Plant Secondary Metabolism: A Primer

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Introduction:

Plants are *photoautotrophs*, and therefore capable of meeting all of their nutritional needs through biosynthesis, using CO_2, H_20, a few minerals from the soil and sunlight. Moreover, plants are sessile, and as such can neither move away from potentially threatening situations, nor towards beneficial ones. As a consequence, over the course of evolution, plants have adapted various structural and (bio)chemical mechanisms to protect themselves and interact with their environment. Not surprisingly, plants are biochemically diverse.

In general, plant metabolism can be divided into two categories: primary metabolism and secondary metabolism. Primary metabolism can be defined as "the pathways for generally modifying and synthesizing carbohydrates, proteins, fats and nucleic acids…found to be essentially the same in all organisms, apart form minor variations".[1] For example, glycolysis, the tricarboxylic acid cycle, β-oxidation of fatty acids, protein synthesis and nucleic acid synthesis are all examples of primary metabolism. By contrast, secondary metabolism refers to the biosynthesis of the myriad of other organic compounds that are found in biological organisms but that either have limited distribution or no apparent *direct* role in the normal growth and development of the organism in which they are found. Obviously the boundary between primary and secondary metabolism is not clearly defined since many developmentally important compounds (e.g., steroidal hormones) have limited taxonomic distribution. Does this make them secondary metabolites? Or does their obvious role in growth and development make them primary metabolites? To make matters worse many primary and secondary metabolites share common structural features and metabolic precursors (see below). It is perhaps more appropriate to distinguish between primary and secondary metabolism using a functional definition in which primary metabolites are those defined as participating in nutrition and essential metabolic processes, while secondary metabolites are defined as those that influence the ecological interactions between a plant and its environment.[2]

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[1] P.M. Dewick, 1997, *Medicinal Natural Products: A Biosynthetic Approach*. (Wiley & Sons, New York,) pp. 5.
[2] R. Croteau, T.M. Kutchan and N.G. Lewis (2000) *Natural Products*. In, Biochemistry and Molecular Biology of Plants (B.B. Buchanan, W. Greuissem and R.L. Jones, Eds),(American Society of Plant Physiologists, Rockville MD). Chapter 24, pp 1251.

Plant Secondary Metabolism

Secondary metabolites in plants are generally divided into three main categories: terpenoids, alkaloids and phenylpropanoids. This division is primarily based on biosynthetic origins. For example, all terpenoids are derived from the same five-carbon precursor, isopentenyl pyrophosphate, while most alkaloids are derived from ornithine and lysine, and phenylpropanoids from the aromatic amino acids phenylalanine and tyrosine (and in some cases the acetate pathway as well). Figure 1 illustrates this point, as well as the intimate association between primary and secondary metabolism.

From Figure 1 it is obvious that virtually all secondary metabolites can be traced back to simple sugars produced by photosynthesis. It should be reiterated that by definition, secondary metabolites are taxonomically limited in distribution, and that the pathways depicted in Figure 1 represent a composite. Moreover, many of the individual pathways are compartmentalized. For example, glycolysis is cytoplasmic while the Kreb's cycle of course occurs in mitochondria. Similarly, the reactions of aromatic amino acid biosynthesis (e.g., the shikimate pathway) are found exclusively in plastids (e.g., chloroplasts), while the biosynthesis of phenylpropanoids is largely cytoplasmic. Likewise, the main precursors to the alkaloids (lysine and ornithine) are biosynthesized in plastids while alkaloids are biosynthesized in the cytoplasm. Terpenoids are unique in that there are two pathways leading to isopentenyl pyrophosphate. The mevalonate pathway (depicted in Figure 1), which utilizes acetyl-CoA as a carbon source, is cytoplasmic, while a newly discovered "plastid-localized" pathway (not shown in the Figure) uses pyruvate and glyceraldehyde-3-phosphate as precursors. For all three classes of secondary metabolites, however, the building blocks are biosynthesized from readily available primary metabolites.

The biosynthesis of secondary metabolites follows a fairly simple process. In fact, there are a relatively limited number of building blocks that are employed in the process, and once a basic carbon skeleton is in place, the types of modifications that take place are also limited in scope. But don't be fooled! The potential for structural diversity in secondary metabolites is enormous, and to date approximately 25,000 terpenoids, 12,000 alkaloids and 8,000 phenolics have been described. Thus, starting with some of the main building blocks depicted in Figure 1, carbon and nitrogen can be added very selectively via a number of typical building blocks such as:

- C_1 units added via S-adenosylmethionine
- C_2 units added via acetyl-CoA or malonyl-CoA
- C_3 and C_4 units via simple sugars
- C5 units added via isopentenyl pyrophosphate
- C_6C_3 units from phenylalanine or tyrosine (after de-amination; side chain degradation yields C_6C_2 and C_6C_1 units)
- C_6C_2N units from decarboxylated phenylalanine or tyrosine
- indole units from tryptophan
- C_4N units from ornithine
- C_5N units from lysine

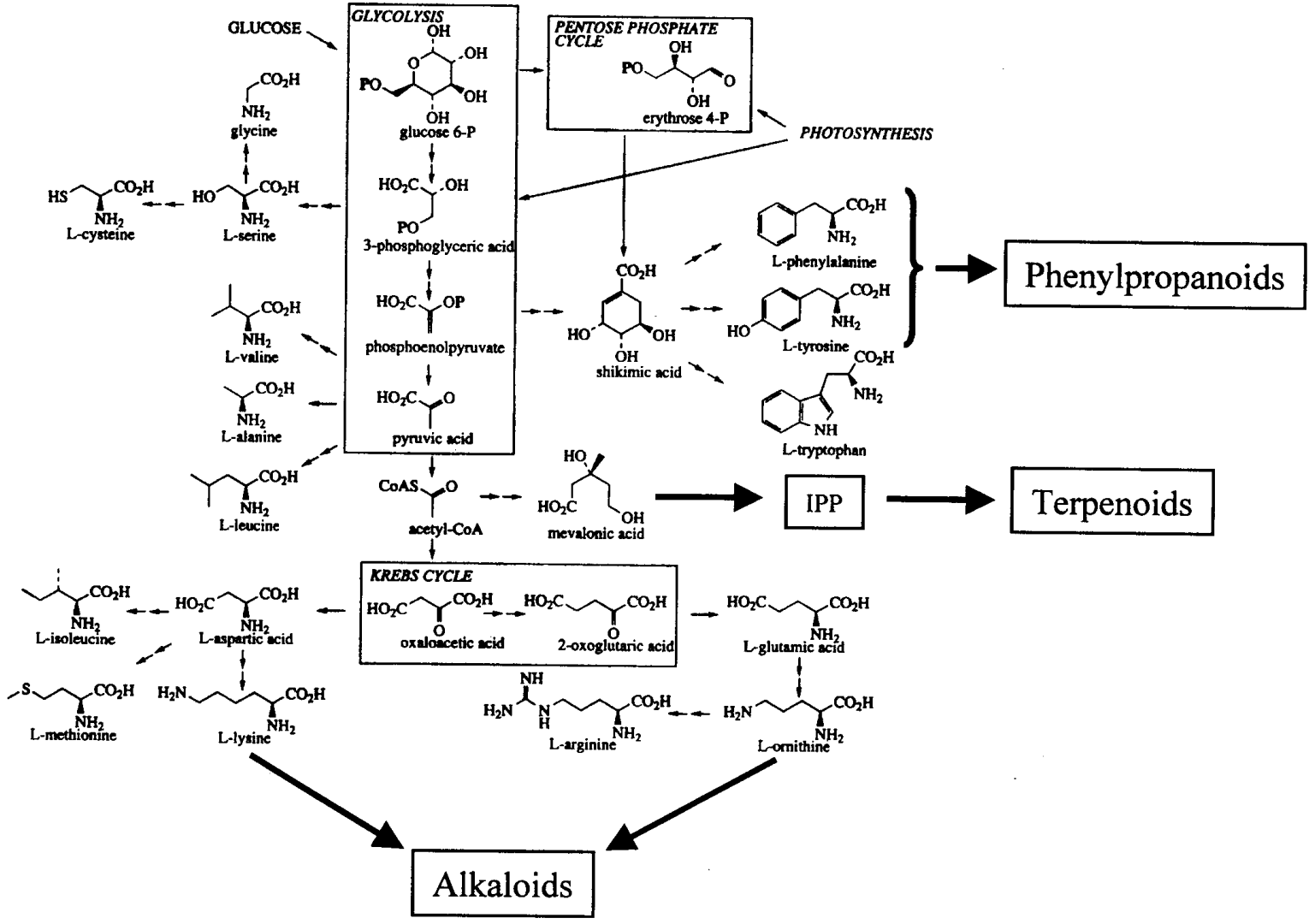

Figure 1. Biosynthetic connections between primary and secondary metabolism. IP = isopentenyl pyrophosphate. Adapted from P.M. Dewick, 1997, *Medicinal Natural Products: A Biosynthetic Approach.* (Wiley & Sons, New York,) pp. 6.

In general, modifications to basic carbon skeletons fall into one of the following categories:

- alkylation reactions (addition of C_1 or C_5 units)
- aldol and Claisen reactions (condensation reactions)
- Schiff's base formation
- transamination
- decarboxylation
- oxidation/reduction (including oxygenation reactions cytochrome P-450 oxygenases)
- oxidative (free radical) coupling
- glycosylation

The biosynthesis of silibin, a flavanolignan with hepatoprotective properties from milk thistle (*Silybum marianum*) (Figure 2) illustrates several of these basic carbon skeleton modifications. For example, the coniferyl alcohol moiety is derived from phenylalanine through deamination, hydroxylation, methylation and reduction reactions. Similarly, the dihydroflavanol, taxifolin, is derived from phenylalanine via deamination, hydroxylation, claison (condensation) and ring closure reactions. Finally the two pieces are coupled through a one-electron oxidation-mediated free radical coupling process. Each step is catalyzed by a unique enzyme, although in many cases these are assembled into multi-protein complexes.

The Role of Secondary Metabolites

When first identified, secondary metabolites were thought to represent useless metabolites that served no real purpose. However, as more became known about them, it became readily apparent that they account for a majority of the aroma, flavour and pharmacological properties of plants. Today it is well known that secondary metabolites provide plants with the very key to their survival. Thus far it has been well documented that secondary metabolites are involved in:

- floral colour (e.g., anthocyanins)
- pollinator attraction (e.g., monoterpenes)
- UV protection (e.g., flavonoids, carotenoids)
- herbivor deterance (e.g., tannins, phenolics, alkaloids, glucosinolates)
- seed dispersal (e.g., aromatics, flavours)
- defense against pathogens (e.g., flavonoids, terpenoids)
- adaptation to water stress
- adaptation to cold
- cell-cell signaling (e.g., flavonoids, phenolics)

While this list is certainly not exhaustive, it serves to illustrate the diverse ecological roles played by secondary metabolites. The biological activity of secondary metabolites is obvious in many of the stated roles they play, and in most cases it is a serendipitous bonus that this biological activity often translates into pharmcacologically relevant activity. In the following sections specific examples of many of these roles will be presented.

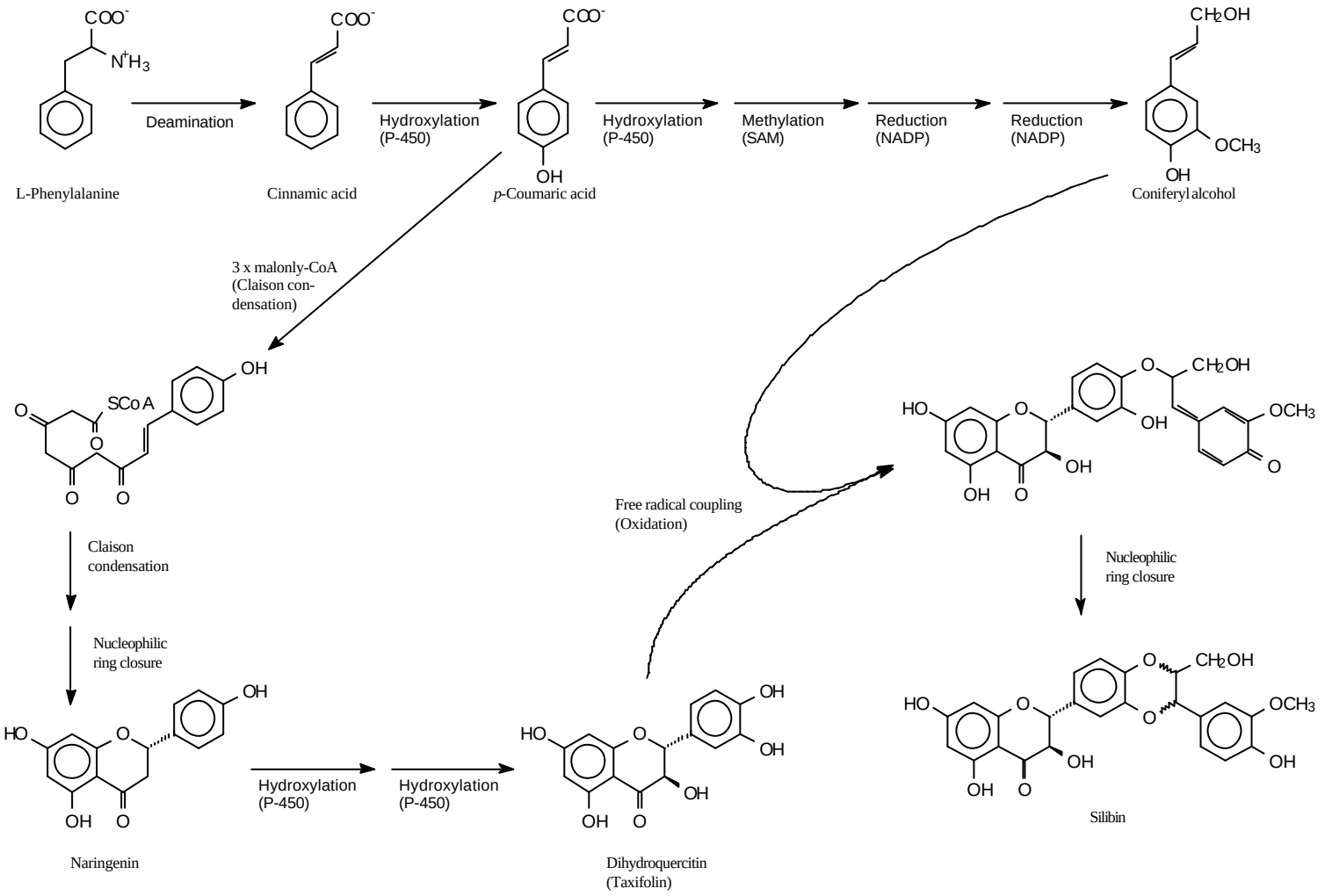

Figure 2. The biosynthesis of silibin in milk thistle. Two separate pathways to form taxifolin (a dihydroflavanol) and coniferyl alcohol converge via one electron oxidation-mediated free radical coupling. The main product (silibin) is formed as a diasteriomeric pair. This biosynthetic scheme illustrates many of the common biotransformations found in secondary metabolism, including hydroxylation, methylation, acylation and oxidation-reduction reactions.

The Terpenoids

As indicated above, all terpenoids are derived from the 5-carbon building block isopentenyl pyrophosphate (IPP). Figure 3 outlines the basic pathway to the main terpenoid carbon skeletons. The nomenclature is such that coupling of one IPP and one DMAPP yields a geranyl pyrophosphate (GPP), the precursor to all *monoterpenes* (C-10) (by themselves, IPP and DMAPP are considered *hemiterpenes*). Monoterpenes are generally volatile and are represented by such commonly known compounds as menthol and the pinenes. Addition of another IPP to GPP yeilds farnesyl pyrophosphate (FPP), immediate precursor to the *sequiterpenes* (C-15). The antimicrobial compounds Solanaceous plants are of this type. Addition of a third IPP unit yields geranyl geranyl pyrophosphate (GGPP), the precursor to the *diterpenes* (C-20). The well known anticancer drug paclitaxel (Taxol) is based on a highly modified diterpenoid carbon skeleton. Coupling of two FPP units yields squalene, from which all *triterpenoids* (C-30) are derived. The triterpenoids include the steroids and many pharmacologically relevant compounds such as ginsenosides. The synthesis C-40 terpenoids (the *tetraterpenoids*) initiates from the coupling of two GGPP units. The carotenes are derived from tetraterpenes. From these simple beginnings, more than 25,000 structures are derived through the types of modifications indicated above.

As for their role *in planta*, terpenoids are known to act as:

- growth regulators (e.g., gibberellins, abscisic acid)
- photosynthetic pigments (e.g., phytol, carotenoids)
- electron carriers (e.g., ubiquinone, plastoquinone)
- membrane components (e.g., sterols)
- defense compounds (e.g., attractants, phytotoxins, antibiotics, toxins, repellents)

Industrially, terpenoids have been used as:

- solvents (e.g., terpentine)
- flavourings (e.g., menthol, spearmint)
- fragrances (e.g., essential oils)
- adhesives
- coatings
- synthetic intermediates (e.g., rubber)
- agrochemicals (e.g., pyrethrins, azadirachtin)
- pharmaceuticals

Figure 4 illustrates some biologically active terpenoids, including some with pharmaceutical properties.

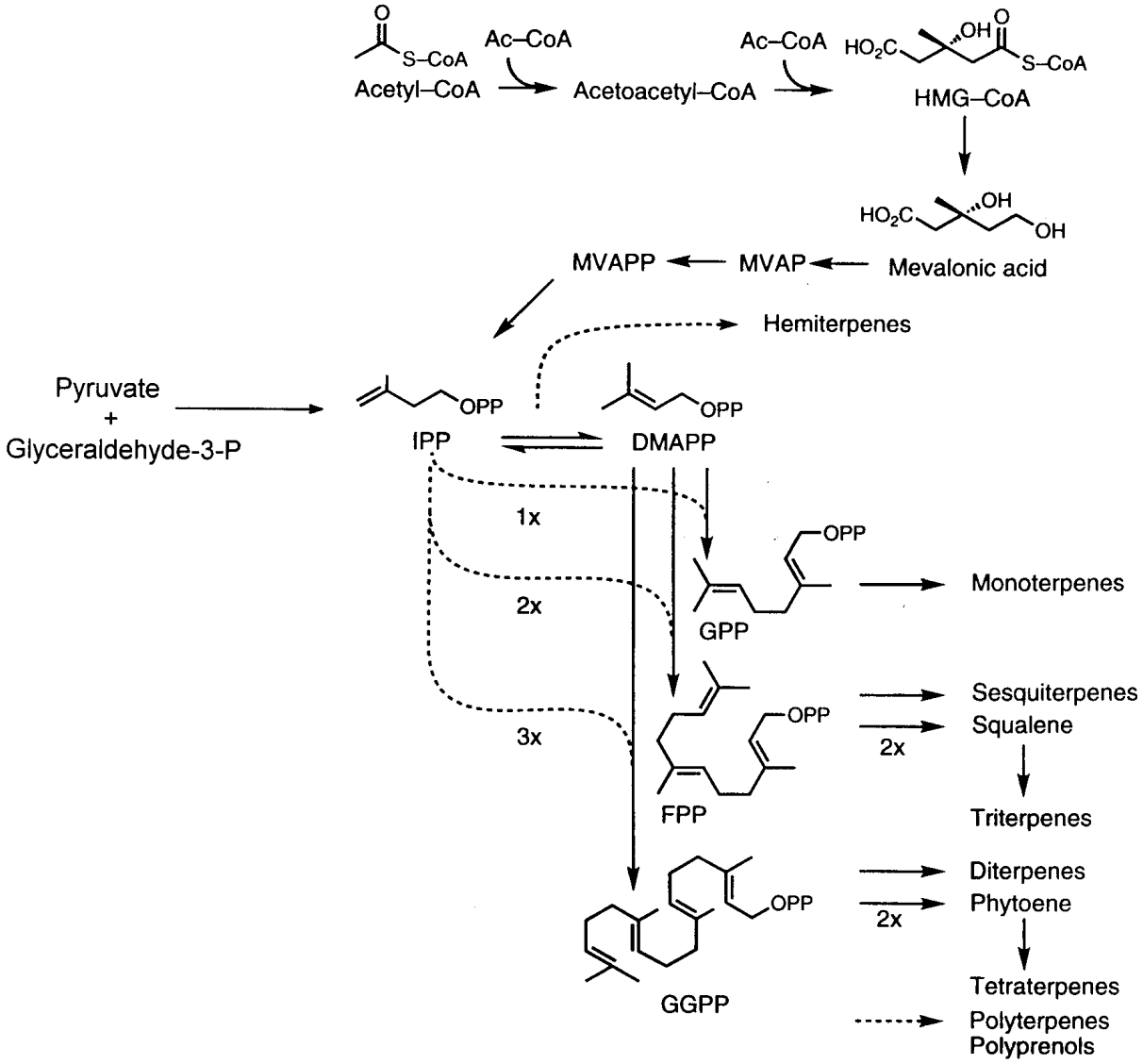

Figure 3. The biosynthetic origin of the major carbon skeletons of the terpenoids. The 1x, 2x and 3x at left refer to the number of IPP units added to a single DMAPP unit to make the parent structure. At right, 2x refers to the number of FPP or GGPP units used to synthesize squalene and phytoene, respectively. Adapted from McGarvey and Croteau (1995) *The Plant Cell* **7**:1015.

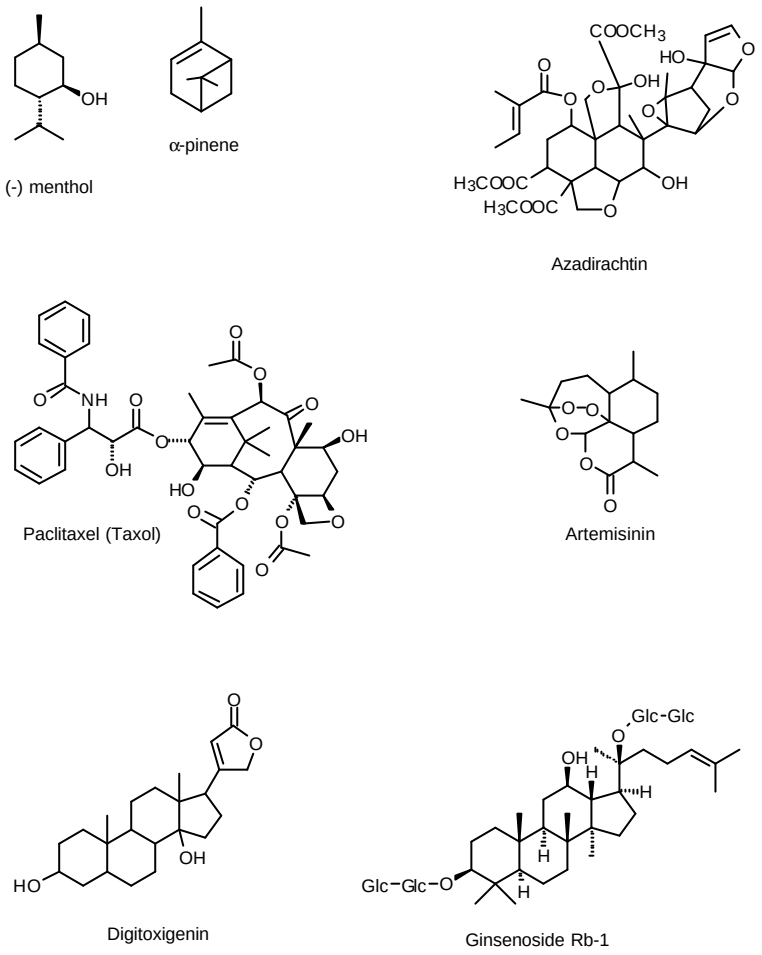

Figure 4. Examples of biologically active terpenoids. Examples include menthol from the essential oil of peppermint, α-pinene, an insecticidal compound from *Pinus* spp., azadirachtin A, a potent insect antifeedant from the neem tree (*Azadirachta indica*), paclitaxel, an anticancer agent from yew (*Taxus brevifolia*), artemisinin, an potent antimalarial from annual wormwood (*Artemesia annua*), digitoxigenen, the aglycone of the cardenolide digitoxin from the foxglove (*Digtalis* spp.), and ginsenoside Rb-1, a steroidal sponin from ginseng (*Panax* spp.).

The Alkaloids

Originally, the term alkaloid was used to refer to "pharmacologically active nitrogen-containing basic compounds from plants"[3] (alkaloid derived from alkali), but now encompasses all cyclic nitrogen-containing secondary metabolites (note that this definition excludes acyclic amines and primary metabolites such as amino acids and nucleotides). While fewer in number than terpenoids, they are more structurally diverse. Alkaloids are classified according to the nitrogen-containing ring structure, which is determined by the amino acid from which they are made (e.g., L-lysine, L-ornithine, L-phenylalanine, L-tyrosine, L-trpytophan, nicotinic acid, anthranilic acid and L-histidine). Typical classifications include the piperidine, pyrrolizidine and tropane alkaloids (L-ornithine-derived), indole alkaloids (tryptophan-derived), piperidine and quinolizidine alkaloids from L-lysine, the pyridine alkaloids from nicotinic acid, the isoquinaloline alkaloids from L-tyr and the imidazole alkaloids derived from L-histidine. It is beyond the scope of this paper to try and summarize alkaloid biosynthesis. What is important to remember is that the different classifications of alkaloids derive from different amino acids, and that the same modifications to these building blocks as we saw above apply.

Despite the large number of structures, alkaloids are limited in taxonomic distribution. In fact, only about 20 % of flowering plants produce alkaloids. *In planta*, alkaloids play a major role as feeding deterents, as many are bitter tasting or toxic. Because of their physiological activity, many alkaloids are used in modern medicine. Figure 5 illustrates some common alkaloids. Note the degree of structural diversity evident in the examples shown in Figure 5, and that α-solanine is actually a hybrid based on a terpenoid (e.g., steroid), modified by the addition of a nitrogen-containing moiety.

The Phenolics

Phenolics are universally distributed throughout the plant kingdom. Interestingly, this wide distribution does not result in a greater structural diversity: there are over 8,000 known phenolic compounds. By definition, phenolics are compounds with at least one aromatic ring bearing one or more hydroxyl groups. Biosynthetically they are derived from one of three pathways:

- Phenylalanine via the Shikimate pathway
- Polyketide pathways (chain elongation via malonyl-CoA)
- Isoprenoid pathway (i.e, aromatic terpenoids)

By far the majority of phenolics are derived all or in part from the deamination of phenylalanine (or in some cases tyrosine), which yields the C_6C_3 building block *trans*-cinnamic acid. Table 1, and the accompanying set of structures illustrate the major classes of phenolics in plants. Most phenolics display some form of biological activity: principally they are antioxidants, but many are also toxic or bitter (antifeedants), antimicrobial, antineoplastic *etc*.

[3] R. Croteau, T.M. Kutchan and N.G. Lewis (2000) *Natural Products*. In, Biochemistry and Molecular Biology of Plants (B.B. Buchanan, W. Greuissem and R.L. Jones, Eds),(American Society of Plant Physiologists, Rockville MD). Chapter 24, pp 1269.

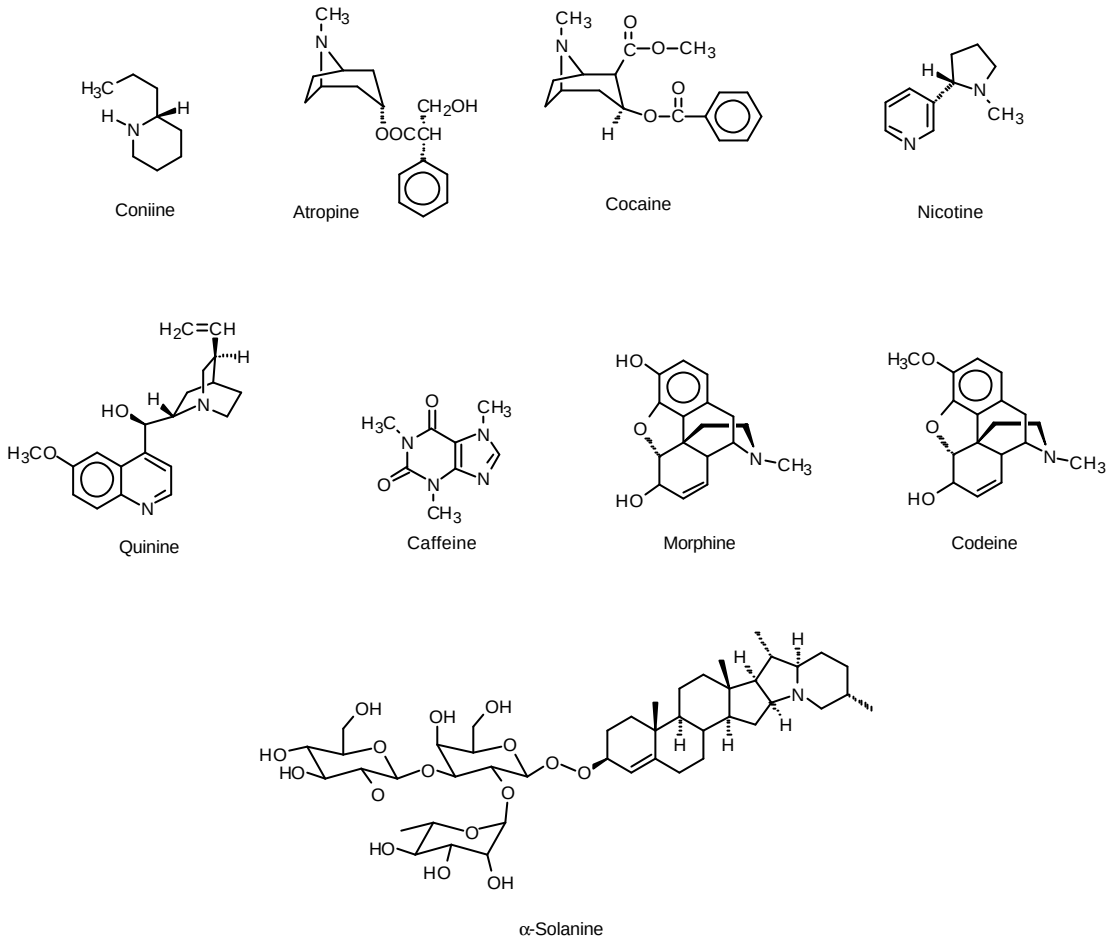

Figure 5. Pharmacologically active alkaloids. The piperidine alkaloid coniine, from the poisinous hemlock (*Conium maculatum*) is extremely toxic. The tropane alkaloids atropine (from *Hyoscymus niger*) and cocaine (from *Erythroxylon coca*) are structurally related. Atropine is an anticholinergic agent, while cocaine is a topical anesthetic, potent CNS stimulant and adrenergic blocker. Nicotine is the addictive agent in tobacco (*Nicotianum tobacum*), a pyridine alkaloid. Quinine, an indole alkaloid from *Chinchona officinalis*, is an potent antimalarial agent. Caffeine is a purine alkaloid found in *Coffea arabica* (and several other plant species). It is a CNS stimulant. Morphine and codeine, two analgesics, are benzyl-tetrahydroisoquinoline alkaloids (a group of isoquinoline alkaloids derived from tyrosine), found in the opium poppy (*Papaver somniferum*). α-Solanine is a toxic steroidal alkaloid found in potato tubers (*Solanum tuberosum*). (Adapted from R. Croteau, T.M. Kutchan and N.G. Lewis (2000) *Natural Products*. In, Biochemistry and Molecular Biology of Plants (B.B. Buchanan, W. Greuissem and R.L. Jones, Eds), (American Society of Plant Physiologists, Rockville MD) Chapter 24).

Table 1. The major classes of phenolic compounds in plants.(Adapted from Dey and Harborne (1997) *Plant Biochemistry* (Academic Press, New York). Chapter 10.

C No.	C Skeleton	Compound class	Compound example	Example of compound occurrence
6	C_6	Simple phenols	Hydroquinone	Arbutin in Rosaceae and Ericaceae
			Catechol	Urushiol in *Toxicodendron radicans*
7	C_6-C_1	Hydroxybenzoates	4-Hydroxybenzoate	Mainly wall-bound in most plant families
8	C_6-C_2	Acetophenones	4-Hydroxyacetophenone	Picein in *Picea abies*
		Phenylacetates	4-Hydroxyphenylacetate	In *Taraxacum officinale*
9	C_6-C_3	Hydroxycinnamates	Caffeate	Chlorogenate in Solanaceae
		Phenylpropenes	Eugenol	In essential oils of various families
		Coumarins	Esculetin	Cichoriin in *Cichorium intybus*
		Chromones	2-Methyl-5-hydroxy-7-methoxychromone	Eugenin in *Eugenia aromatica*
10	C_6-C_4	Naphthoquinones	Juglone	As 5-O-glucoside in Juglandaceae
13	C_6-C_1-C_6	Xanthones	1,3,6,7-Hydroxyxanthone	Mangiferin in *Mangifera indica*
14	C_6-C_2-C_6	Stilbenes	Resveratrol	In *Eucalyptus* trees
		Anthraquinones	Emodin	Emodin 6-O-glucoside in *Rheum palmatum*
15	C_6-C_3-C_6	Flavonoids	Quercetin	Rutin in various families
18	$(C_6$-$C_3)_2$	Lignans	Pinoresinol	In *Picea* and *Pinus* trees
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids	Amentoflavone	In most gymnosperms
n	$(C_6)_n$	Catechol melanins	Naphthalene polymer	In the ascomycete *Daldinia concentrica*
	$(C_6$-$C_1)_n$: Glc	Hydrolyzable tannins	Gallotannins	Chinese Tannin in galls of *Rhus semialata*
	$(C_6$-$C_3)_n$	Lignins	Guaiacyl lignins	Lignins in gymnosperms
			Guaiacyl-syringyl lignins	Lignins in angiosperms
	$(C_6$-C_3-$C_6)_n$	Condensed tannins	Catechin polymers	Tannin in the bark of *Quercus robur*

$(C_6$-$C_1)_n$: Glc = polyester with glucose (Glc) as the common central polyol moiety; arbutin = hydroquinone-O-β-glucoside; urushiol = catechol with a C_{15} hydrocarbon side chain [-$(CH_2)_7CH=CH(CH_2)_5CH_3$]; picein = 4-glucosyloxyacetophenone; chlorogenate = 5-O-caffeoylquinate; cichoriin = esculetin 7-O-β-glucoside; eugenin = 2-methyl-5-hydroxy-7-methoxychromone; mangiferin = 1,3,6,7-tetrahydroxyxanthone 2-C-β-glucoside; rutin = quercetin 3-O-rutinoside.

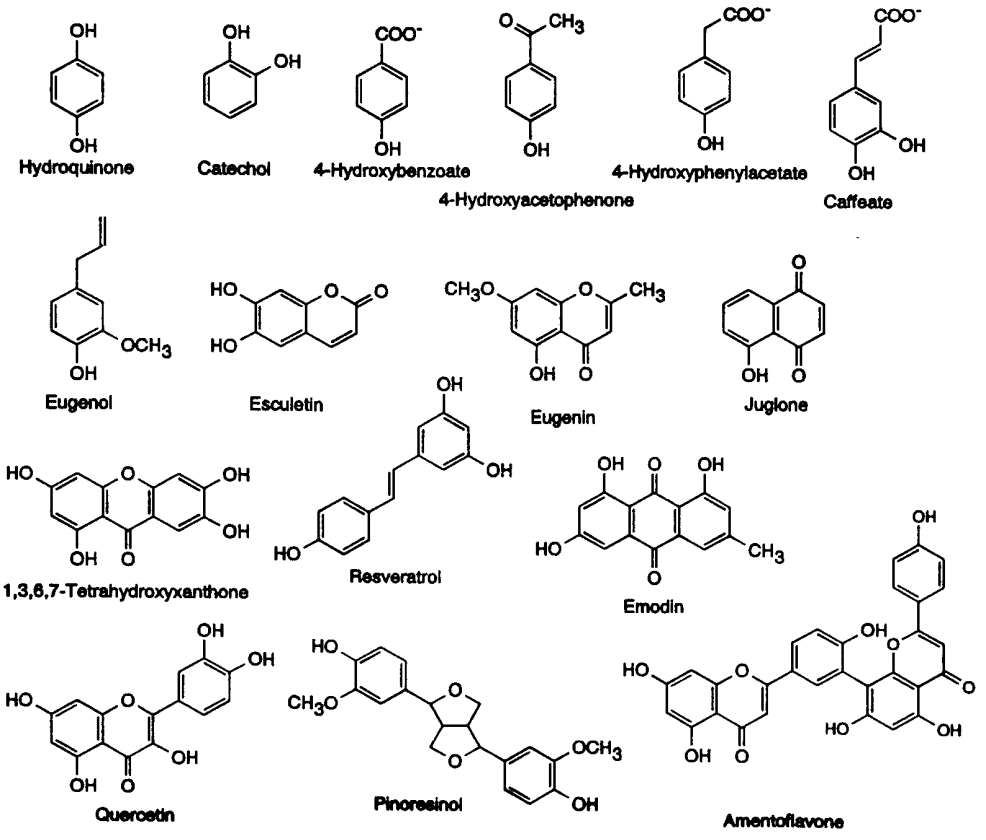

Figure 2 outlines the formation of hydroxycinnamic acids (exemplified by *p*-coumaric acid in the figure) and illustrates their central role as a precursor to many phenolic compounds (flavonoids and monolignols are depicted in Figure 2). Figure 6 expands on the central role of hydroxycinamic acids and once again illustrates the general types of modifications that occur to basic building blocks to give rise to the structural variety found in secondary metabolites.

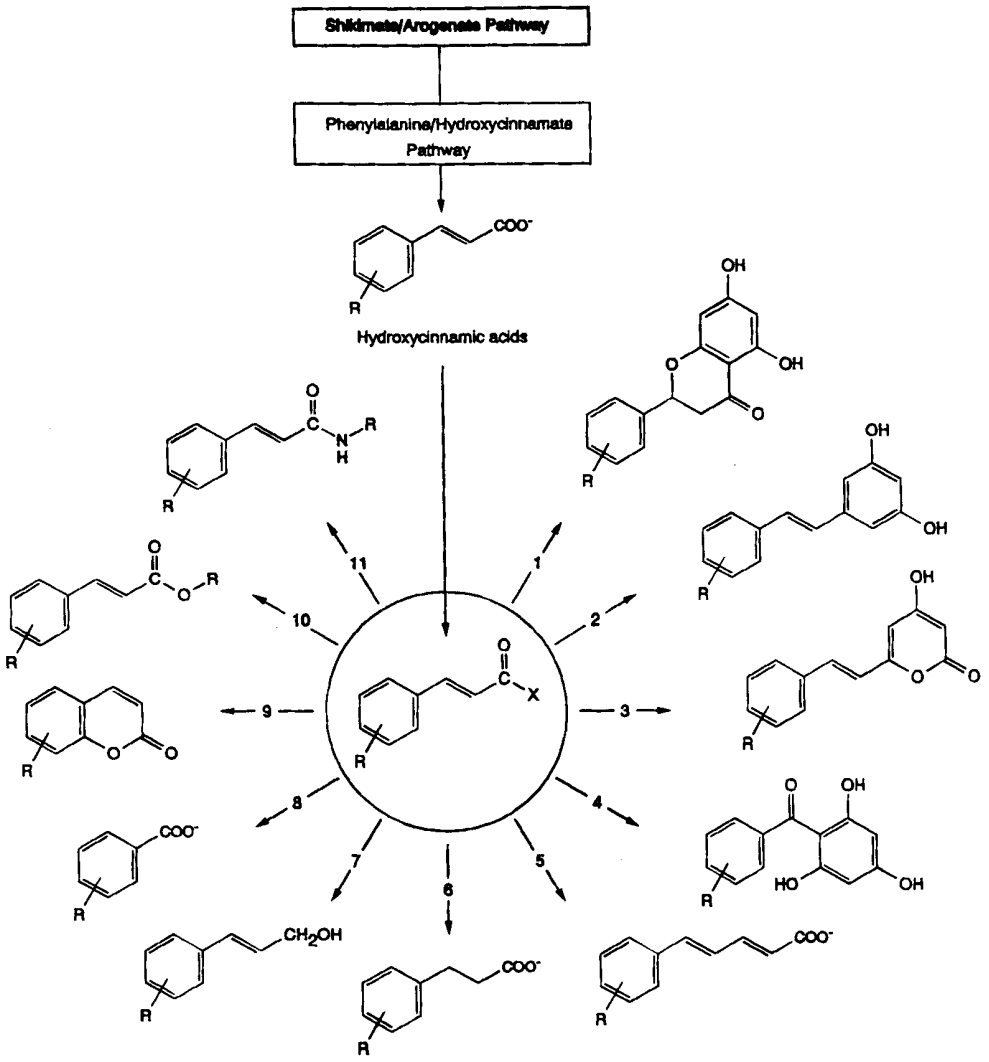

Figure 6. The central role of hydroxycinnamates in phenolic biosynthesis. The types of modifications that occur include: chain elongation leading to flavonoids (1), stilbenes (2), styrylpyrones (3), benzophenones (4) and elongated hydroxycinnamates (5); side chain reduction leading to dihydrocinnamates (6) and hydroxycinnamyl alcohols (monolignols; 7); side chain degradation leading to hydroxybenzoates (8); 2-hydroxylation and lactonization leading to hydroxycoumarins (9); conjugation leading to hydroxycinnamate esters (10) and amides (11). X = OH, SCoA or glucose. Adapted from Dey and Harborne (1997) *Plant Biochemistry* (Academic Press, NY) Chapter 10.

In planta, phenolics play many roles ranging from signal molecules to structural components of the cell walls (e.g., lignin). Phenolics play a significant role in plant defense against pathogens (e.g., isoflavonoid and pterocarpin phytoalexins) and herbivors (e.g., the astringency caused by tannins). They also act as pollinator attractants (e.g., floral colour and UV "runways") and UV protectants (especially flavonoids). Pharmacologically, three groups of phenolics are particularly relevant: flavonoids, lignans and tannins, examples of which are provided in Figure 7.

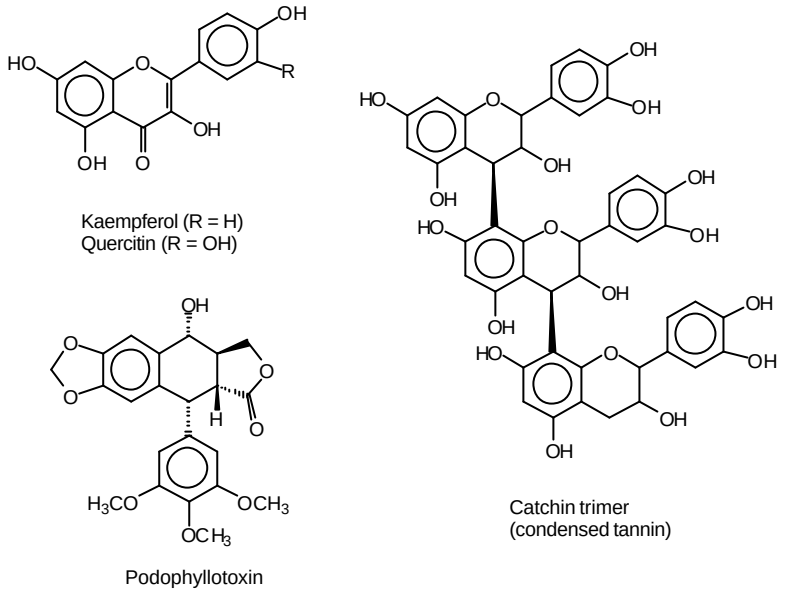

Figure 7. Pharmacologically active phenolics. The flavonoids kaempherol and quercitin, as well as condensed tannins afford the antioxidant properties of red wines (not to mention the astringency). Lignans, which are dimers of hydroxycinnamyl alcohols, display many properties, such as the antineoplastic activity of podophyllotoxin, isolated from the may apple (*Podophyllum peltatum*).