Phenolic Compounds: Introduction

50

Vincenzo Lattanzio

Contents

Abstract

"Plant phenolics" and "polyphenols" are secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway, which directly provides phenylpropanoids, or the "polyketide" acetate/malonate pathway, which can produce simple phenols, or both, thus producing monomeric and polymeric phenols and polyphenols, which fulfill a very broad range of physiological roles in plants. Higher plants synthesize several thousand known different phenolic compounds. The ability to synthesize phenolic compounds has been selected throughout the course of evolution in different plant lineages, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time.

Plant phenolics are considered to have a key role as defense compounds when environmental stresses, such as high light, low temperatures, pathogen infection, herbivores, and nutrient deficiency, can lead to an increased production of free radicals and other oxidative species in plants. Both biotic and abiotic stresses stimulate carbon fluxes from the primary to the secondary metabolic pathways,

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thus inducing a shift of the available resources in favor of the synthesis of secondary products. An interesting link between primary and secondary metabolism couples the accumulation of the stress metabolite proline with the energy transfer toward phenylpropanoid biosynthesis via the oxidative pentose phosphate pathway. The alternating oxidation of NADPH by proline synthesis and reduction of $NADP^+$ by the two oxidative steps of the oxidative pentose phosphate pathway lead to the simultaneous accumulation of phenolic compounds.

1   Introduction

There are approximately 300,000 documented species of higher plants on the planet, which synthesize an enormous number of chemicals of diverse structure and class (more than 200,000 isolated and identified individual chemical entities). These compounds can be further divided into primary and secondary metabolites. The primary metabolites include metabolites such as sugars, fatty acids, amino, and nucleic acids, as well as chemicals considered ubiquitous to all plants for growth and development [1, 2]. Secondary metabolites are structurally and chemically much more diverse than the primary metabolites and refer to compounds present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism but are thought to be required for plants' survival in the environment. Plants have metabolic pathways leading to tens of thousands of secondary products capable of effectively responding to stress situations imposed by biotic and abiotic factors. These pathways, often recruited from essential primary metabolism pathways upon initial gene duplication leading to duplicated genes showing new functions and optimized and diversified roles in new pathways, are an integral part of the developmental program of plants. Accumulation of secondary metabolites often marks the onset of developmental stages. A strict spatial and temporal control of gene expression ensures the correct accumulation pattern of various secondary products. The required transport of metabolic intermediates constitutes an additional level of regulation. Ontogeny and circadian clock-controlled gene expression are also important features of plant secondary metabolism, as are master regulatory transcription factors [3–8]. Secondary metabolites apparently act as defense (against herbivores, microbes, viruses, or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protecting the plant from ultraviolet radiation and oxidants. The pattern of secondary metabolites in a given plant is complex; it changes in a tissue- and organ-specific way; differences can be seen between different developmental stages

(e.g., organs important for survival and reproduction have the highest and most potent secondary metabolites), between individuals, and between populations. These secondary metabolites are classified into several groups according to their biosynthetic routes and structural features [9–14].
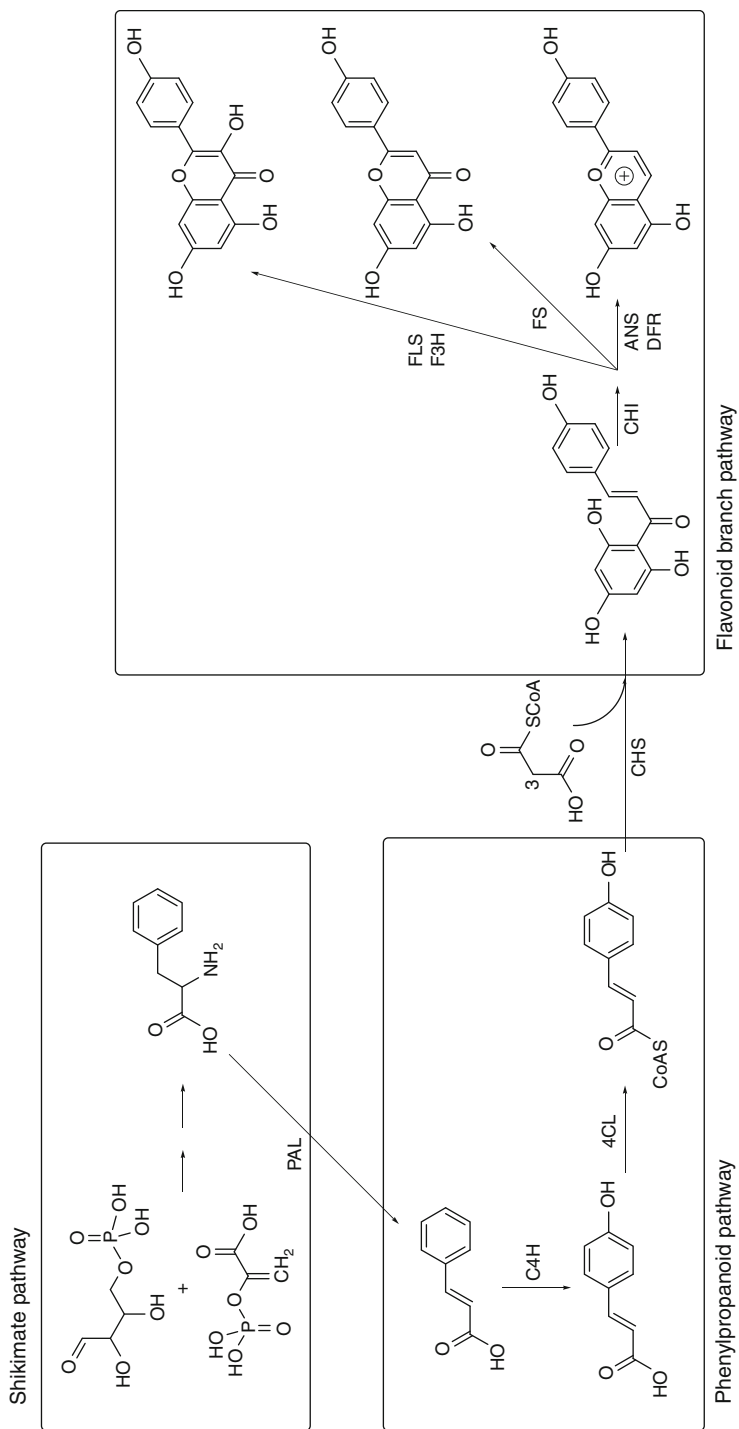
Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom, even if the type of compound present varies according to the phylum under consideration. Phenolics are uncommon in bacteria, fungi, and algae. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenols is found [15, 16]. It is estimated that about 2% of all carbon photosynthesized by plants is converted into flavonoids or closely related compounds [17]. Higher plants synthesize several thousand known different phenolic compounds, and the number of those fully characterized is continually increasing. Leaves of vascular plants contain esters; amides and glycosides of hydroxycinnamic acids; glycosylated flavonoids, especially flavonols; and proanthocyanidins and their relatives. Lignin, suberin, and pollen sporopollenin are examples of phenolic-containing polymers. Some soluble phenolics are widely distributed, for example, chlorogenic acid, but the distribution of many other structures is restricted to specific genera or families, making them convenient biomarkers for taxonomic studies.
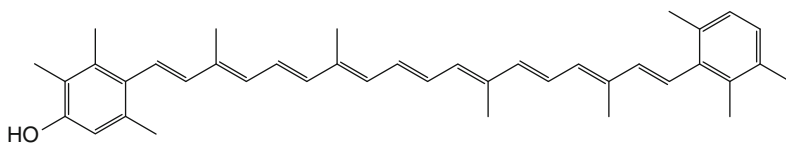
The ability to synthesize phenolic compounds has been selected throughout the course of evolution in different plant lineages when such compounds addressed specific needs, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time [18–21]. For example, the successful adaptation to land of some higher members of the Charophyceae – which are regarded as prototypes of amphibious plants that presumably preceded true land plants when they emerged from an aquatic environment onto the land – was achieved largely by massive formation of "phenolic UV light screens" [9, 15, 22, 23]. The phenylpropanoid pathway leading to lignins involves a common set of biochemical reactions in vascular plants already present 400 million of years ago with the emergence of erect vascular land plants. From an evolutionary point of view, these metabolic backbones have been progressively enriched to provide specific adaptations to different plant families and the remarkable biochemical diversity we can observe [21]. Accounting for about 40% of organic carbon circulating in the biosphere, these phenolic compounds are biosynthetically formed by way of either the shikimic acid pathway or the malonate/acetate pathway, also known as the polyketide pathway, and related biochemical pathways. Furthermore, it is their reassimilation back to carbon dioxide during biodegradation (mineralization) that presents the rate-limiting step in recycling biological carbon [24, 25].

Plant phenolics are, in themselves, a fascinating group of substances and have attracted some of the most distinguished of the Nobel-Prize-winning organic chemists, including Emil Fischer, who studied chemical substances used in tanning; Richard M. Willstätter, who in 1913 proposed the first chemical hypothesis concerning blue flower color development; Robert Robinson (copigmentation
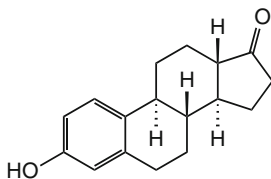
theory); Richard L.M. Synge (interested in interactions of tannins with proteins); and Alexander R. Todd who worked from 1931 to 1934 on anthocyanins and other coloring matters together with Sir Robert Robinson [26–29]. The continuing fascination of polyphenols for chemists involved with plant constituents is shown by the fact that a "plant phenolics group" was established in France (Narbonne 1970: the first meeting; Avignon-Montfavet 1971: the first International Conference on Polyphenols). This "Groupe Polyphénols" was constituted in an international society in Narbonne (France) in 1972 with the aim of promoting research on plant polyphenols, while providing members worldwide with a unique forum to exchange information on all aspects of these fascinating natural products, from their most basic and fundamental biophysicochemical properties to their most diverse applications in food and agricultural, pharmaceutical, and cosmetic sciences and technologies (http://www.groupepolyphenols.com/).

Broadly, as far as the definition of plant phenolics is concerned, the term 'phenol' is a chemical term that defines a phenyl ring bearing one or more hydroxyl substituents. The term 'polyphenol' could thus be used to define natural products featuring at least two phenyl rings bearing one or more hydroxyl substituents, including their functional derivatives (e.g., esters and glycosides), but in the context of plant phenolics such a definition is not satisfactory, since it would include compounds such as the gossypol, the phenolic carotenoid 3-hydroxyisorenieratene (I) or the phenolic female sex hormone oestrone (II), which are principally terpenoid in origin [30]. Thus, as a general rule recently proposed by Quideau et al. [31], the terms 'plant phenolics' should be strictly used to refer to secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway, which directly provides phenylpropanoids (Scheme 50.1), or the 'polyketide' acetate/malonate pathway, and which fulfill a very broad range of physiological roles in plants [30, 31]. In fact, although the bulk of these compounds play cell wall structural roles, plant tissues synthesize a vast array of nonstructural constituents that have various roles in plant growth and survival. Thus, the expression "plant phenolics" embraces a highly diverse group, the chemically known members of which can be counted in several thousand with a large range of identified structures: monomeric, dimeric, and polymeric phenolics have been identified. Several classes of phenolics have been categorized on the basis of their basic skeleton: C_6 (simple phenol, benzoquinones), C_6—C_1 (phenolic acid), C_6—C_2 (acetophenone, phenylacetic acid), C_6—C_3 (hydroxycinnamic acid, coumarin, phenylpropanes, chromones), C_6—C_4 (naphthoquinones), C_6—C_1—C_6 (xanthones), C_6—C_2—C_6 (stilbenes, anthraquinones), C_6—C_3—C_6 (flavonoids, isoflavonoids, neoflavonoids), $(C_6$—C_3—$C_6)_{2,3}$ (bi-, triflavonoids), $(C_6$—$C_3)_2$ (lignans, neolignans), $(C_6$—$C_3)_n$ (lignins), $(C_6)_n$ (catechol melanins), and $(C_6$—C_3—$C_6)_n$ (condensed tannins). Low-molecular-weight phenolics occur universally in higher plants; some of them are common in a variety of plant species and others are species specific. Higher-molecular-weight proanthocyanidins (also called condensed tannins) are the most abundant polyphenols in woody plants but are usually absent in herbaceous plants. Hydrolyzable tannins have a more restricted occurrence than proanthocyanidins, being found in only 15 of the 40 orders of dicotyledons [16, 32–40].

Scheme 50.1 Schematic of the major branch pathways of (poly)phenol biosynthesis. *PAL* phenylalanine ammonia-lyase, *C4H* cinnamate-4-hydroxylase, *4CL* 4-coumaroyl:CoA-ligase, *CHS* chalcone synthase, *CHI* chalcone isomerase, *ANS* anthocyanidin synthase, *DFR* dihydroflavonol reductase, *FS* flavone synthase, *FLS* flavonol synthase, *F3H* flavanone 3-hydroxylase

I

II

2　The Major Classes of Phenolics in Plants

Examples of simple phenols (C_6) include catechol and phloroglucinol. Although most of the more complex plant polyphenols contain these two simple phenols as a parts of their structures, catechol and phloroglucinol are uncommon in plant tissues. Catechol has been found in leaves of *Gaultheria* species, while phloroglucinol has been found as glucoside in the peel of various *Citrus* fruits. Arbutin (**III**) is found in leaves of various *Vaccinium* spp., such as blueberry, cranberry, cowberry, and pear trees (*Pyrus communis* L., *Rosaceae*) [41–43].
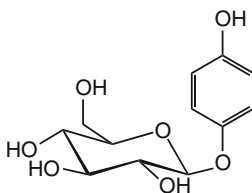
III

Phenolic acids (C_6—C_1) are commonly represented by gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids. Phenolic acids are usually present in the bound soluble form conjugated with sugars or organic acids and are typically components of complex structures such as lignins and hydrolyzable tannins. Gallic acid is the base unit of gallotannins, whereas gallic acid and hexahydroxydiphenoyl moieties are both subunits of the ellagitannins, which are classified as hydrolyzable tannins. Free and bounded phenolic acids are found in cereals. Hydroxybenzoic acid glycosides are also characteristic of some herbs and spices. Aldehydes related to C_6—C_1 acids are also found in plants. Salicylaldehyde, *p*-hydroxybenzaldehyde (*Sorghum* spp.), *p*-anisaldehyde (*Vanilla*, *Mimosa*), and *p*-protocatechualdehyde

(*Cichorium intybus*) are also quite common in plants. Vanillin, the odor principle occurring in *Vanilla* pods, is certainly of very widespread origin [42, 44]. Ellagic acid has been reported to be present in berries, particularly raspberries (*Rubus idaeus*), strawberries, and blackberries. However, free ellagic acid is normally present in low levels in berries that more commonly contain ellagitannins [38, 45].

Among the less common C_6—C_2 compounds, phenolic ketones have occasionally been found as plant constituents. Picein (**IV**) the main component of all investigated spruce needles (*Picea abies* (L.) Karst.), also occurs in *Larix decidua* Mill., *Populus balsamifera*, and *Salix* spp. *p*-Hydroxyphenylacetic acid occurs free and as a glucoside in bamboo shoots. Xanthoxylin, a phloroacetophenone derivative, has been found in *Xanthoxylum* spp. [42, 46–48].

IV
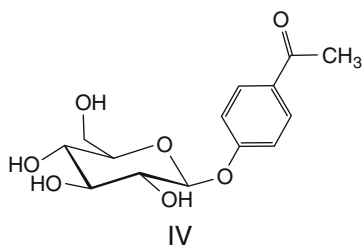
A ubiquitous phenolic unit in plants is one with an aromatic ring attached to a C_3 aliphatic side chain. Such phenylpropanoids occur universally in plants and are also the precursors of many other classes of plant phenolics. For example, by *o*-hydroxylation and subsequent cyclization, *p*-coumaric acid can give rise to hydroxycoumarins. On reduction, it yields *p*-coumaryl alcohol, which is one of the monomeric building rocks of the lignin. β-Oxidation of *p*-coumaric acid yields *p*-hydroxybenzoic acids. Dimerization of *p*-coumaryl alcohol can give rise to a lignin. Finally, the *p*-coumaric moiety is an important unit in the structure of flavonoids, stilbenoids, and xanthones. The most widely distributed hydroxycinnamates (C_6—C_3) are *p*-coumaric, caffeic, ferulic, and sinapic acids, which usually occur in various conjugated forms and are seldom found in the free state except as artifact due to chemical or enzymic hydrolysis during tissue extraction. In aqueous solvents, they isomerizes from the more stable *E* (*trans*) form to the *Z* (*cis*) form, especially under the action of UV light. Hydroxycinnamates frequently accumulate as their respective tartrate esters, coutaric, caftaric, and fertaric acids. Quinic acid conjugates (Fig. 50.1) of caffeic acid, namely, 3-, 4-, and 5-*O*-caffeoylquinic acid, are commonly found in fruits and vegetables. Green coffee beans (*Coffea arabica*) are one of the richest dietary sources of caffeoylquinic acids. Chlorogenic acid (5-*O*-caffeoylquinic acid) (**V**) is the dominant caffeoylquinic derivative accounting for 50% of the total [49, 50]. Coumarins, which are also C_6—C_3 derivatives, are benzo-α-pyrones (lactones) formally derived from *o*-hydroxycinnamic acids by cyclization and ring closure between the *o*-hydroxy and carboxyl groups. This group of phenolic compounds can be found free in nature or in combined form with sugars as heterosides and glycosides in many dicotyledonous
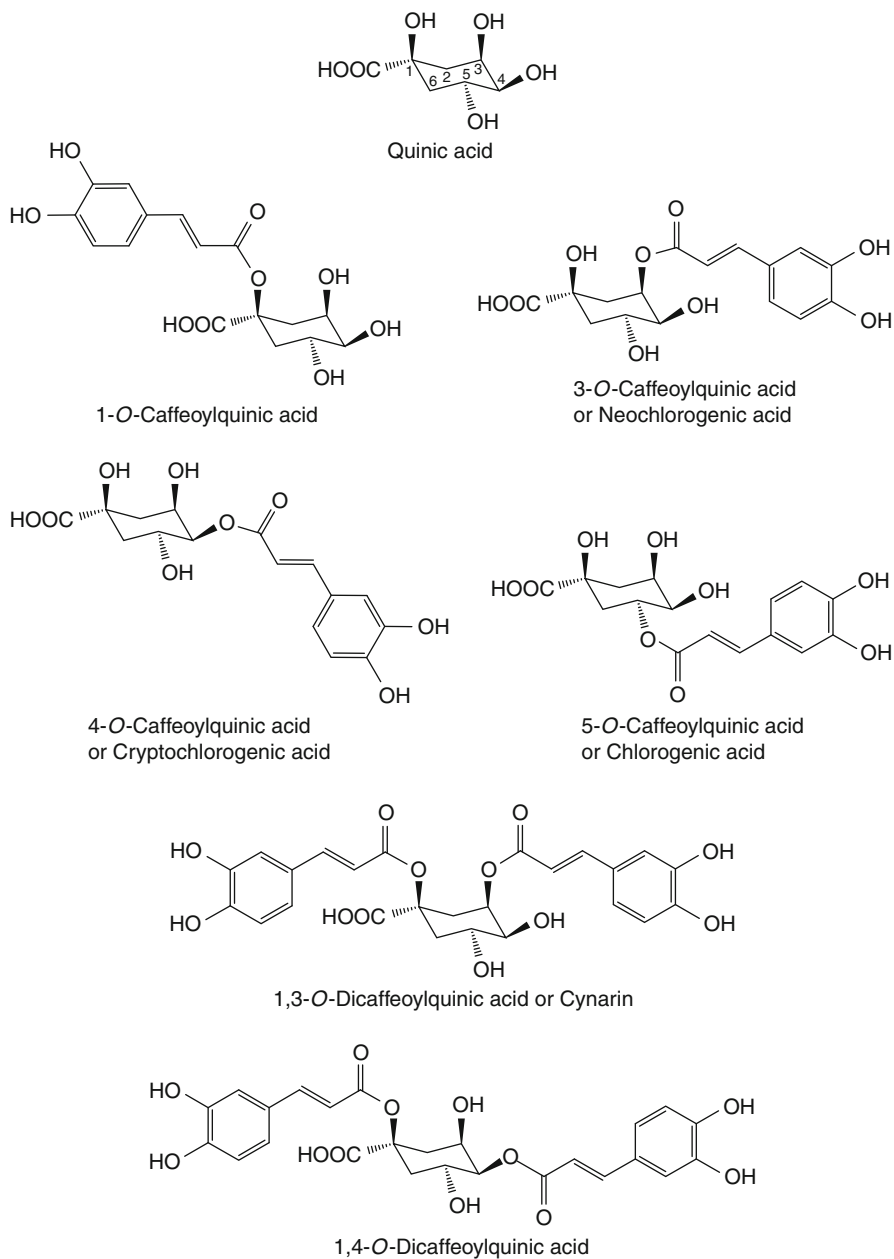
Quinic acid

1-*O*-Caffeoylquinic acid

3-*O*-Caffeoylquinic acid
or Neochlorogenic acid

4-*O*-Caffeoylquinic acid
or Cryptochlorogenic acid

5-*O*-Caffeoylquinic acid
or Chlorogenic acid

1,3-*O*-Dicaffeoylquinic acid or Cynarin

1,4-*O*-Dicaffeoylquinic acid
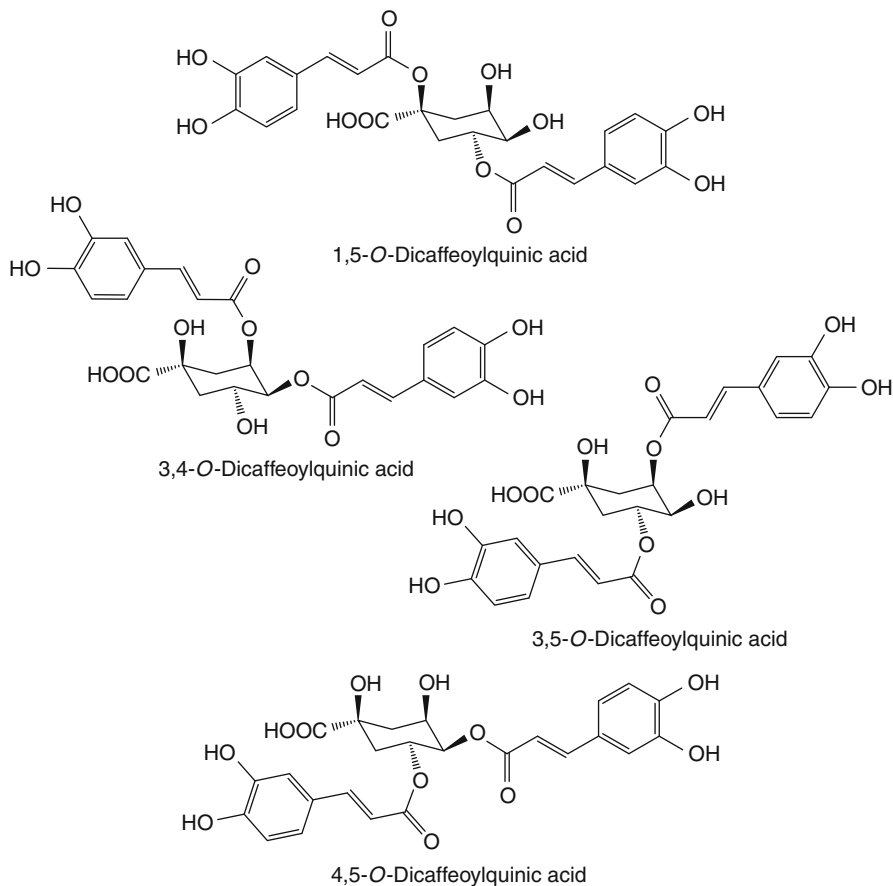
Fig. 50.1 (continued)

Fig. 50.1 Mono- and di-caffeoylquinic acids nomenclature according to IUPAC rules 1976 [149]

families, including the *Apiaceae*, *Asteraceae*, *Fabaceae*, *Moraceae*, *Rosaceae*, *Rubiaceae*, and *Solanaceae*. Although mainly synthesized in the leaves, coumarins occur in high levels in fruits, roots, and stems. Coumarins can be roughly categorized as (i) simple hydroxycoumarins, the most common ones being umbelliferone, esculentin, and scopoletin (**VI**), which occur naturally as their β-O-glucosides; (ii) furanocoumarins (bergaptene, (**VII**)) that consist of a five-membered furan ring attached to the coumarin nucleus; and (iii) finally the pyranocoumarins (calanolide B, (**VIII**)) that are analogous to furanocoumarins but contain a six-membered pyran ring [51, 52]. Chromones are isomeric with coumarins but differ in the biosynthesis and position of the keto group. Their biosynthesis seems to proceed *via* the polyketide pathway by the condensation of five acetate units and subsequent *O*- and *C*-methylation. Most naturally occurring chromones contain a methyl group or an alkyl group at C-2 and hydroxyl or

alkoxyl groups at C-5 and C-7. Only few simple chromones are known to occur naturally. Eugenin (**IX**) is an example of a simple 2-methyl-chromone present in *Eugenia aromatica* [49].
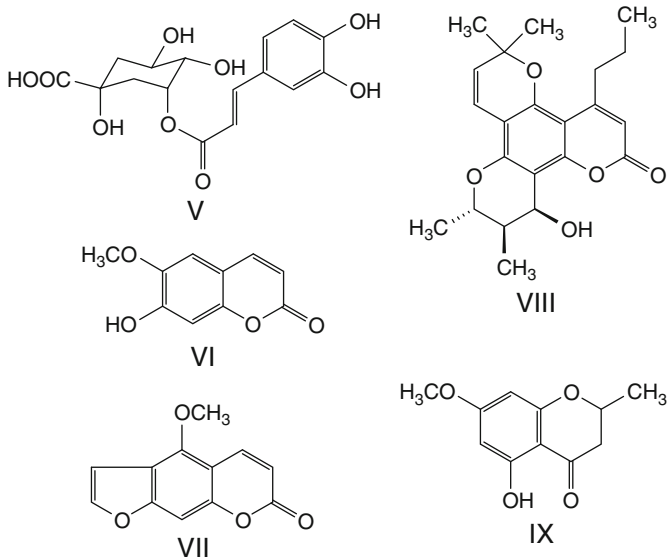
Naphthoquinones (C_6—C_4) represent a class of quinone pigments widespread in nature. The most important higher plant families containing naphthoquinones are *Avicenniaceae*, *Bignoniaceae*, *Boraginaceae*, *Droseraceae*, *Ebenaceae*, *Juglandaceae*, *Nepenthaceae*, and *Plumbaginaceae*. They are biosynthesized *via* a variety of pathways including polyketide pathway (plumbagin, **X**) shikimate/succinyl-CoA combined pathway [lawsone (2-hydroxy-1,4-naphthoquinone)], and shikimate/mevalonate pathway (alkannin, **XI**). Although intensely colored naphthoquinones are not often apparent as plant-coloring matters. They are often present in heartwood or bark, where their presence is masked. When present in living tissues, leaves, and roots, they are usually in colorless form, and color is produced only after extracts have been treated with acid to bring about hydrolysis of sugar linkages and oxidation of quinol to quinone as observed for plumbagin, an orange pigment identified in *Plumbago capensis* [16, 53–55].
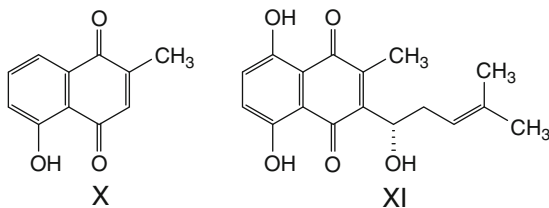
Xanthones (C_6—C_1—C_6) are a class of plant phenolics occurring in a few higher plant families (Gentianaceae, Guttiferae, Loganiaceae, Podostemaceae, and Polygalaceae); therefore, they have a high taxonomic value in such families. The majority of natural xanthones have been found in just two families of higher plants, Guttiferae and Gentianaceae. Xanthones may be classified into five major groups: simple oxygenated xanthones (this group can further be subdivides into six groups according to the degree of oxygenation), xanthone glycosides, prenylated and related xanthones, xanthonolignoids, and miscellaneous. As far as the biosynthetic pathways to xanthones is concerned, ring A and attached carbonyl group are provided by the shikimic acid pathway, whereas ring B arises via the acetate-malonate polyketide route. Mangiferin is unique among the natural xanthones in having a much wider natural occurrence than that of any of the others. This 2-*C*-glucoside of 1,3,6,7-tetrahydroxyxanthone was first found in the leaves of *Mangifera indica*. It has since been found in many ferns and angiosperms such as *Asplenium* spp., *Hypericum* spp., *Cratoxylum pruniflorum*, *Senecio* spp., and *Dahlia* spp. [56–59].

The members of the stilbene family have the C_6—C_2—C_6 structure and are widely distributed in the plant kingdom, although some structures are character-istic of particular plant families. They are found in liverworts, in some ferns, in gymnosperms, and in many dicotyledonous angiosperms, ranging from the unsubstituted *trans*-stilbene from *Alnus* and *Petiveria* to the hexasubstituted combretastatin A-1 from *Combretum caffrum* [60]. There are also increasing numbers of reports of prenylated stilbenes, stilbene glycosides, and polymeric stilbenes. Stilbenes are biosynthesized via phenylpropanoid-polymalonate pathway. The first part of the pathway is common to both stilbenoids and flavonoids, the two biosynthetic routes diverging at the point of cyclization of a styryl-3,5,7-triketoheptanoic acid (Scheme 50.2). A C-acylation produces a chalcone, and subsequent modifications lead to the flavonoids, while an aldol condensation of the same intermediate polyketide produces a stilbene-2-carboxylic acid that is an unstable intermediate in pathways to a range of stilbenoids [61]. The main physiological roles of stilbenes include those of phytoalexins and growth regulators. Preexisting stilbenes may help to protect plant tissues from the attack by fungi, insects, and other organisms. In addition, synthesis of a number of antifungal stilbenes can be induced by infections by appropriate organisms, or by a number of abiotic stimuli such as UV light. These stilbene phytoalexins include resveratrol and its derivatives (**XII**) in *Trifolium*, *Arachis*, and members of Vitaceae [54].


XII
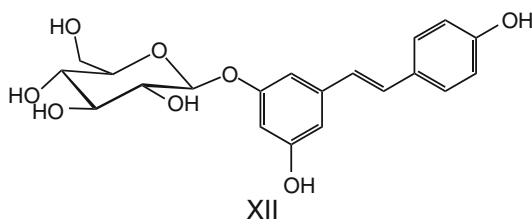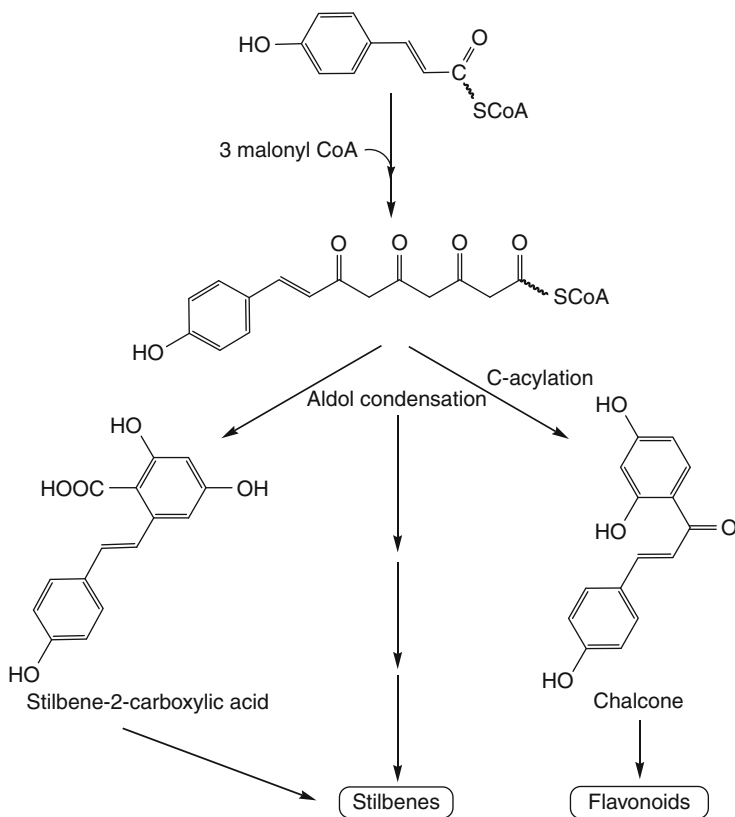
Scheme 50.2 Schematic of stilbene biosynthesis

The tricyclic C_6—C_2—C_6 anthraquinones represent the largest group of natural quinones, and a wealth of structures with varying numbers of hydroxyl groups and other substituents have been identified. More than half of the natural anthraquinones are found in lower fungi, particularly in *Penicillium* and *Aspergillus* species, and in lichens. Over 200 structures have been found in flowering plants, especially in the families of Leguminosae, Liliaceae, Polygonaceae, Rhamnaceae, Rubiaceae, and Scrophulariaceae. Anthraquinones have been encountered in all parts and organs of plants. Thus, they have been isolated from leaves and stems, pods, seed coats, and embryos of *Cassia* plants and from leaves and roots of *Digitalis* spp. Anthraquinones may either be formed via the acetate-malonate pathway in Polygonaceae and Rhamnaceae or *O*-succinylbenzoic acid in the Bignoniaceae and Verbenaceae. In plants, anthraquinones are mostly present as glycosides but the free aglycones are widely distributed as well. Among the most common naturally occurring anthraquinone aglycones in higher plants are emodin (**XIII**), rhein, chrysophanol, aloe-emodin, and physcion. The anthraquinone emodin (1,3,8-trihydroxy-6-methylanthraquinone), mainly reported in three plant families, Fabaceae

(*Cassia* spp.), Polygonaceae (*Rheum*, *Rumex*, and *Polygonum* spp.), and Rhamnaceae (*Rhamnus* and *Ventilago* spp.), is present in both vegetative organs (stem, foliage) as well as in reproductive organs (flower, fruit, seeds, pods) [16, 62, 63].

XIII
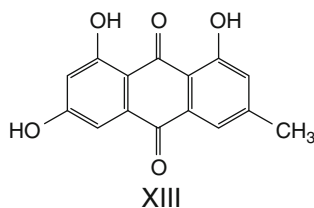
Flavonoids and their conjugates form a very large group of natural products; over 8,000 different flavonoids have been identified. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structures of this class of compounds are based on a C_6—C_3—C_6 skeleton. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylben-zopyrans), the isoflavonoids (3-benzopyrans), and the neoflavonoids (4-benzopyrans). In addition, these chemical structures may differ in the saturation of the heteroatomic ring C and in the overall hydroxylation patterns. All these groups usually share a common chalcone precursor and, therefore, are biogenet-ically and structurally related. The flavonoids may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core. Various subgroups of flavonoids are classified according to both the substitution pattern and the degree of oxidation and saturation present in the heterocyclic C-ring. The flavonoids *sensu stricto* may be divided into 13 subgroups (Fig. 50.2), while isoflavonoids are subdivided into 11 subgroups (Fig. 50.3). The neoflavonoids, structurally and biogenetically closely related to the flavonoids and the isoflavonoids, comprise the 4-arylcoumarins (4-aryl-2*H*-1-benzopyran-2-ones), 3,4-dihydro-4-arylcoumarins, and neoflavenes [35, 39, 64–69].

Together with the proanthocyanidins, the bi- and triflavonoids constitute the two major classes of complex C_6—C_3—C_6 plant phenolics. These compounds arise from the oxidative coupling of various flavonoid structures and thus predominantly possess a carbonyl group at C-4 or its equivalent in every constituent unit. Anyhow, it should be emphasized that the terms bi- and triflavonoids are used loosely and that there is no commonly accepted trivial nomenclature for these classes of flavonoids. A multitude of compounds that do not arise via the phenol oxidative coupling of monomeric flavonoid structures possessing C-4 carbonyl functional groups are also classified as bi- and triflavonoids. For example, the bichalcone (**XIV**) isolated from *Dorstenia zenkeri* is a biflavonoid generated via an intramolecular Diels-Alder process [70].
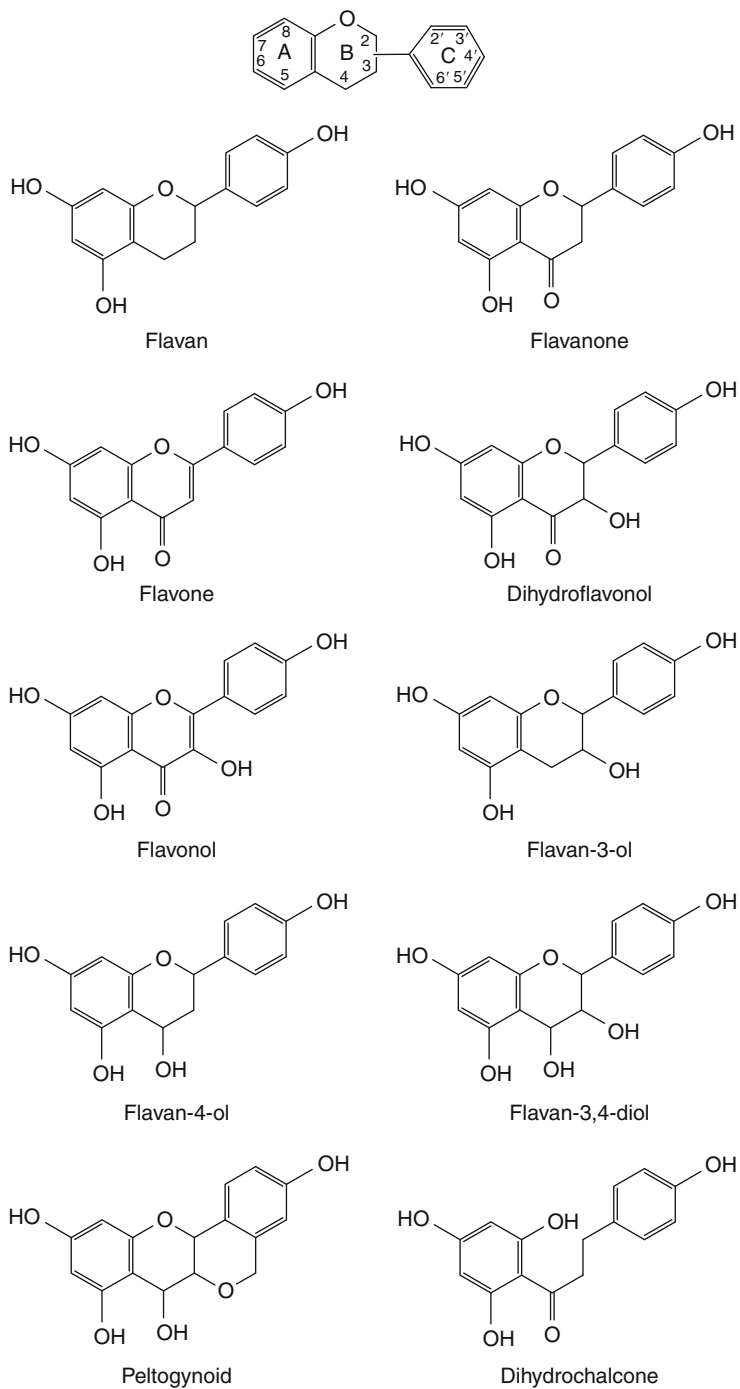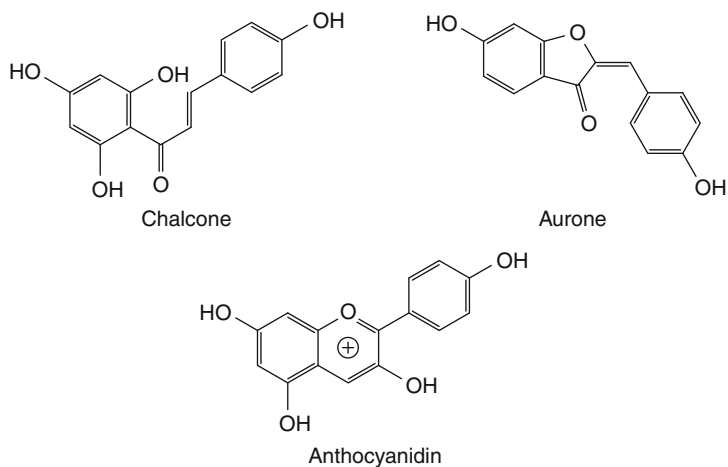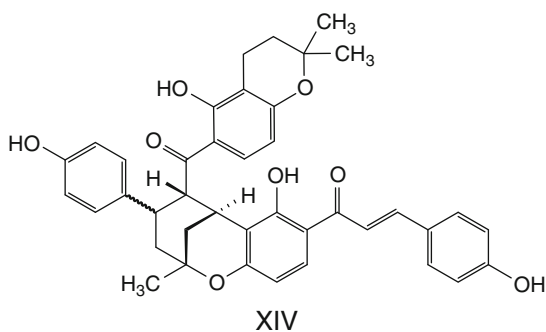
Fig. 50.2 (continued)

Fig. 50.2 Flavonoid subgroups

Lignans and neolignans (C_6—C_3)$_2$ are a large and varied group of plant phenolics produced by the oxidative dimerization of two phenylpropanoid units, which occur in a wide range of plant species. For the purpose of numbering the various parent structures, the C_6—C_3 unit is numbered 1–9 where the α position is the 9, β is the 8, and γ is the 7. When the two C_6—C_3 units are linked by a β,β'-bond (or 8,8'-bond), the parent structure lignane is used as the basis for naming the lignan. If the two C_6—C_3 units are linked by a bond other than a β,β'-bond, the parent structure, neolignane, is used as the basis for naming the neolignan (Fig. 50.4) [71]. (+)-Pinoresinol (**XV**), for example, is a lignan derived by a tail-to-tail linkage in the β-position of two coniferyl alcohol residues, which has been extracted from various plant sources such as *Saussurea medusa*, *Pinus*, and *Picea*. Related dimers, called neolignans, can be formed by other condensations between two C_6—C_3 units, for example, joining head-to-tail
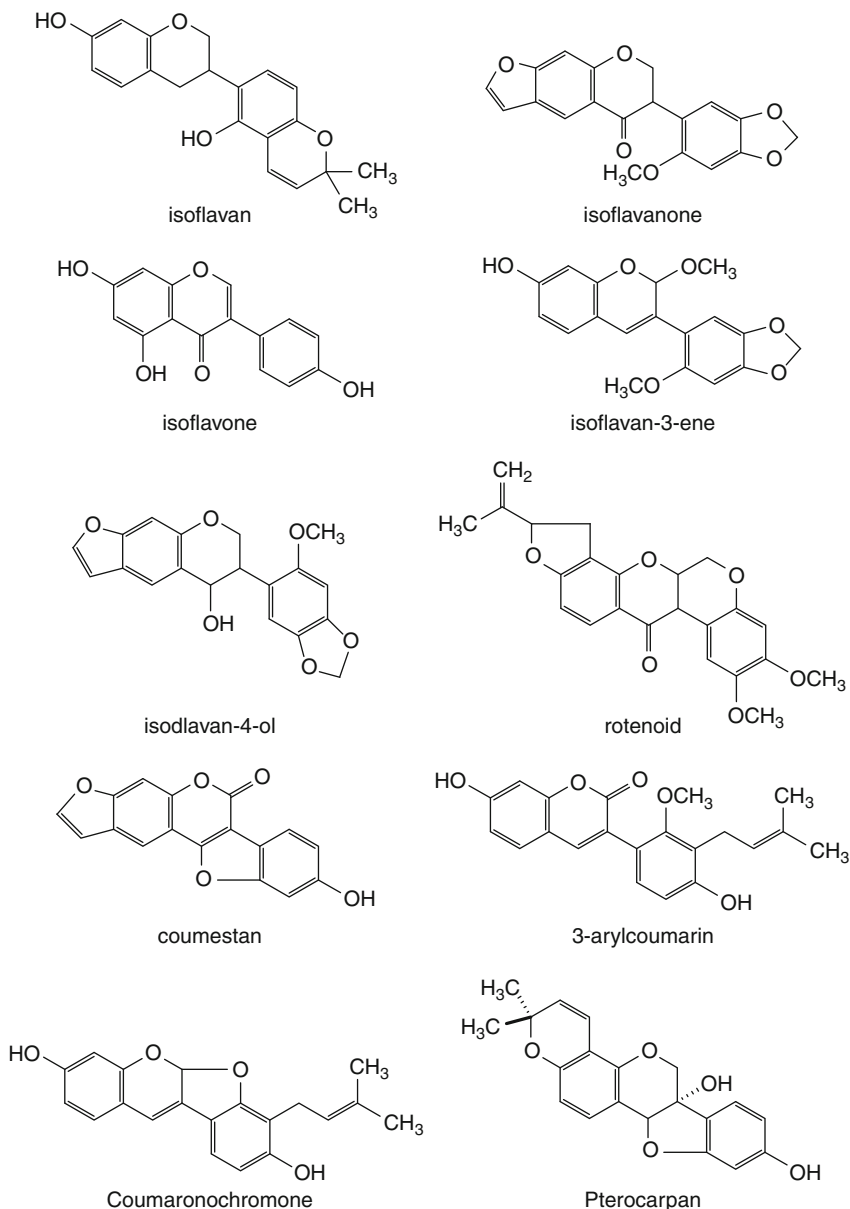
Fig. 50.3 Isoflavonoid subgroups

instead of tail-to-tail. One example of dimer formed by other condensations between two C_6—C_3 units, for example, joining head-to-tail instead of tail-to-tail, is eusiderin (**XVI**). This and related structures occur in heartwoods of Magnoliaceae, Piperaceae, and Lauraceae [72, 73].
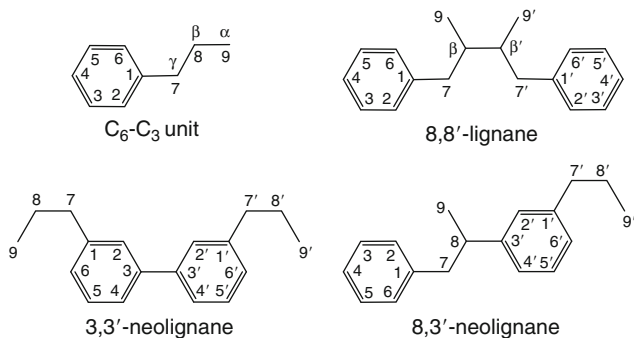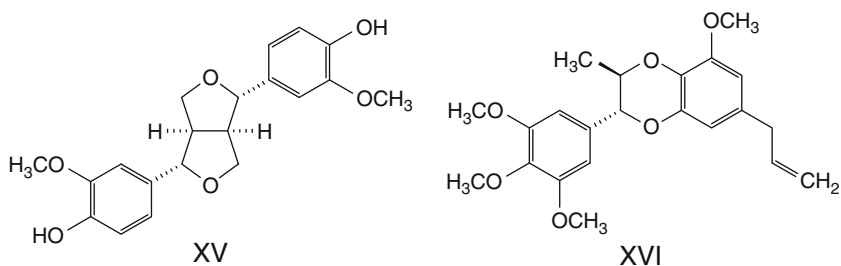
**Fig. 50.4** Parent structures of lignane and neolignanes

The plant *tannins* are a unique group of phenolic compounds of relatively high molecular weight which have the ability to complex strongly with carbohydrates and proteins. The name "tannin" is derived from the French "tanin" (tanning substance) and is used for a range of natural polyphenols. Probably the most acceptable definition of vegetable tannins is still that of Bate-Smith and Swain, formulated in 1962. They adopted the earlier ideas of White and classified these higher plant metabolites as "water-soluble phenolic compounds having molecular weights between 500 and 3,000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins." In higher plants, tannins consist of two major groups of metabolites: the hydrolyzable tannins and condensed tannins. More recently, a third class of tannin, the phlorotannins, has been isolated in several genera of algae. Hydrolyzable tannins are split by acids, bases, and in some cases by hydrolytic enzymes (tannase) into sugars (usually D-glucose) or related polyols and a phenolic acid. In the case of gallotannins, this is gallic acid, and the vegetable tannins are simply polygalloyl esters. Ellagitannins (**XVII**) can be defined in a narrow sense as hexahydroxydiphenoyl esters of carbohydrates or cyclitols. When the hexahydroxydiphenoyl group is cleaved from the molecule, the parent acid rapidly lactonizes to yield the insoluble dilactone ellagic acid. The oligomeric and polymeric proanthocyanidins (*syn.* condensed tannins) (**XVIII**) constitute one of the most ubiquitous groups of all plant phenolics.

The proanthocyanidins usually originate by coupling at C-4 (C-ring) of an electrophilic flavanyl unit, presumably generated from a flavan-3,4-diol or a flavan-4-ol, most commonly to C-8 or C-6 (A-ring) of a nucleophilic flavanyl unit, for example, a flavan-3-ol. This classification of the two major groups of tannins suggested by Freudenberg, based on structural considerations, also represents a subdivision based on biogenetic origins: proanthocyanidins are products of the flavonoid pathway of biosynthesis (polyketide and cinnamate) and gallo- and ellagitannins are both derived from the shikimate pathway. Phlorotannins consist of phloroglucinol units linked to each other in various ways and are of wide occurrence among marine organisms, especially brown and red algae. Based on the means of linkage, phlorotannins can be classified into four subclasses, namely, phlorotannins (i) with an ether linkage (fuhalols and phlorethols), (ii) with a phenyl linkage (fucols), (iii) with an ether and a phenyl linkage (fucophlorethols), and (iv) with a dibenzodioxin linkage (eckols and carmalols). 3-Phloroeckol (**XIX**), isolated from *Eisenia arborea*, is an example of phlorotannins with a dibenzodioxin linkage [37, 74–81].
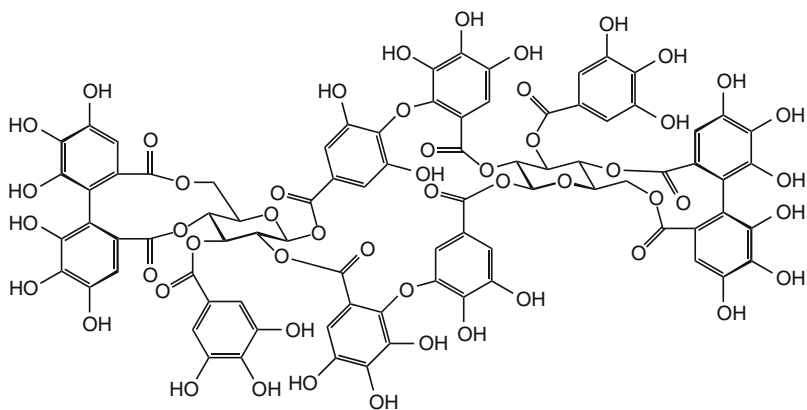
XVII
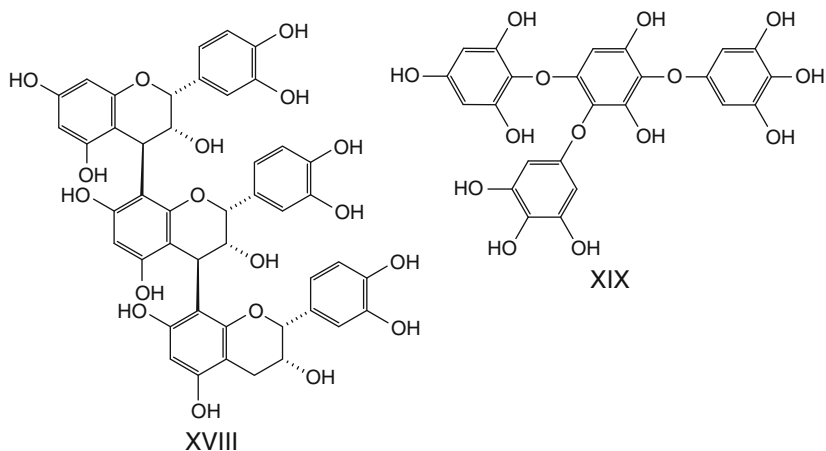
XVIII

XIX

Lignin (Latin: *lignum* = wood) [(C$_6$—C$_3$)$_n$], the essential structural polymer of wood and second only to cellulose as the most abundant organic substance in plants, is found as an integral cell wall constituent of all vascular plants including herbaceous species. 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. As a biomacromolecule, lignin is unusual in having a complex network-type structure that at a first glance appears chaotic. In fact, it is optically inactive. "Normal" biomolecules, such as cellulose and proteins, generally are synthesized in the active site of an enzyme (or enzyme complex) in nucleotide triphosphate-driven condensations, and the polymers formed have very defined structures. It is unlikely that lignin is synthesized in this way. First, lignin polymerization from monolignols is not a condensation. Second, many different types of bonds connect the monolignol residues in a somewhat random pattern. Third, lignin is racemic, which does not accord with enzyme-controlled biosynthesis.

As far as in vivo formation of lignins is concerned, 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. 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. Freudenberg [82] demonstrated in a classic work that a polymer with the same types of chemical bonds as those in lignin could be obtained through the oxidation of coniferyl alcohol to resonance-stabilized radicals by a peroxidase. He suggested that the coupling of two unpaired electrons formed the covalent bonds. The polymerization could continue if a phenolic group on the lignin polymer became oxidized to a radical, either by a peroxidase or by a peroxidase-generated monolignol radical, and the phenolic radical on the polymer underwent coupling to a second monolignol radical. 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%). 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 predominate [83–85]. Currently, there are two models for coupling radicals to produce a functional lignin molecule. One, the random coupling model, which emerged during the early studies on the structure of lignin, centers on the hypothesis that since lignins are not optically active, the optical centers must be generated randomly, that is, under simple chemical control, and then lignin formation proceeds through coupling of individual monolignols to the growing lignin polymer in a near-random fashion. In this view, the amount and

type of individual phenolics available at the lignification site and normal chemical coupling properties regulate lignin formation (Fig. 50.5) [86, 87]. The second model, the dirigent protein model proposed by Lewis group, is more recent and suggests that a protein putatively harboring an array of dirigent (monolignol radical binding) sites assembles primary lignin chains. Such chains are then hypothesized to act as templates for replication of the chain. The rationale for this new model is the belief that nature would not leave the formation of such an important molecule as lignin "to chance." It is argued that the only way to explain the high proportion of 8—O—4 linkages in lignin would be through regulation by specific dirigent proteins [88, 89].

Suberin is an example of hydroxycinnamic acid-derived polymer that comprises both a poly(aliphatic) and a poly(aromatic) lignin-like domain. Suberized tissues are formed as multilamellar domains consisting of alternating polyaliphatic and polyaromatic layers. The aliphatic domain (aliphatic suberin) consists of a glycerol-based fatty acid–derived polyester that, on transesterification, releases small amounts of p-hydroxycinnamic acid (mainly ferulic) together with aliphatic monomers and glycerol. The major aliphatic constituents are esterified ω-hydroxy fatty acids and 1,ω-dicarboxylic acids. Structural knowledge of the aromatic part is not well established. Although it has been reported that esterified hydroxycinnamic acids are the major constitutive components, a lignin-like polymer of oxidatively cross-linked phenolics has also been postulated for the polyaromatic domain. Suberin is an abundant, complex, intractable plant cell wall polymeric network that forms both protective and wound-healing layers. Suberin is found in specialized plant cell walls (e.g., in dermal tissues of most underground plant organs, the periderm of aerial tissues that undergo secondary thickening, and the Casparian band of the endodermis), where it is laid down between the primary wall and plasmalemma. It is essential for water retention by plants and functions in the overall control of water movement through the apoplastic stream, as well as in providing a physical barrier to opportunistic pathogens. From an evolutionary perspective, suberization was of utmost importance in plant adaptation to living on land and may even have preceded lignifications [90–93].

Finally, melanins are pigments of high molecular weight formed by the oxidative polymerization of phenolic compounds and usually are dark brown or black. They are widely distributed in the living world. In general, they are conjugated polymers of ortho-dihydroxyphenols. The individual residues of polymeric melanin likewise contain two *ortho* oxygens. Melanins are among the most stable, insoluble, and resistant biochemical materials, and they enhance the survival and competitive abilities of organism in certain environments. Melanins constitute a mechanism of defense and resistance to stress such as UV radiations, free radicals, gamma rays, dehydration, and extreme temperatures and contribute to the fungal cell wall resistance against hydrolytic enzymes in avoiding cellular lysis. It has also been shown to chelate metal ions, to function as a physiological redox buffer, to provide structural rigidity to cell walls, and to help to store water and ions. The more general
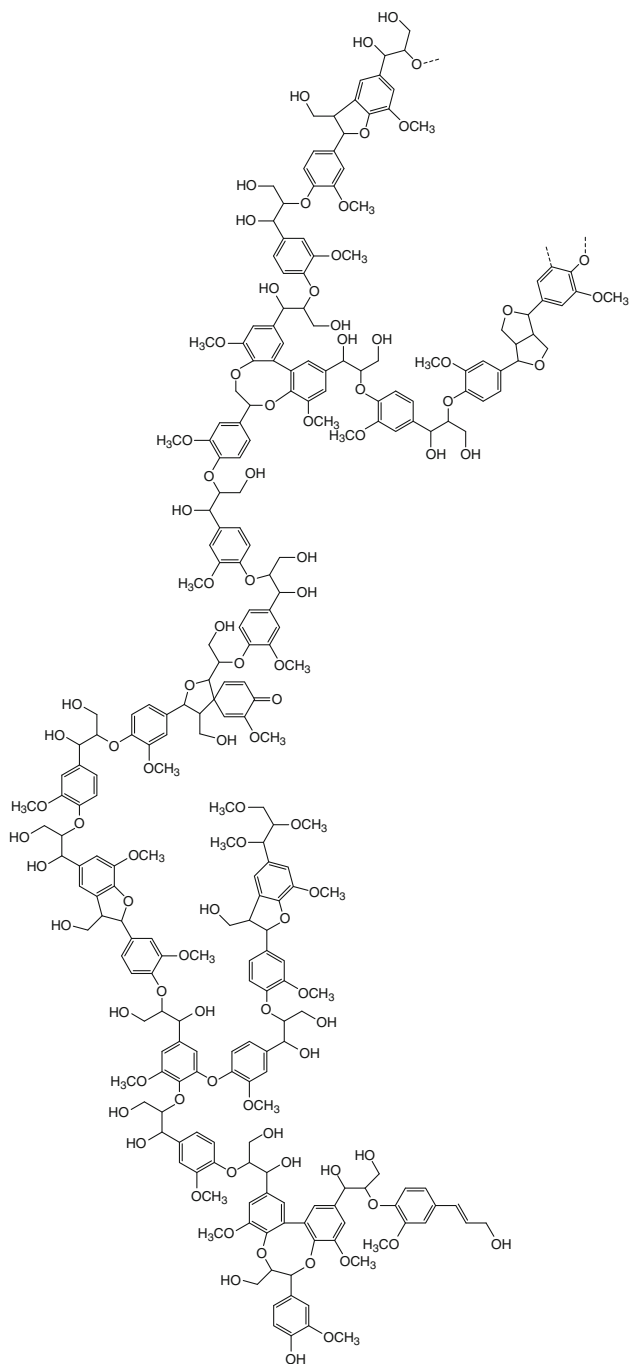
Fig. 50.5 Spruce lignin model (Redrawn from [87])

classification of such compounds, including all their types in pro- and eukaryota, contains three main types of such polymers:

1. Eumelanins (black or brown) that are produced in the course of oxidation of tyrosine (and/or phenylalanine) to 3,4-dihydroxyphenylalanine (DOPA) and dopaquinone, which further undergoes cyclization to 5,6-dihydroxyindole or 5,6-dihydroxyindole- 2-carboxylic acid
2. Pheomelanins (yellow, red, or brown) that are initially synthesized just like eumelanins, but DOPA undergoes cysteinylation, directly or by the mediation of glutathione, then polymerizes
3. Allomelanins (black), the most heterogeneous group of polymers, which emerge through oxidation/polymerization of dihydroxynaphthalene or tetrahydroxynaphthalene, homogentisic acid, γ-glutaminyl-4-hydroxybenzene, 4-hydroxyphenylacetic acid, as well as of catechols

The eumelanins and pheomelanins are found mainly in animal species, whereas the allomelanins are found in plants. For example, they constitute an important part of the black protective coating of many ripe seeds, such as seeds of sunflower and watermelon, and fungi, where they are found primarily in the gills and spores, as in mushrooms or in the black hyphae of molds. The characteristic feature of allomelanins is that they appear to be polymers of simple phenols, such as catechol, and their quinones and are considered as catechol melanins $[(C_6)_n]$. The available evidence indicates that, in the presence of the enzyme polyphenol oxidase (PPOs, EC 1.14.18.1 and EC 1.10.3.1), extensive condensation may occur between catechol units in various stages of oxidation to give polymeric structures in which the monomeric units are joined by C—C and C—O linkages, for example, partial structures such as (**XX**). Broadly, since the polymerization does not follow a precise pattern, as does the synthesis of most other biopolymers, a given sample of melanin contains molecules with various structures; therefore any diagramed structure represents an oversimplification. However, catechol also appears to be the main degradation product of plant allomelanins. The allomelanin of the fungi *Aspergillus niger*, *Ustilago maydis*, and *Daldinia* appears to contain perylene (**XXI**) units derived from 1,8-dihydroxynaphthalene (**XXII**) [94–99].

XX   XXI   XXII
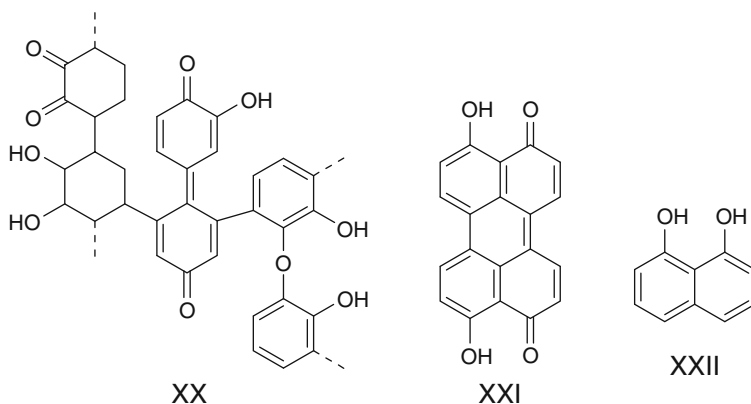
Unless they are completely esterified, etherified, or glycosylated, plant phenolics are normally soluble in polar organic solvents. Most phenolic glycosides are water-soluble, but the corresponding aglycones are usually less so. With a few exceptions, water solubility increases with the number of hydroxyl groups present. Some phenolics are solubilized by sodium hydroxide and sodium carbonate, but in alkaline media their oxidation is enhanced, and therefore treatment with alkaline solvents should either be performed under N_2 or, preferably, avoided. Phenolics with only a few hydroxyl groups are soluble in ether, chloroform, ethyl acetate, methanol, and ethanol. Methanol, ethanol, water, and alcohol–water mixtures are most commonly used for dissolving phenolic compounds for analytical purposes. All phenolic compounds exhibit intense absorption in the UV region of the spectrum and those that are colored absorb strongly in the visible region as well. Each class of phenolic compounds has distinctive absorption characteristics. For example, phenols and phenolic acids show spectral maxima in the range 250–290 nm, cinnamic acid derivatives have principal maxima in the range 290–330 nm, flavones and flavonols exhibit absorption bands of approximately the same intensity at about 250 and 350 nm, chalcones and aurones have an absorption peak of high intensity above 350 nm and a much less intense band at 250 nm, and anthocyanins and betacyanins show rather similar absorption in the visible region (475–560 nm and 535–545 nm, respectively) and a subsidiary peak at about 270–275 nm [14, 100–102].

3   Physiological and Ecological Role of Plant Phenolics

The highly ordered interactions between plants and their biotic and abiotic environments have been a major driving force behind the emergence of specific natural products. In this connection, the accumulation of phenolics in plant tissues is considered a common adaptive response of plants to adverse environmental conditions, therefore increasing evolutionary fitness. Plant phenolics are considered to have a key role as defense compounds when environmental stresses, such as high light, low temperatures, pathogen infection, herbivores, and nutrient deficiency, can lead to increased production of free radicals and other oxidative species in plants. A growing body of evidence suggests that plants respond to these biotic and abiotic stress factors by increasing their capacity to scavenge reactive oxygen species. The induction of secondary metabolism gene expression by biotic and abiotic stress is often mediated by integrating signaling molecules such as salicylic acid, jasmonic acid, and their derivatives [8, 103, 104].

Nonfreezing low temperature stress on phenolics metabolism has been considered in several papers. These studies have shown that the phenolic metabolism is enhanced under chill stress and that the behavior of the same metabolism is further dependent on the storage temperature. There is a low critical temperature below which an increase of phenolic metabolism is stimulated, and this temperature is related to the threshold temperature at which chilling injury is also induced. It has been also observed that the low temperature effect involves a cold-induced stimulation of the phenylalanine ammonia-lyase activity (PAL, EC 4.3.1.5) as well as other enzymes important in the

phenolic biosynthetic pathway. Cold treatments caused an increase in levels of hydroxycinnamic acid derivatives in artichoke heads (*Cynara cardunculus L.* subsp. *scolymus (L.) Hayek*) as well as of phloretin and quercetin glycosides in Golden Delicious apples (*Malus domestica* Borkh). These responses in phenolic metabolism to low temperature (increase in the activity of the enzymes, as well as in the level of phenolic compounds) could combine with the temperature-dependent phase changes in the cellular membrane, to affect the shelf life of stored fruit and vegetables by providing an adequate substrate to the browning reactions [105–107].

Plant growth depends on the supply of recycled nutrients; external nutrient inputs generally contribute only with a minor proportion to the total requirement. Nutrient mineralization by soil microorganisms is generally viewed as the rate-limiting step in the nutrient cycle, and the factors involved in the control of this process include climate, substrate (litter) quality, and decomposer organisms. Polyphenols, which enter the soil mainly as leachates from above- and below-ground parts of plants and/or within above- and belowground plant litter, have been recognized as regulators of soil processes, where it has been suggested that they could control the pool and the form of nutrients available for plants and/or microbes. For example, phenolic compounds can directly affect the composition and activity of decomposer communities, thus influencing the rates of decomposition and nutrient cycling. Different types of soluble polyphenols, such as ferulic acid, gallic acid, or flavonoids, have been found to either stimulate or inhibit spore germination and hyphal growth of saprotrophic fungi. Plant mycorrhizal infection, nutrient uptake, and plant growth can be impaired by specific phenolics released by competitors in a process referred to as allelopathy [10]. Furthermore, plants depend on the ability of roots to communicate with microbes. The converse is also true; many bacteria and fungi are dependent on associations with plants that are often regulated by root exudates. For example, isoflavonoids and flavonoids present in the root exudates of a variety of leguminous plants activate the rhizobium genes responsible for the nodulation process and might be responsible for vesicular–arbuscular mycorrhiza colonization. Nodule formation is initiated by the host plant roots exuding phenolic flavonoid compounds into the rhizosphere. The exudate partly determines the specificity of the symbiotic relationship as each rhizobia species responds to specific flavonoids. The flavonoid perception attracts the bacteria to the root and activates the rhizobia *nod* (nodulation) gene expression, leading to the production and secretion of strain-specific lipo-chito-oligosaccharides, also known as nod factors (NF). NFs have an oligosaccharide backbone of *N*-acetyl-D-glucosamine units with a fatty acyl group attached to the nonreducing sugar. A major determinant of host–symbiont specificity is attributed to the different NF substituents attached to the oligosaccharide backbone. The presence of compatible rhizobia species and their corresponding NF is generally sufficient to trigger nodule development [108–111].

In general, plants are rooted and are unable to demonstrate mobility. However, a variety of plants are able to move in certain ways. Some plants are known to open their leaves in the daytime and "sleep" at night with their leaves folded. This circadian rhythmic leaf movement known as nyctinasty is widely observed in

Potassium lespedezate
(Leaf-opening compound)

Potassium 5-*O*-β-D-glucopyranosyl gentisate
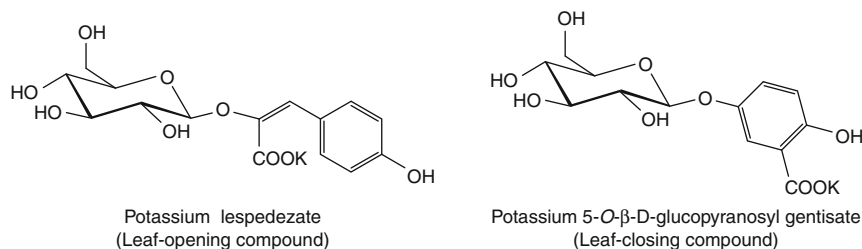(Leaf-closing compound)

Fig. 50.6 Leaf-movement factors from nyctinastic plants

leguminous plants. Nyctinastic leaf movement is induced by the swelling and shrinking of motor cells in the pulvinus, an organ located in the joint of the leaf. A flux of potassium ions across the plasma membranes of the motor cells is followed by a massive water flux, which results in the swelling and shrinking of these cells. At the heart of such a mechanism is the regulation of the opening and closing of the potassium channels involved in the nyctinastic leaf movement, a process which is under metabolic control. Many attempts have been made to isolate the endogenous bioactive substances that control nyctinasty. It has been found that nyctinastic plants have a pair of endogenous bioactive substances that control the nyctinastic leaf movement. One of these is a leaf-opening factor that "awakens" the plant leaves, and the other is a leaf-closing factor that reverses this process so that the plant leaves "sleep." Figure 50.6 shows a pair of bioactive substances showing a phenolic structure. Five sets of leaf-closing and -opening factors in five different nyctinastic plants have been identified. All the leaf-opening factors have a common structural feature, the *p*-coumaroyl moiety, and this result suggests that this structural feature would be deeply involved in the common mechanism for leaf-opening [112–114].

Anthocyanins represent a class of flavonoids providing the red and blue/purple colors familiar in many flowers and fruits. These compounds are synthesized as visual cues, to attract pollinators and other animals for seed dispersal, as well as molecular cues protecting plants from various stress conditions, and are stored in the acidic vacuole of specialized cells. Anthocyanins are also the pigments responsible for spectacular displays of variable red to reddish-orange color in the leaves of deciduous trees. Leaf color change is not simply a side effect of leaf senescence, and, in the past decade, several hypotheses have emerged to explain the evolution of autumn colors. Autumn colors are due mainly to carotenoids (yellow-orange) and anthocyanins (red-purple). Although carotenoids are present all year round in the leaves, they are masked in mature leaves by the green of chlorophyll; in autumn, they become visible because of the breakdown of green chlorophyll molecules into colorless metabolites, but there is no evidence for a de novo synthesis. Anthocyanins, by contrast, are newly generated in autumn, shortly before the leaf fall. Thus, red is produced actively in autumn and is not simply the side effect of leaf senescence. Why leaves that are about to be shed turn red? The adaptive value of the autumn colors of leaves is still a matter of controversy. Red may protect the leaf from the damaging effects of light at low temperatures (photoinhibition and

photooxidation), allowing a more efficient resorption of nutrients, especially nitrogen ("photoprotection theory"). Alternatively, red might be a warning signal of the status of the tree (indicating high levels of defenses or low nutritional capacity) to animals, particularly feeding insects like aphids ("coevolution theory"). During winter, the combination of cold, dry, and bright sunlight conditions can result in excess energy capture relative to processing, photoinhibition of photosynthesis, formation of ROS, and greater photooxidative damage. Red pigments are thought to alleviate these stress factors by intercepting green sunlight (light attenuation) and/or neutralizing ROS directly as antioxidants. On the other hand, recent studies on autumn and juvenile leaves suggest that red pigments reduce the leaf damage either by making leaves less palatable or less visible to animals lacking a red visual receptor (camouflage) or by signaling a low leaf quality. According to the coevolution theory, red is a signal of the status of the tree to insects that migrate to (or move among) the trees in autumn. Migrating insects avoid red leaves and colonize preferentially green leaves. Trees with red leaves have better chemical defenses or a worst nutritional capacity that induces a lower fitness in the insects. In this scenario, therefore, color and preference coevolve in an arms race: autumn colors are an adaptation of the trees to reduce their parasite load, and insect preference for green is an adaptation to find the most suitable host trees [115–120].

Plants encounter numerous pests and pathogens in the natural environment. An appropriate response to the attack by such organisms can lead to tolerance or resistance mechanisms that enable the plant to survive. Resistance mechanisms refer to traits that inhibit or limit attack, while tolerance strategies do not limit attack but reduce or offset consequences on the plant fitness by adjusting its physiology to buffer the effects of herbivory or diseases. In this connection, plants produce a broad range of phenolic metabolites that serve a dual function of both repelling and attracting different organisms in the plant's surroundings. The role of plant phenolics in chemoecology, especially on the feeding behavior of herbivores, has been recognized since 1959 when Fraenkel [121] described phenolic compounds as "trigger" substances, which induce or prevent the uptake of nutrients by animal herbivores. They act as protective agents, inhibitors, natural animal toxicants, and pesticides against invading organisms, that is, herbivores, nematodes, phytophagous insects, and fungal and bacterial pathogens [54, 122, 123]. Preformed antibiotic compounds that occur constitutively in healthy plants are likely to represent inbuilt chemical barriers to herbivorous and fungal enemies and may protect plants against attacks by a wide range of potential pests and pathogens. In contrast, induced defense compounds are synthesized in response to biotic stresses as a part of the plant defense response. These induced defense mechanisms are expressed at the site of attack (hypersensitive response) as well as at a distance (signaled by salicylates, jasmonates) from the site of primary infection and protect the plant from the spread of infection and future attacks. Induced resistance is regulated by a network of interconnecting signal transduction pathways in which phenolic acids are key signaling molecules [124, 125].

In an environment rich in potentially harmful microbes, plant survival depends on an efficient microbe perception and fast defense responses. Plant immunity relies

on the ability of each cell to recognize pathogens. A first level of microbe recognition is performed by membrane proteins termed pattern recognition receptors (PRRs), which perceive the molecular signatures characteristic of a whole class of microbes, termed pathogen-associated molecular patterns (PAMPs). Phenolics are synthesized when plant PRRs recognize potential pathogens by conserved PAMPs, leading to a PAMP-triggered immunity [126–132]. The plant's recognition of pathogens induces its endogenous multicomponent defense system. The multicomponent defense response induced after the pathogen attack requires a substantial commitment of the cellular resources, including extensive genetic reprogramming, because the induced expression of a large number of defense-related genes is essential for plants to counter the pathogen attack. Many defense-related genes encode enzymes that catalyze defense metabolites, known as phytoalexins, for example, antimicrobial, low-molecular-weight secondary metabolites that are both synthesized by and accumulated in plant cells as a result of the interaction between the metabolic systems of the host and a fungal parasite. The structures of the phytoalexins are often unique at the family level: the majority of phytoalexins produced by the members of the family Leguminosae are isoflavonoids, and the commonest isoflavonoid subclass is the pterocarpans such as medicarpin and glyceollin II (**XXIII**), whereas phytoalexins from Vitaceae seem to constitute a rather restricted group of molecules belonging to the stilbene family, the skeleton of which is based on the *trans*-resveratrol structure (3,5,4'-trihydroxystilbene). Dicotyledonous species represent the majority of plants from which such compounds have been identified. Some monocotyledonous species producing phenolic phytoalexins are rice (*Oryza sativa*) (sakuranetin, **XXIV**), oat (*Avena sativa*) (avenalumin I, **XXV**), and sorghum (*Sorghum bicolor*) (3-deoxyanthocyanidins). Sorghum phytoalexins have been identified as 3-deoxyanthocyanidins: luteolinidin, 5-methoxyluteolinidin, apigeninidin, caffeic acid ester of arabinosyl 5-*O*-apigeninidin (**XXVI**), and 7-methoxyapigeninidin [36, 54].

XXIII

XXIV

XXV

XXVI
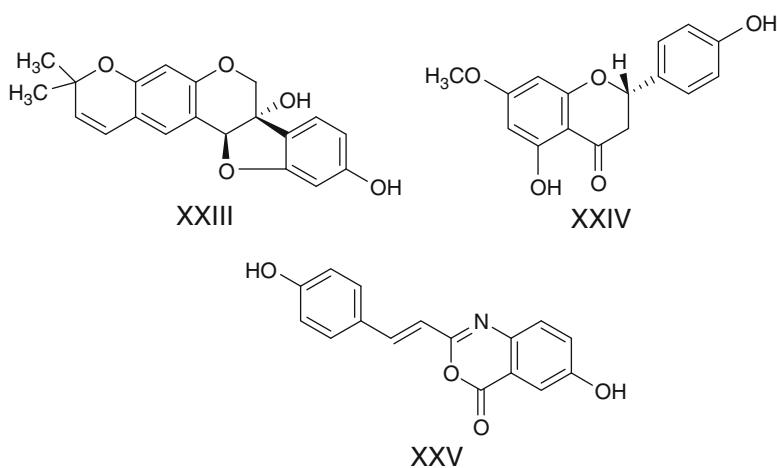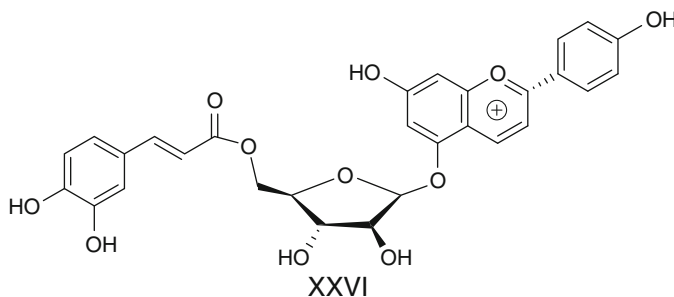
The ecological relationship between plants and insects is a complex one with physical as well as chemical interactions. This relationship is also affected by plant factors, insect factors, and by some insect–plant factors, including hypersensitive reaction and plant resistance to insect-borne diseases. Plant constituents that make unpalatable a host are secondary metabolites in sufficient concentration to exert an undesirable physiological effect. It is now generally accepted that plant phenolics play a role in protecting plants from insects. For example, some cotton flavonoids are feeding stimulants for the boll weevil, *Anthonomus grandis* [133], or oviposition stimulants of a *Citrus*-feeding swallowtail butterfly, *Papilio xuthus* L. [134] or, finally, antibiotics effective against phytophagous insects [135]. Tannins, also, may affect the growth of insects in three main ways: they have an astringent taste which affects palatability and decreases feed consumption; they combine with proteins to form complexes of reduced digestibility; and they act as enzyme inactivators. Recent work by Raymond Barbehenn and coworkers about tannin oxidation in insects suggests that tannin activity cannot be explained quite this simply, as tannin oxidation should also be taken into account as a defense mechanism for plants [136–138].

4 Carbon Fluxes from the Primary to the Secondary Metabolic Pathways

Chemical defenses represent a main trait of the plant innate immune system. Besides regulating the relationship between plants and their ecosystems, plant phenolics are involved both in resistance against pests and pathogens and in tolerance toward abiotic stresses. Both biotic and abiotic stresses stimulate carbon fluxes from the primary to the secondary metabolic pathways, thus inducing a shift of the available resources in favor of the synthesis of secondary products, for example, reallocation of host resources. Therefore, defensive chemicals are considered to be costly for plants, reducing the fitness of the host in the absence of disease, because resistance genes might impose metabolic costs on plants (e.g., lower growth rates than their sensitive counterparts) [18]. When resistant genotypes have relatively low fitness in the absence of pests, it is often assumed that

their poor performance must be explained by the energetic drain involved in building and maintaining a chemical or structural defense, metal hyperaccumulation, and temporal or spatial escape: all these mechanisms include allocation costs (the costs due to diversion of limited energy and resources away from primary metabolism), storage costs, ecological (i.e., environmental) costs (negative effects of resistance on one of the myriad of interactions between a plant and its environment that affect a plant's fitness under natural growing condition), etc. One way for a plant to reduce these costs is to synthesize defense compounds only after there has been some degree of initial damage by a pathogen or insect: this strategy is inherently risky because the initial attack may be too rapid or too severe for an effective defense response. Therefore, plants that are likely to suffer frequent and/ or serious damage may be better off investing mainly in constitutive defenses, whereas plants that are attacked rarely may rely predominantly on induced defenses [139–142].

The negative correlation between concentrations of secondary metabolites and plant growth rate is assumed to indicate a trade-off between plant growth and the production of defensive compounds. Plants, in fact, have limited resources to support their physiological processes; hence, all requirements cannot be met simultaneously and more carbon is diverted from growth toward secondary metabolism when plant growth is restricted by any physiological and/or ecological constraint.

An interesting link between primary and secondary metabolism has been recently proposed by Lattanzio et al. [143] which couples the accumulation of the stress metabolite proline with the energy transfer toward phenylpropanoid biosynthesis via the oxidative pentose phosphate pathway [144] (Fig. 50.7). In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses, such as water deprivation, salinization, high/low temperature stress, heavy metal toxicity, pathogen infection, nutrient deficiency, atmospheric pollution, and UV irradiation. In this connection, it has been also suggested that the value of stress-induced proline accumulation may be mediated largely *via* the effects of its synthesis and degradation on cellular metabolism [145–148]. Proline synthesis is accompanied by the oxidation of NADPH. An increased $NADP^+$/NADPH ratio is likely to enhance the activity of the oxidative pentose phosphate pathway providing precursors for the phenolic biosynthesis via the shikimic acid pathway. The alternating oxidation of NADPH by proline synthesis and reduction of $NADP^+$ by the two oxidative steps of the oxidative pentose phosphate pathway serve to link both pathways and thereby facilitate the continuation of high rates of proline synthesis during stress and lead to a simultaneous accumulation of phenolic compounds.

Furthermore, it has been also shown that an exogenous application of proline to the nutrient medium of *in vitro* grown oregano plants elicited the accumulation of rosmarinic acid and other phenolic compounds in that plant. This suggests that mitochondrial proline oxidation could drive the oxidative pentose phosphate pathway by recycling glutamic acid into the cytosol to generate a proline redox cycle.
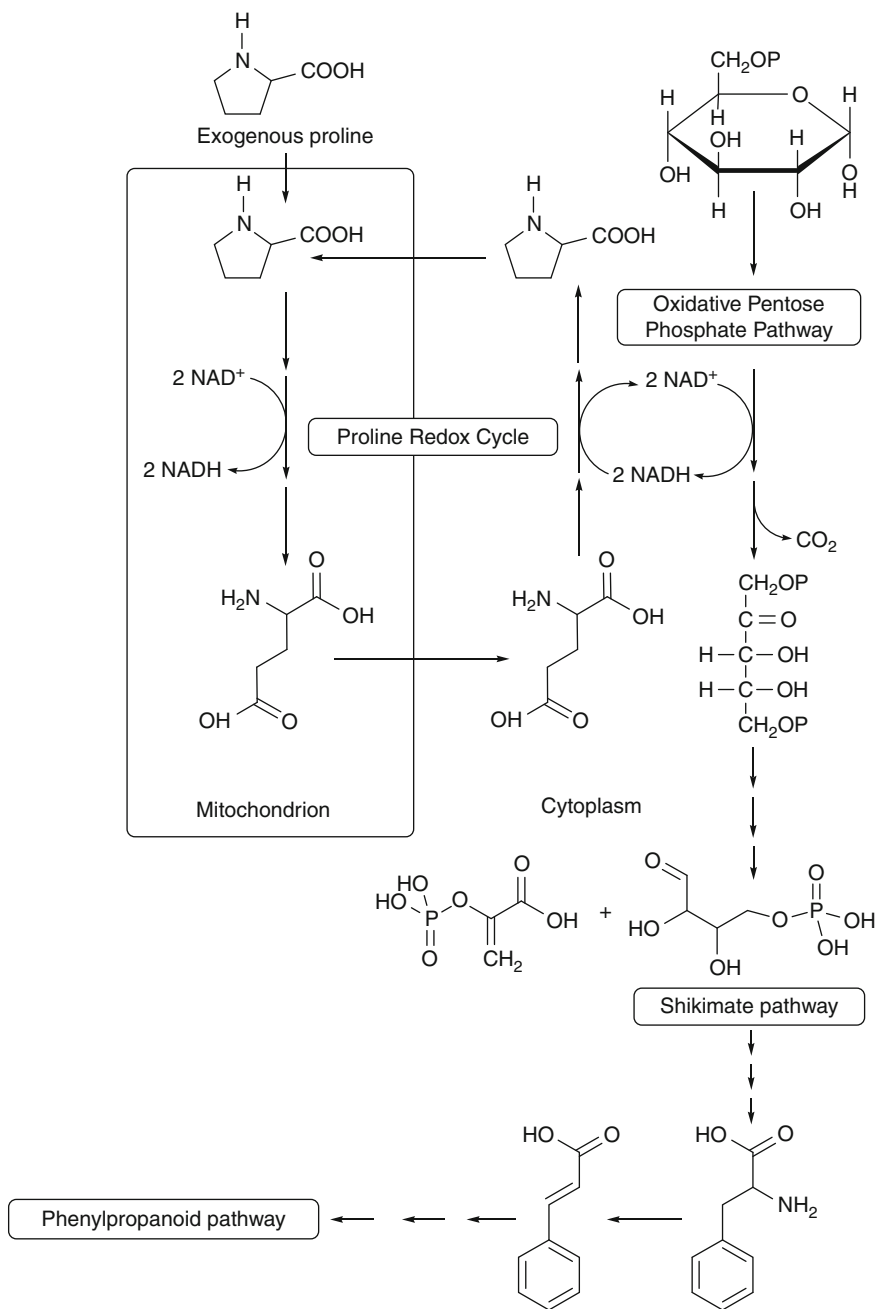
Fig. 50.7 Relationship among proline redox cycle, oxidative steps of cytosolic pentose phosphate pathway, and phenylpropanoid pathway

5   Conclusions

(Poly)phenols are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. They constitute a large reservoir of natural chemical diversity that encompasses an enormous range of compounds and enzymes and a wide spectrum of mechanisms of gene regulation and of transport of metabolites and enzymes. Levels of phenolic compounds in plants are both environmentally induced as well as genetically controlled. Despite a basic knowledge of the main biosynthetic pathways, some open questions, such as the biosynthesis, the intracellular transport, and functions of plant phenolics, need additional study. Until now, it appears that the phenolic patterns of higher plants have been shaped by a dialog between plants and their environment for the benefits of plants and their better adaptation to external conditions. Plants adapt themselves their phenolic patterns to a changing environment through the emergence of new genes brought about by gene duplication and mutation and subsequent recruitment for adaptation to specific functions. Plants also have the ability to synthesize specific chemical compounds which can act as toxins and deterrents to pathogens/ herbivores and other competitors and are also able to attract needed symbionts for procreative purposes. Primary metabolism is an important source of precursors for the synthesis of secondary phenolic metabolites. Central metabolism requires high levels of limited plant resources, and during intense growth, the synthesis of phenolic metabolites may be substrate- and/or energy-limited. On the other hand, either abiotic or biotic stresses divert substantial amounts of substrates from primary metabolism into secondary defensive product formation, and this could lead to constraints on growth. Adjustments in resource allocation are the major mechanism by which plants respond to environmental constraints. The allocation pattern of a plant defines its ecological roles and is therefore an important factor in understanding plant distribution and adaptation. On the other hand, as far as the development of a new strategy to enable the production of useful secondary metabolites on a commercial scale is concerned, any progress made in the basic understanding of metabolic pathways and regulatory mechanisms may be addressed to exploit the plant cell and tissue culture potentials to produce food additives, such as antioxidant phenolics.

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