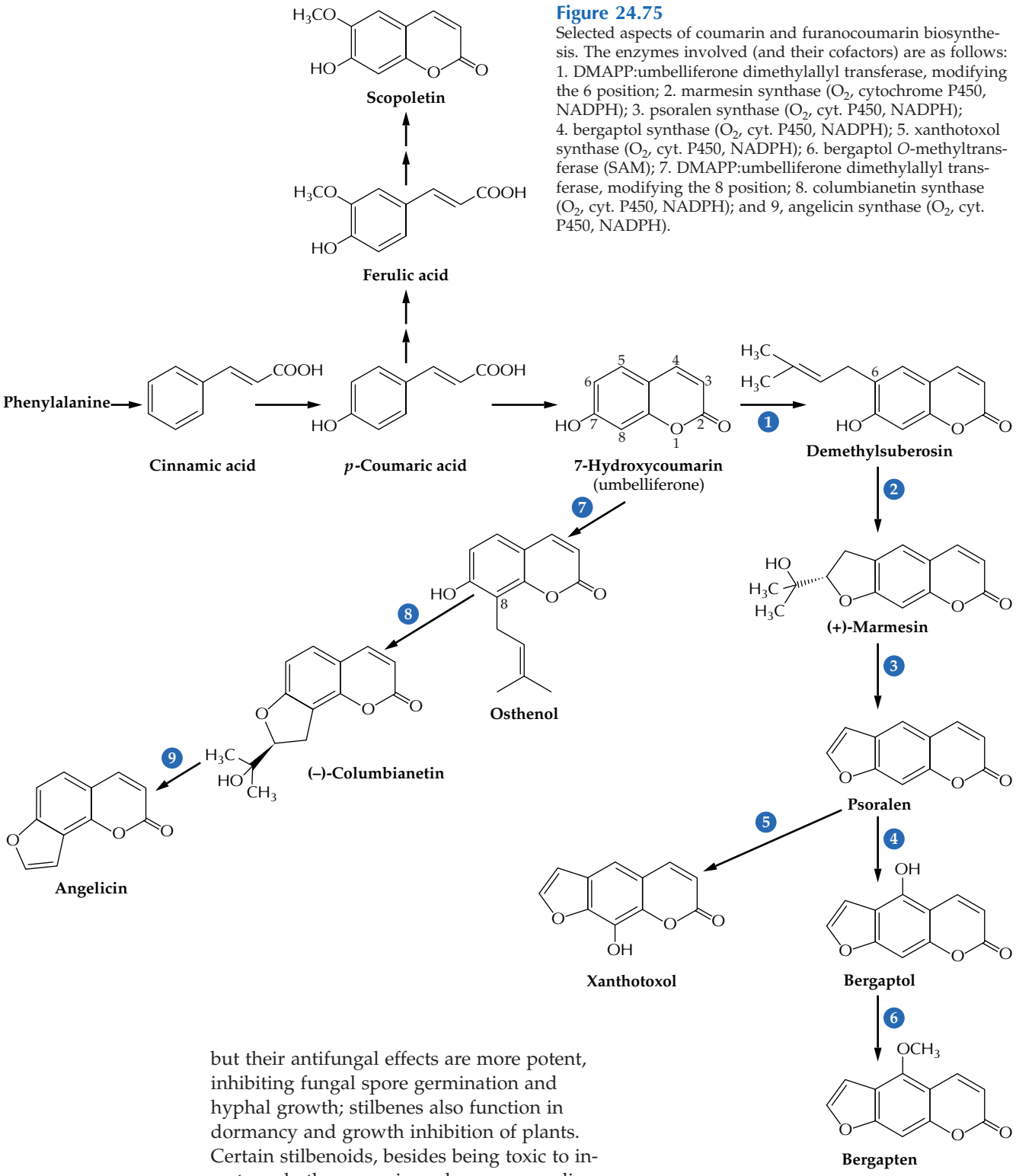


Figure 24.75
Selected aspects of coumarin and furanocoumarin biosynthesis. The enzymes involved (and their cofactors) are as follows: 1. DMAPP:umbelliferone dimethylallyl transferase, modifying the 6 position; 2. marmesin synthase (O_2, cytochrome P450, NADPH); 3. psoralen synthase (O_2, cyt. P450, NADPH); 4. bergaptol synthase (O_2, cyt. P450, NADPH); 5. xanthotoxol synthase (O_2, cyt. P450, NADPH); 6. bergaptol *O*-methyltransferase (SAM); 7. DMAPP:umbelliferone dimethylallyl transferase, modifying the 8 position; 8. columbianetin synthase (O_2, cyt. P450, NADPH); and 9, angelicin synthase (O_2, cyt. P450, NADPH).

but their antifungal effects are more potent, inhibiting fungal spore germination and hyphal growth; stilbenes also function in dormancy and growth inhibition of plants. Certain stilbenoids, besides being toxic to insects and other organisms, have mammalian antifeedant and nematicidal properties. Stilbenoid formation can be induced by insect attack, as illustrated by the colored deposits formed in radiata pine sapwood when attacked by the *Sarix* wasp (see photographs in Box 24.5).

From a pharmacological perspective, the stilbene combretastatin has important antineoplastic activities, and resveratrol, present in red wine, helps suppress tumor formation (Fig. 24.77).

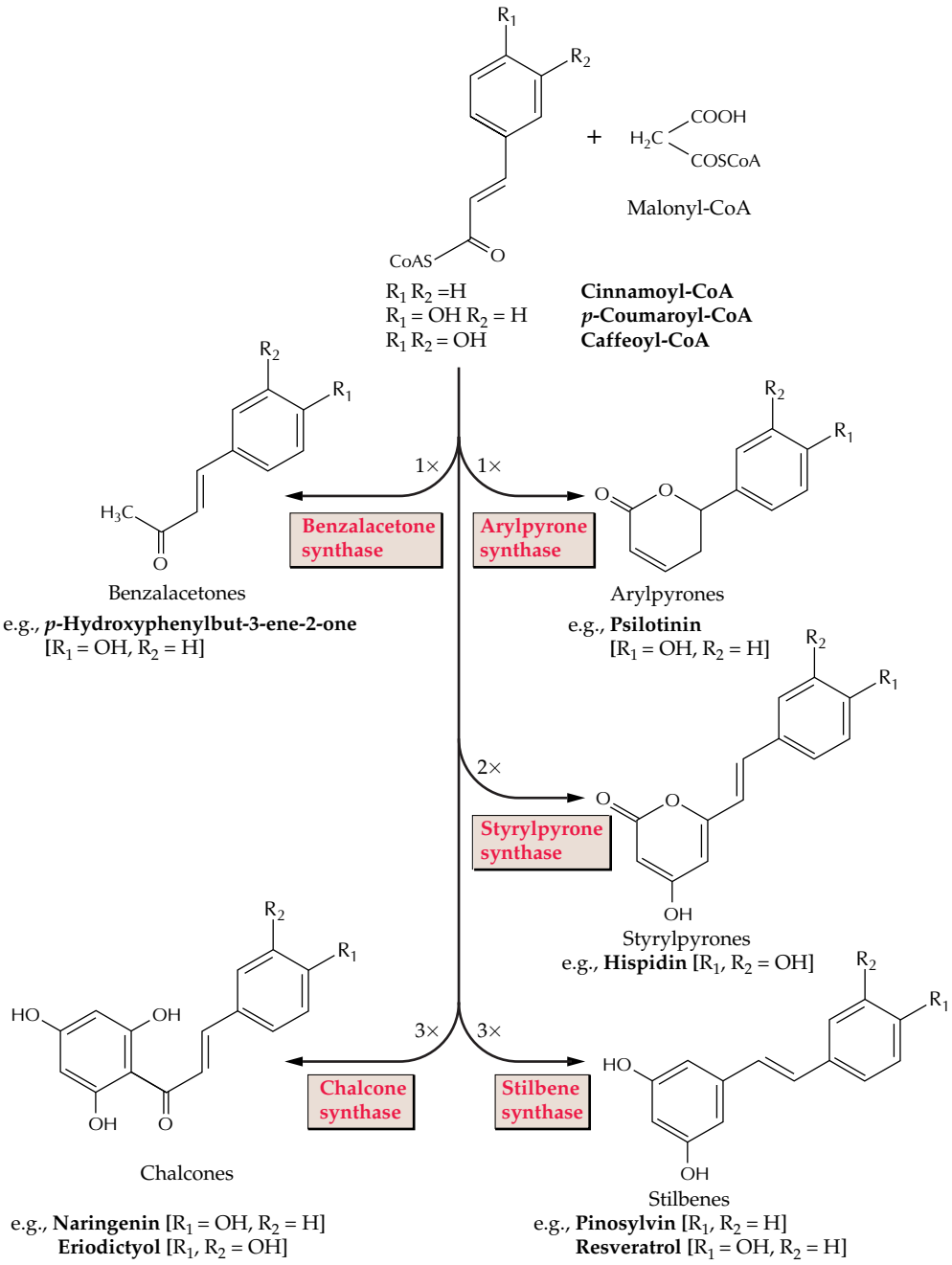


Figure 24.76
Cinnamoyl-CoA, *p*-coumaroyl-CoA, and caffeoyl-CoA, precursors for the biosynthesis of arylpyrones, styrylpyrones, and stilbenes. The designation 1×, 2×, and 3× refers to the number of molar equivalents of malonyl-CoA required.

24.14 Metabolic engineering of phenylpropanoid production: a possible source of enhanced fibers, pigments, pharmaceuticals, and flavoring agents

The biochemical, chemical, and molecular characterization of how plants produce various metabolic substances is essential to understanding the very basis of the biodiversity and life of plants. This pursuit also has economic implications, affording new opportunities for systematic modification of commercially important plants to engineer or specify particular traits that can benefit humanity.

Many biotechnological possibilities await our manipulation of plant phenolic metabolism: plants with increased resistance to pathogens; improvements in the quality of wood and fiber products; new or improved

Box 24.5 Postlignification metabolism and heartwood formation require nonstructural plant phenolic compounds.

Heartwood represents more than 95% of the merchantable bole of harvested wood. The heartwood of commercially important woody plants accounts for more than 60% of the revenues generated from harvesting plant materials before further factory processing. Heartwood serves as the main source of raw material for lumber, solid wood products, fine furniture, paper, and many miscellaneous applications. Despite its economic significance, however, the general mechanism responsible for its formation is one of the most poorly studied and poorly understood areas of plant science today.

Heartwood is formed by the species-specific deposition of distinct and varied metabolites that frequently alter the color, durability, texture, and odor of particular woods relative to that of sapwood. Heartwoods contain strikingly distinctive colored metabolites that can readily be observed by inspecting cross-sections of woody stems of plants, such as tamarack (see panel A of figure), western red cedar (reddish- or pinkish-brown to dark brown), ebony (jet black), and southern pine (yellow-orange). In contrast, spruce wood, highly valued for pulp and paper manufacture, contains less of the highly colored heartwood metabolites and hence has a pale whitish-yellow color. Indeed, the pale color and the very high lignin content of this wood (about 28%) indicate that lignin biopolymers themselves are nearly colorless.

Heartwood production is a postsecondary xylem-forming process, whereby nonstructural highly colored phenolics (primarily lignan, stilbene, and flavonoid-derived compounds) and other characteristic substances (e.g., terpenes or alkaloids) are infused into wood that has already been lignified. Substances similar (if not identical, in some cases) to those in heartwood can also be formed in regions where insects or pathogens have attacked sapwood, but these are manifested as a more-localized containment response. For example, panel B of the figure shows sapwood of radiata pine into which a *Sirex noctilio* wasp has bored, forming two tunnels, one for the wasp's eggs and one for a fungus, *Amylostereum noctilio,* that serves as a foodstuff for the larvae. The attacked plant responds by increasing the deposition of various phenolic substances, in this case stilbenes, which are primarily localized in the affected regions, making them appear lighter-colored than the background in the stained wood section shown in panel C of the figure.

Constitutive heartwood formation, on the other hand, follows several years or decades of sapwood growth and development. According to biochemical details only now becoming known, heartwood metabolites are first deposited in the central (pith) region of the lignified woody stem tissues, which primarily consist of dead, lignified cells. Over years of subsequent growth, heartwood formation gradually extends radially, until almost all of the woody xylem tissue is encompassed. A transition zone sometimes visible between the heartwood and sapwood is presumed to be involved in the final stages of biosynthesis of heartwood metabolites preceding cell death. The composition of heartwood metabolites varies extensively among species. For example, Douglas fir accumulates flavonoids and lignans in its heartwood, whereas yellow poplar deposits lignans, terpenoids, and alkaloids.

Given that wood is composed largely of dead cells, how are heartwood metabolites deposited? Investigators recognized 50 years ago that ray parenchyma cells remain living in lignified sapwood. As their last function before death, these cells accumulate or biosynthesize substances (often in complex species-specific mixtures) that are then infused into lignified woody secondary xylem by way of pit apertures (see figure, panel D). This infusion process may explain why many of the heartwood substances also occur at much lower concentrations in sapwood, where the ray parenchyma cells

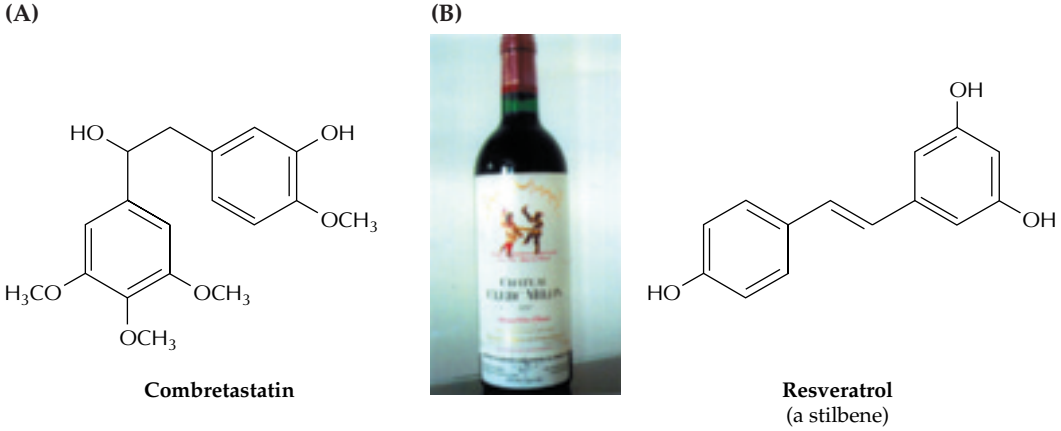
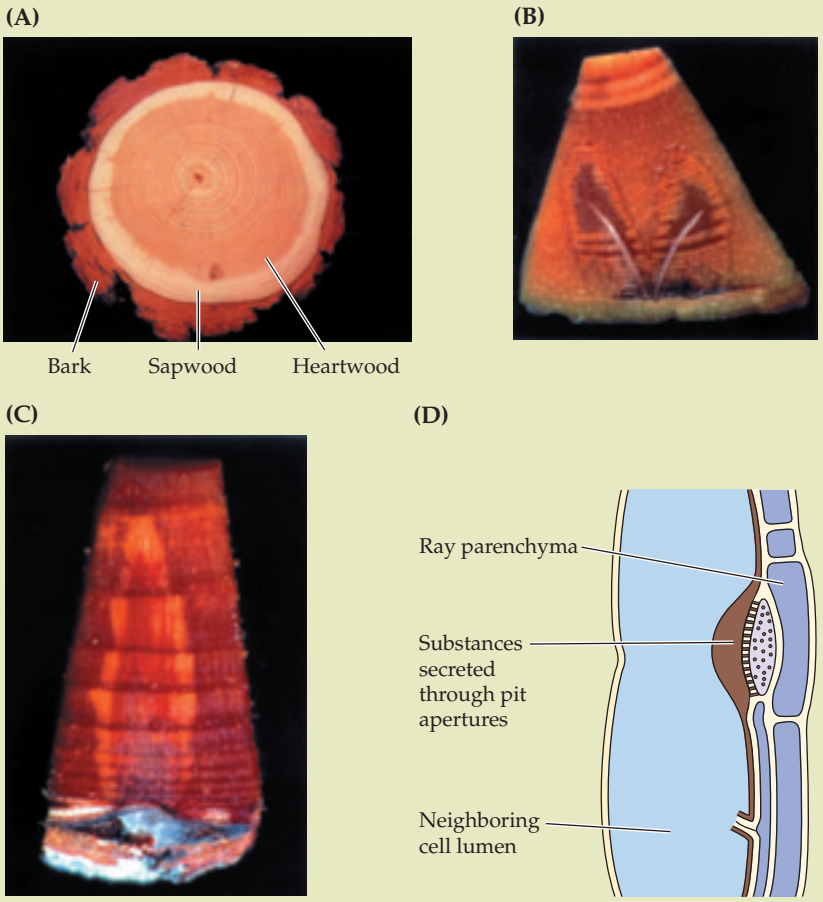


Figure 24.77
(A) The stilbene, combretastatin, has antineoplastic activity. (B) Another stilbene, resveratrol, present in red grapes and red wine, has potent antitumor properties.



are located. At some point during heartwood formation, the infused phenolic substances apparently undergo oxidation to form nonstructural oligomeric and polymeric components, some of which can be removed only under the conditions typically used for lignin extraction. With the further sealing of bordered pits, heartwood ceases to function in conducting nutrients and water and essentially becomes a protective structural tissue, highly durable and resistant to rot.

sources of pharmaceuticals, nutriceuticals, pigments, flavors, and fragrances; and selective adjustments to the taste and odor of selected plant species. Indeed, a biotechnological revolution is now being witnessed in the plant sciences. The combined use of molecular genetic techniques and conventional plant breeding approaches is expected to produce a new generation of plants that are even further optimized for human use.

By far the largest and economically most significant deployment of plant materials is as a fiber source, whether for pulp/paper, lumber for housing and shelter, wood for furniture, or other applications. Accordingly, many biotechnological strategies are directed toward improving fiber and wood properties by manipulating the biochemical processes responsible for cell wall biosynthesis and associated metabolic functions. This approach could involve modification of lignin, either to render it more susceptible to removal, or to increase its content, thereby increasing the strength and rigidity of certain fragile crops. Modifying heartwood metabolite formation may allow researchers to tailor traits such as rot resistance, texture, color, and durability in various commercially important woody plant heartwoods. These goals require further study of the fundamental mechanisms controlling both macromolecular assembly patterns involved in the biosynthesis of plant cell wall polymers and exploration of how and where the heartwood-forming metabolites are generated.

To this point, most of the biotechnological emphasis placed on attempting to engineer lignin content and composition has involved

Box 24.6 Phenolics flavor our world.

Phenylpropanoid-derived plant phenolics contribute significantly to imparting specific fragrances/odors, flavors, and tastes to various plants widely utilized in the food and beverage industries today (see figure). Although the biochemistry of their formation is scarcely addressed in this brief chapter, their importance cannot be discounted.

The capsaicinoids, such as capsaicin, are responsible for the pungent properties of the red peppers, whereas the piperinoids flavor black pepper. The delightful tastes of cinnamon and ginger are imparted by various cinnamate and gingerol derivatives, respectively; and allylphenols establish the characteristic tastes and odors of oil of cloves, widely used in toothache treatment, and of the spices nutmeg and mace. Vanillin, from the vanilla bean, is used extensively in both baking and confectionery. In most instances, precise biochemical pathways to these compounds are not yet established at the levels of either the enzymes or the genes.

Plant phenolics are important components of the characteristic aromas, flavors, and colors of many beverages, whether for alcoholic or nonalcoholic consumption. Chlorogenic acid, for example, constitutes about 4% of the coffee bean and is thus ingested daily by millions. Green and black tea leaves contain other plant phenolics, such as (*epi*-)catechins and various other tannins that impart characteristic tastes to these popular beverages. Most drinks consumed today would be watery indeed if not for various phenolics, such as vanillin, ferulic acid, certain flavonoids, tannins, and others. An important endeavor of the flavor and fragrance industry is to define or identify the mixtures of various phenolic substances that create pleasing flavors ranging from maple syrup to whisky.

using antisense and sense strategies to target the genes that encode various enzymatic steps in the pathway from phenylalanine to the monolignols (see Fig. 24.49). This work has focused primarily on cinnamyl alcohol dehydrogenase and cinnamoyl-CoA reductase and has targeted such plants as tobacco, poplar, and eucalyptus. Although the effects on lignin formation per se have often been quite small, the transgenic plant tissues produced were highly colored, unlike the original wild-type plants. Whether these transgenic plants will have any beneficial properties, for example, greater ease of lignin removal for pulp/paper applications, is unclear. The pigmentation effects observed were not anticipated by the researchers involved and point to the fact that attempts to alter lignin-forming processes must also take into account the related biochemical pathways that utilize the same substrates.

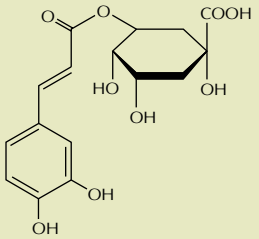
The finding that lignin formation proper is somehow temporally and spatially associated with various presumedly proline-rich proteins and dirigent sites holds much promise. Full details of the influence of these proteins on lignin structure may result in the design of new strategies for modifying both lignin deposition and structure. The discovery of dirigent proteins, pinoresinol/lariciresinol reductases, and their corresponding genes also affords the opportunity to pursue various interesting questions, including how heartwood is formed.

Advances in lignan and (iso)flavonoid biochemistry and molecular biology offer the opportunity to modify concentrations of health protectants and pharmacologically active species in particular plants of choice. Eventually, we should be able to engineer the formation of secoisolariciresinol, matairesinol, daidzein, genistein, and similar compounds in staple crops that do not ordinarily produce them in significant quantities. The corresponding transgenic plants thus would provide long-term health benefits as sources of cancer preventives. A similar target for enhanced production might be podophyllotoxin, one of a handful of plant anticancer compounds already in use today.

The potential being unleashed is perhaps most vividly demonstrated by the impressive advances in plant metabolic engineering seen in the manipulation of flower color by application of sense/antisense technologies. Several laboratories in Europe and New Zealand have successfully transformed various plants such as petunia to alter petal color.

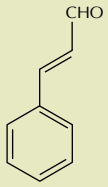
Lastly, knowledge of these pathways will eventually lead to the systematic modification and improvement of plant flavors and fragrances, the properties of which define the very essence of many of our foodstuffs, such as pepper, ginger, and vanilla.

Coffee beans



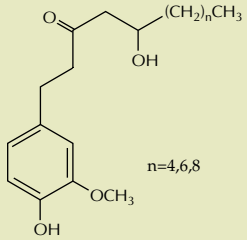
Chlorogenic acid

Cinnamon bark



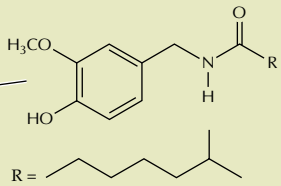
Cinnamaldehyde

Ginger rhizome



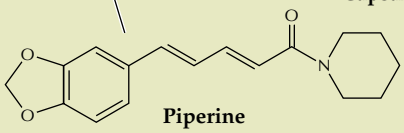
Gingerols

Red and black peppers



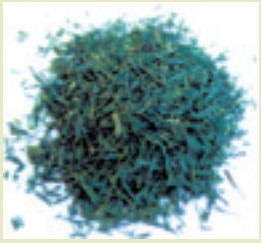
Nordihydrocapsaicin

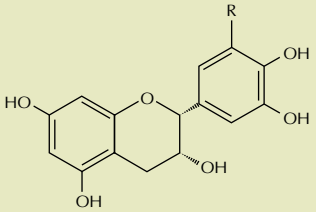
R = **Capsaicin**



Piperine

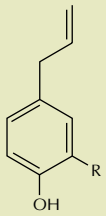
Green tea





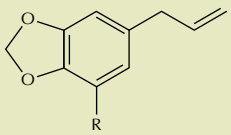
R = H **(–)-Epicatechin**
R = OH **(–)-Epigallocatechin**

Cloves



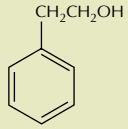
R = H **Chavicol**
R = OCH_3 **Eugenol**

Nutmeg



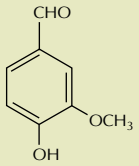
R = H **Safrole**
R = OCH_3 **Myristicin**

Orchid



Phenylethyl alcohol

Vanilla



Vanillin

These modifications will ultimately impact the quality of many of our alcoholic and nonalcoholic beverages, which in turn are often largely determined by their aromatic phenolic constituents (Box 24.6).

Summary

Plants produce a great variety of organic compounds that are not directly involved in primary metabolic processes of growth and development. The roles these natural products or secondary metabolites play in plants have only recently come to be appreciated in an analytical context. Natural products appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as attractants of pollinators and seed dispersers. They may also act to create competitive advantage as poisons of rival species.

Most natural products can be classified into three major groups: terpenoids, alkaloids, and phenolic compounds (mostly phenylpropanoids). Terpenoids are composed of five-carbon units synthesized by way of the acetate/mevalonate pathway or the glyceraldehyde 3-phosphate/pyruvate pathway. Many plant terpenoids are toxins and feeding deterrents to herbivores or are attractants of various sorts. Alkaloids are synthesized principally from amino acids. These nitrogen-containing compounds protect plants from a variety of herbivorous animals, and many possess pharmacologically important activity. Phenolic compounds, which are synthesized primarily from products of the shikimic acid pathway, have several important roles in plants. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens. In addition, lignins strengthen cell walls mechanically, and many flavonoid pigments are important attractants for pollinators and seed dispersers. Some phenolic compounds have allelopathic activity and may adversely influence the growth of neighboring plants.

Throughout the course of evolution, plants have developed defenses against herbivory and microbial attack and produced other natural products to aid competitiveness. The better-defended, more-competitive plants have generated more progeny, and so the capacity to produce and safely store such ecologically useful metabolites has become widely established in the plant kingdom. Pressures from herbivores and pathogens, as well as constant competition, continue to select for new natural products. In cultivated species, however, such chemical defenses have often been artificially selected against.

Study of the biochemistry of plant natural products has many practical applications. Biotechnological approaches can selectively increase the amounts of defense compounds in crop plants, thereby reducing the need for costly and potentially toxic pesticides. Similarly, genetic engineering can be utilized to increase the yields of pharmaceuticals, flavor and perfumery materials, insecticides, fungicides, and other natural products of commercial value. Although many natural products and their functions have been described in this chapter, the metabolism of natural products in most plant species remains to be elucidated. A great deal of fascinating biochemistry remains to be discovered.

Further Reading

Terpenoids

Cane, D. E., ed. (1999) *Comprehensive Natural Products Chemistry, Vol. 2, Isoprenoids Including Carotenoids and Steroids.* Pergamon/Elsevier, Amsterdam.

Chappell, J. (1995) Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46: 521–547.

Eisenreich, W., Schwarz, M., Cartayrade, A., Arigoin, D., Zenk, M. H., Bacher, A. (1998) The deoxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem. Biol.* 5: R221–R233.

Gershenzon, J., Croteau, R. (1993) Terpenoid biosynthesis: the basic pathway and formation of monoterpenes, sesquiterpenes and diterpenes. In *Lipid Metabolism in Plants,* T. S. Moore, Jr., ed. CRC Press, Boca Raton, FL, pp. 339–388.

Harborne, J. B., and Tomas-Barberan, F. A., eds. (1991) *Ecological Chemistry and Biochemistry of Plant Terpenoids.* Clarendon Press, Oxford, UK.

Langenheim, J. H. (1994) Higher plant terpenoids: a phytocentric overview of

their ecological roles. *J. Chem. Ecol.* 20: 1223–1280.
Lichtenthaler, H. K. (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 47–65.
McGarvey, D., Croteau, R. (1995) Terpenoid biosynthesis. *Plant Cell* 7: 1015–1026.

Alkaloids

Cordell, G., ed. (1997) *The Alkaloids,* Vol. 50. Academic Press, San Diego.
Rosenthal, G. A., and Berenbaum, M. R., eds. (1991) *Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. 1: The Chemical Participants,* 2nd ed. Academic Press, San Diego.
Southon, I. W., and Buckingham, J., eds. (1989) *Dictionary of Alkaloids.* Chapman and Hall, London.

Suberin

Bernards, M. A., Lewis, N. G. (1998) The macromolecular aromatic domain in suberized tissues: a changing paradigm. *Phytochemistry* 47: 583–591.

Lignins and lignans

Gang, D. R., Costa, M. A., Fujita, M., Dinkova-Kostova, A. T., Wang, H.-B., Burlat, V., Martin, W., Sarkanen, S., Davin, L. B., Lewis, N. G. (1999) Regiochemical control of monolignol radical coupling: a new paradigm for lignin and lignan biosynthesis. *Chem. Biol.* 6: 143–151.
Lewis, N. G., Davin, L. B. (1999) Lignans: biosynthesis and function. In *Comprehensive Natural Products Chemistry, Vol. 1, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 639–712.
Lewis, N. G., Davin, L. B., Sarkanen, S. (1999) The nature and functions of lignins. In *Comprehensive Natural Products Chemistry, Vol. 3, Carbohydrates and Their Derivatives Including Tannins, Cellulose and Related Lignins,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 617–745.
Sarkanen, S., Lewis, N. G. (1998) Biosynthesis of lignins and lignans. *ACS Symp. Ser.* 697: 1–421.
Timell, T. E. (1986) *Compression Wood in Gymnosperms,* Vols. 1–3. Springer-Verlag, Berlin.

Flavonoids

Dixon, R. A. (1999) Isoflavonoids: biochemistry, molecular biology and biological functions. In *Comprehensive Natural Products Chemistry, Vol. 1, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 773–824.
Forkmann, G. (1991) Flavonoids as flower pigments: the formation of the natural spectrum and its extension by genetic engineering. *Plant Breed.* 106: 1–26.
Forkmann, G., Heller, W. (1999) Biosynthesis of flavonoids. In *Comprehensive Natural Products Chemistry, Vol. 1, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 713–748.
Harborne, J. B., ed. (1994) *The Flavonoids: Advances in Research Since 1986.* Chapman and Hall, London.

Coumarins and furanocoumarins

Berenbaum, M. R., and Zangerl, A. R. (1996) Phytochemical diversity: adaptation or random variation. *Rec. Adv. Phytochem.* 30: 1–24.
Keating, G. J., O'Kennedy, R. (1997) The chemistry and occurrence of coumarins. In *Coumarins: Biology, Application and Mode of Action,* R. O'Kennedy and R. D. Thornes, eds. John Wiley & Sons, Chichester, pp. 23–66.
Matern, U., Lüer, P., Kreusch, D. (1999) Biosynthesis of coumarins. In *Comprehensive Natural Products Chemistry, Vol. 1, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 623–638.
Matern, U., Strasser, H., Wendorff, H., Hamerski, D. (1988) Coumarins and furanocoumarins. In *Cell Culture and Somatic Cell Genetics of Plants,* Vol. 5, I. K. Vasil, ed. Academic Press, Orlando, FL, pp. 3–21.

Zobel, A. M. (1997) Coumarins in fruits and vegetables. *Proc. Phytochem. Soc. Eur.* 41: 173–203.

Stilbenes, styrylpyrones, and arylpyrones

Berkert, C., Horn, C., Schnitzler, J.-P., Lehning, A., Heller, W., Veit, M. (1997) Styrylpyrone biosynthesis in *Equisetum arvense. Phytochemistry* 44: 275–283.

Gorham, J., Tori, M., Asakawa, Y. (1995) *The Biochemistry of the Stilbenoids, Vol. 1, Biochemistry of Natural Products Series,* J. B. Harborne, ed. Chapman and Hall, London.

Schröder, J. (1999) The chalcone/stilbene synthase-type family of condensing enzymes. In *Comprehensive Natural Products Chemistry, Vol. 1, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives,* D.H.R. Barton, K. Nakanishi, and O. Meth-Cohn, eds.-in-chief. Elsevier, Amsterdam, pp. 749–772.