Chapter 1

General Introduction

Flavonoids are a large group of polyphenolic secondary metabolite compounds occurring in plants, a group containing more than 8000 known compounds arising from the great structural diversity possible from the various hydroxylation, hydrogenation, methoxylation, malonylation, glycosylation, sulfation and acylation patterns of the core flavonoid structure (Pietta *et al.*, 2003).

Originally, flavonoids were discovered as the pigments responsible for plant colour, ranging from red, orange, yellow to violet in flowering plants. Anthocyanins were the first group of flavonoids documented (Marquart, 1835). Later, flavonoids were discovered as important factors for plant growth, development and defence, for example, attracting animals for pollination and seed dispersal, providing ultraviolet protection, inducing nitrogen fixation (Gould & Lister, 2006). Consumption of flavonoids appears to bestow potential benefits for human health. This has provoked tremendous interest that has developed continuously and is now a large and important research area in food chemistry and nutrition with interactions with pharmacy (Andersen & Markham, 2006).

Flavonoids are believed to be endowed with biological activities, such as anti-inflammatory, anti-allergic, anti-ischemic, anti-platelet, immunomodulatory and anti-tumoral activities (Craig, 1999; Ielpo *et al.*, 2000; Prior & Cao, 2000). They have also been shown to inhibit some enzymes, including lipoxygenases and cyclooxygenases, mono-oxygenases, xanthine oxidase, mitochondrial succinoxidase, reduced nicotinamide-adenine dinucleotide (NADH) oxidase, phospholipase A_2, topoisomerases and protein kinases (Samman *et al.*, 1998; Dugas *et al.*, 2000; Valerio *et al.*, 2001). The biological activities of flavonoids were thought to be mainly due to their antioxidant properties, which are displayed by limiting the production of reactive oxygen species (ROS) and/or scavenging them (Pietta *et al.*, 2003). Now it is thought that inhibition of enzymes has at least an important role.

Flavonoids are components of the diet of herbivores and omnivores, including humans. They are principally found in all type of fruits, vegetables and plant-based food including grains, nuts, stems, leaves and popular drinks such as red wine, tea, coffee, and flavonoid intake may reach 1g/day (Kuhnau, 1976). Flavonoid

consumption may have important implications for human health. In fact, foods like red wine and tea were known for their beneficial effects on health long before flavonoids were isolated, purified and characterised (Nijveldt *et al.*, 2001).

1.1. Basic Structure and Classification

1.1.1. Basic Structure

The flavonoids constitute one of the most characteristic classes of compounds in higher plants, generally categorized as phenols or polyphenols, which are a group of low molecular weight substances. Their chemical structures are based on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2, 3 or 4. The skeleton can be represented as a C6-C3-C6 system. Figure 1-1 shows the general structure and the numbering system used to distinguish the carbon positions around the molecule. The left hand ring, which is derived from the acetate malonate pathway, is referred to as the A ring. The right hand ring, which is derived from the ring carbons of phenylalanine, is referred to as the B ring. The heterocyclic ring between the two rings is referred to as the C ring.

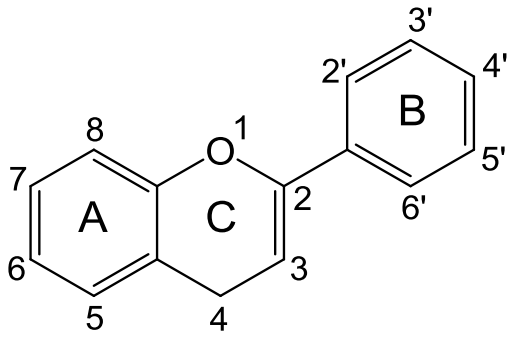
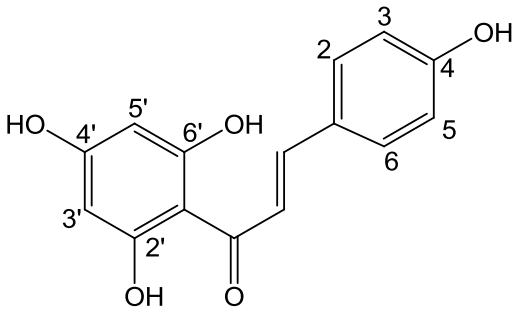


Figure 1-1 Basic monomeric structure of flavonoids

The complete structure is numbered from the heterocyclic oxygen, which is designated as position 1, clockwise round the C and A ring to position 8. The B ring is numbered separately with primed numbers, starting from C ring bond as 1' to the 6'

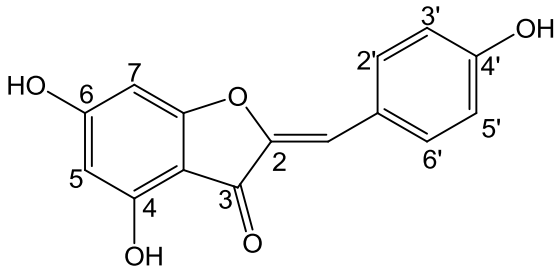
position clockwise. Variations in the chemistry of the C ring give rise to a number of distinct sub-classes of flavonoids, of which 6 are prominent in the human diet.

In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring, for example, chalcone (figure 1-2) and aurone (figure 1-3). Note that the carbon numbering under these conditions is different compared with the basic structure.



2',4',6',4-tetrahydroxychalcone = chalconaringenin

Figure 1-2 Structure of chalcone



4,6,4'-trihydroxyaurone

Figure 1-3 Structure of aurone

1.1.2. Biosynthesis

Flavonoids are ubiquitous non-nutrient secondary metabolites with important functions. Their biosynthesis is probably the best characterized of all the secondary metabolic pathways. The flavonoid pathway is part of the larger phenylpropanoid

pathway, which produces a range of other secondary metabolites, such as phenolic acids, lignins, lignans and stilbenes, and involves numerous enzymes. The key flavonoid precursors are phenylalanine from shikimate and arogenate pathways and malonyl-coenzyme A (CoA) from citrate produced by the TCA cycle (Davies & Schwinn, 2006). Phenylalanine is transformed to *trans*-cinnamic acid and then hydrolysed to 4-coumaric acid. This structure forms the flavonoid B ring and central 3-carbon fragment. The addition of three carbon malonyl-coenzyme A (CoA) units provides the A ring. The resultant molecule is chalcone which is the first flavonoid, formed by chalcone synthase, which may be enzymically isomerised to a flavanone. The flavanone is the main intermediate for forming other sub-classes of flavonoids. The general phenylpropanoid and flavonoid biosynthetic pathways are shown in figure 1-4. The later modification steps including glycosylation, methylation, acylation and sulfation are not shown.

Nearly all flavonoids (except catechins) do not occur in plants in the unsubstituted form. The most frequently occurring forms are the β-glycoside derivatives (Harborne *et al.*, 1975; Havsteen, 1983). Flavonoid molecules not attached to sugar moieties are referred to as aglycones, whereas flavonoid molecules with sugar moieties are called flavonoid glycosides. Glycosylation is either *O*- or *C*- linked, and leads to an increase in the hydrophilicity of the flavonoid molecule. Glycosylation also results in increased complexity of the molecule (Bohm, 1998). The level of glycosylation varies from mono-, di-, or higher. The types of monosaccharides involved include D-glucose, L-rhamnose, glucorhamnose, galactose, xylose, and arabinose (Havsteen, 1983). Glycosylation can occur at any substituted position on the molecule, but certain positions seem to be favoured. For example, the flavones are mainly glycosylated at 7 position while the flavonols at 3 position (Hollman & Arts, 2000). In addition to glycosylation, flavonoids are also commonly found methylated and conjugated to, for example, glucuronic and sulfate acid.

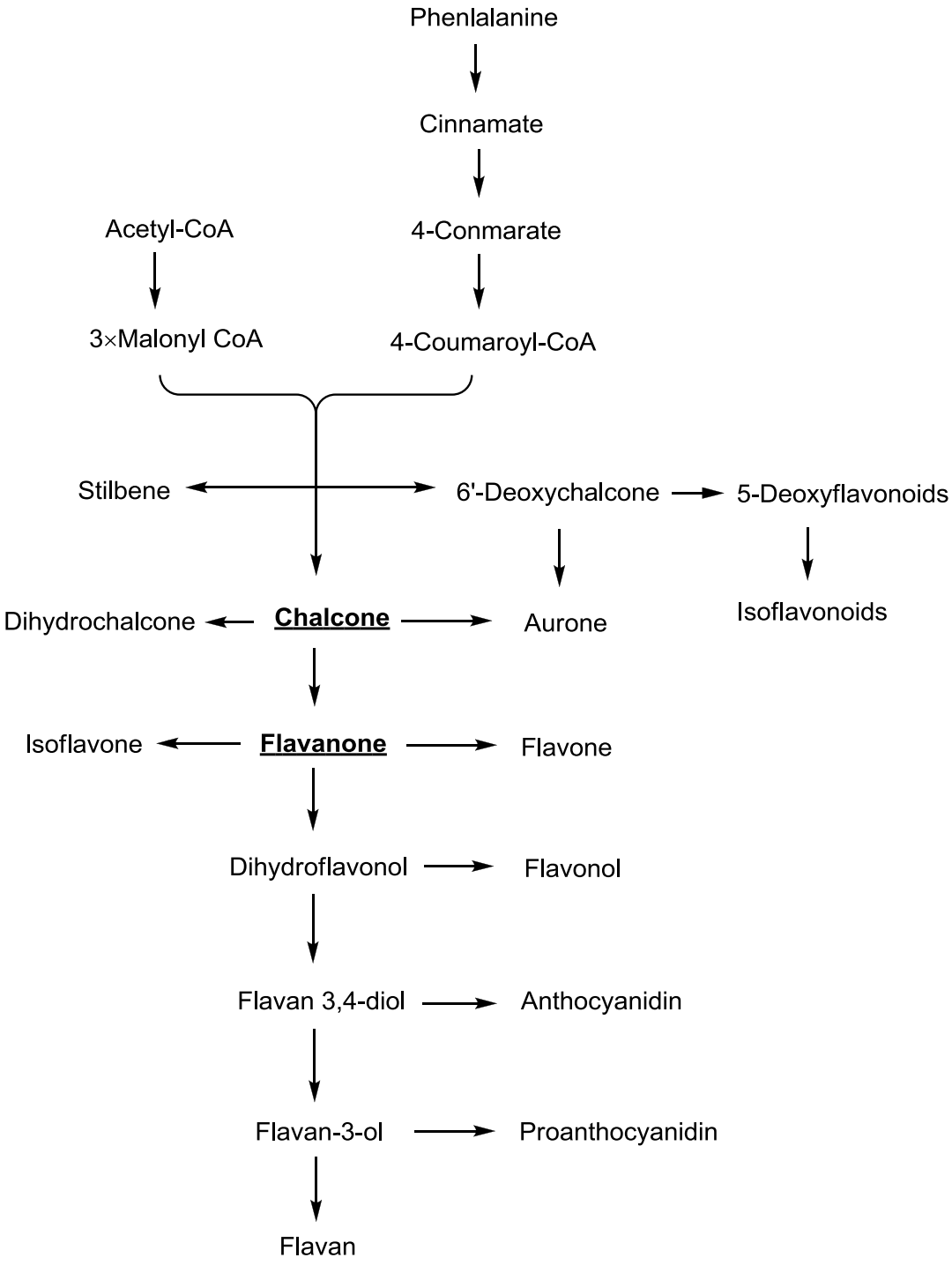


Figure 1-4 General phenylpropanoid and flavonoid biosynthetic pathways (from Harborne & Baxter, 1999a, Davies & Schwinn, 2006)

1.2. Major Sub-Classes of Flavonoids

Flavonoids are a very large group of polyphenolic natural products. There are different ways to classify flavonoids, for example, according to their biosynthetic origin, according to whether the central heterocyclic ring is unsaturated or not, according to their molecular size (Harborne & Baxter, 1999a). The most common way is according to the variation of the heterocyclic C ring. From the flavonoid basic structure, a heterocyclic pyrone C ring can be derivatised to the flavones, flavonoles, flavanones and isoflavones; a pyran C ring produces the flavanols and anthocyanins (Yilmaz & Toledo, 2004). The basic structures of each sub-class are shown in figure 1-5. The variation in the C ring is notable.

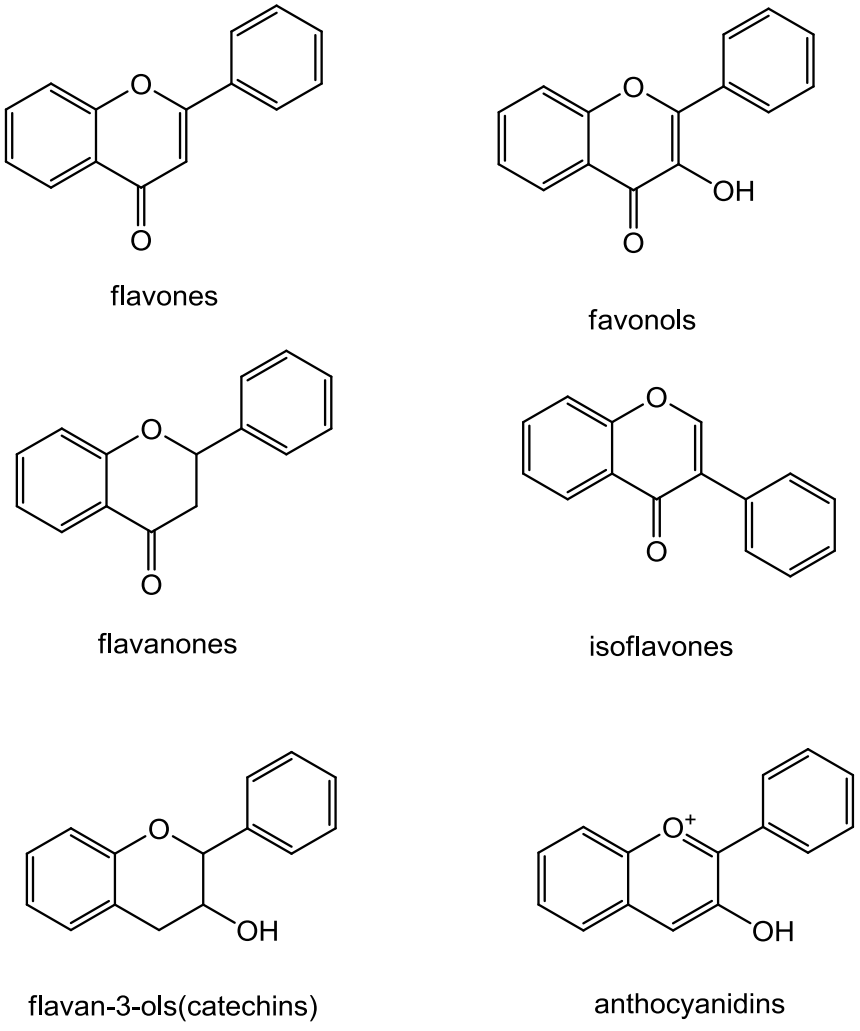


Figure 1-5 Structures of flavonoids sub-classes. Classification is based on variation in the heterocyclic C ring (from Hollman & Arts, 2000).

1.2.1. Flavones

In flavones, the C ring bears a > C = O bond at the 4 position, which is the common structural feature of the flavones, flavonols, flavanones and isoflavones, and a double bond between the 2 and 3 positions.

Common members of the flavones are apigenin and luteolin (figure 1-6), which are most abundant in parsley, celery and capsicum pepper, though flavones are found in many other plants and notably exist in grains and herbaceous families, e.g. labiatae, umbelliferae and compositae (Harborne & Baxter, 1999a). Onion, apple skin, berries, tea, lemon, olive, celery and red pepper are good sources of flavones.

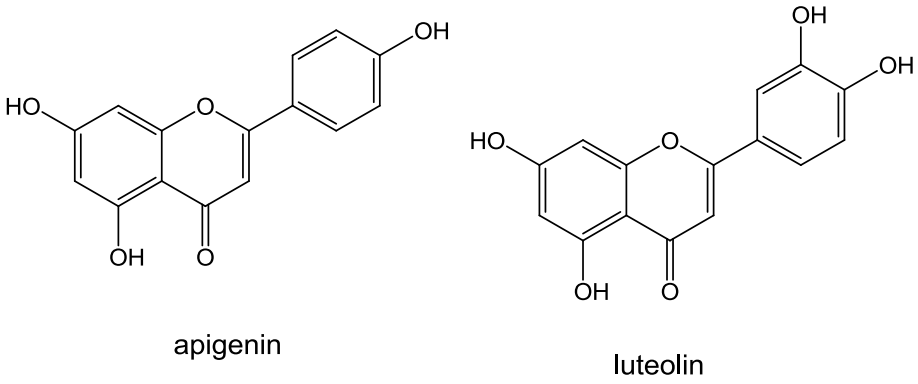


Figure 1-6 Structures of common flavones

Flavones mainly occur as 7-*O* glycosides in plants and the most frequently bound sugar is glucose although a variety of other sugar moieties may be attached to an aromatic carbon atom as *C*-glycosides (Hollman & Arts, 2000).

1.2.2. Flavonols

Flavonols generally exist in woody angiosperms but are also predominant in vegetables, fruits and beverages. Onions, berries, cherries, broccoli, apple, grapefruit, tea and red wine are rich sources of flavonols such as quercetin, myricetin, kaempferol and tamarixetin (Aherne & O'Brien, 2002). Flavonols are closely related to the flavones, their structures differing from flavones only in the presence of a hydroxyl group at the 3 position on the C ring.

Another major difference is the position of glycosylation. Flavones are characteristically present as the 7-glycoside whereas flavonols are generally glycosylated at the 3 position, less frequently the 7 position, and only in rare cases the *C*-4′, *C*-3′ and *C*-5 positions (Hollman & Arts, 2000). Quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavone) is the main representative of the flavonols in the human diet, and it usually occurs as *O*-glycosides, with D-glucose as the most frequent sugar residue. More than 170 different quercetin glycosides have been identified (Valant-Vetschera & Wollenweber, 2006). High concentrations of quercetin can be found in onions as quercetin-3-glucoside, quercetin-4'-glucoside, quercetin-3, 4′-diglucoside and isorhamnetin-4′-glucoside (Price & Rhodes, 1997; Aziz *et al.*, 1998; Fossen *et al.*, 1998). In green beans, quercetin exists mainly as quercetin-3-*O*-glucuronide (Price *et al*., 1998b). Other notable members of this sub-class are kaempferol, found in leafy vegetables and herbs, and myricetin, found in berries and tea (Kyle & Duthie, 2006). Flavonols are nearly always encountered as glycosides in plants, but quercetin may be found as an aglycone in the waxy coating on leaves and leaf buds (Hollman & Arts, 2000). Quercetin and kaempferol are also found in the free form in some species of aquatic algae and bacteria and may be synthesised in large amounts to protect against UV radiation (Rozema *et al.*, 2002). Figure 1-7 shows the structures of the common flavonols.

Flavone and flavonol *O*-glycosides make up one of the largest classes of flavonoids with over 2000 known structures. There were 279 glycosidic combinations of the most common flavonol aglycone, quercetin, and 347 kaempferol *O*-glycosides that had been identified up to December 2003 (Williams, 2006). The group includes any bound form of flavones or flavonols such as acylated and sulfated derivatives and not only those conjugates with sugar. Thus, the number of possible combinations is enormous because of the wide variation in possible substitutions. However, only a few esters of flavones are known. By comparison, a series of mostly monoacylated flavonols is known and recent reports increased the number slightly (Williams, 2006). As a result, many researchers have counted dietary flavonoids based mostly on the content of only 3 flavonols — quercetin, myricetin and kaempferol — and two flavones — apigenin and luteolin (Aherne & O'Brien, 2002).

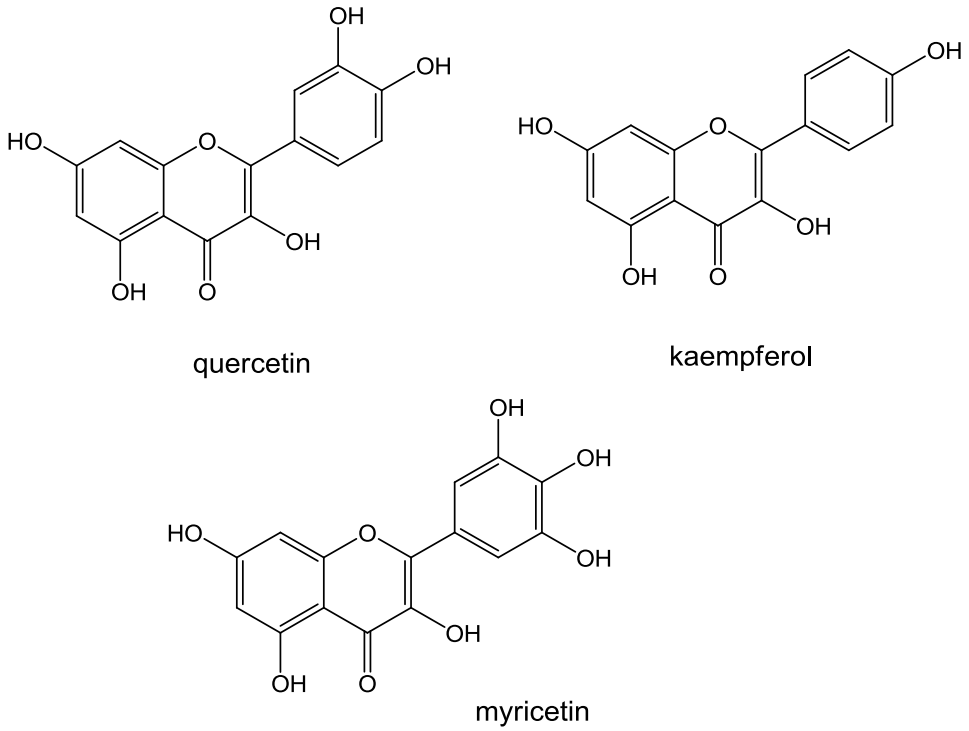


Figure 1-7 Structures of common flavonols

1.2.3. Flavanones and Dihydroflavonols

Flavanones are dihydroflavones, and like dihydroflavonols are referred to as flavanonols (and 3-hydroxylflavanones as well). In the past, flavanones and dihydroflavonols have been put into the category of "minor flavonoids" with chalcones, dihydrochalcones and aurones since there were few compounds belonging to these flavonoid sub-classes. Nevertheless, the term "minor flavonoids" is no longer appropriate because the number of known "minor flavonoids" has increased considerably (Grayer & Veitch, 2006).

Compared to flavones and flavonols, the structural feature of flavanones and dihydroflavonols is the absence of the double bond between the 2 and 3 positions on the heterocyclic C ring, which is present in flavones and flavonols (Tomás-Barberán & Clifford, 2000). Thus, in flavanones, C-2 bears one hydrogen atom in addition to the phenolic B ring, and C-3 two hydrogen atoms. Two stereoisomeric forms of each flavanone structure are possible, since C-2 is a centre of asymmetry (epimeric centre). Consequently, the B ring can be either in the (2*S*)- or (2*R*)-configuration. The great

majority of the flavanones isolated from plants are laevorotatory (–)- or (2*S*)-flavanones, because the enzymatic reaction catalyzing the conversion of chalcones to flavanones is stereospecific (Grayer & Veitch, 2006). In dihydroflavonols, the C-3 atom bears both a hydrogen atom and a hydroxyl group, and is therefore an additional centre of asymmetry (see figure 1-8). Hence, four stereoisomers are possible for each dihydroflavonol structure, (2*R*, 3*R*), (2*R*, 3*S*), (2*S*, 3*R*), and (2*S*, 3*S*). All four configurations have been found in naturally occurring dihydroflavonols, but the (2*R*, 3*R*)-configuration is by far the most common (Grayer & Veitch, 2006).

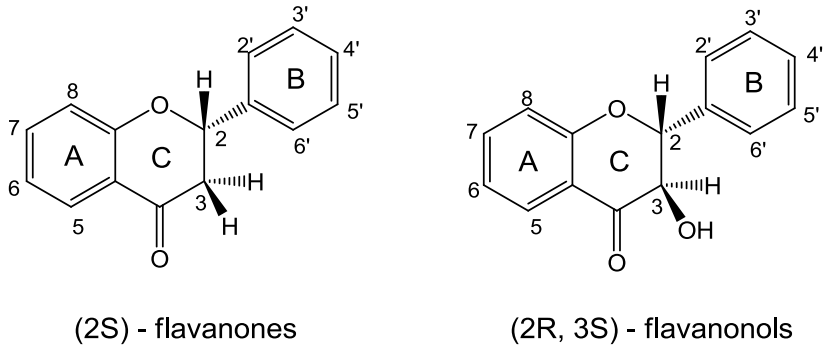


Figure 1-8 Structures of (2*S*)-flavanones and (2*S*, 3*R*)-dihydroflavonols

As in all other flavonoids, there is structural variation in flavanones and dihydroflavonols because of variation in hydroxylation, methoxylation, methylation, prenylation, benzylation, glycosylation, etc. of suitable carbon atoms in the skeleton, i.e., C-5, C-6, C-7, and C-8 of the A-ring, C-2', C-3', C-4', C-5', and C-6' of the B ring, and C-2 of the C ring in both flavanones and dihydroflavonols (Grayer & Veitch, 2006). In addition, the hydroxyl group at C-3 in dihydroflavonols can be methylated, glycosylated, or esterified. Flavanones substituted by hydroxy, methoxy, methylenedioxy, and *C*-methyl or related groups could conveniently be called "simple flavanones," in contrast to flavanones bearing more complex substituents such as prenyl and benzyl groups (Grayer & Veitch, 2006). Flavanones and dihydroflavonols are mainly conjugated as *O*-substitutions, but *C*-conjugations are also known. Flavanones exist in citrus fruits such as grapefruit, oranges and lemons. The most common members are naringin, hesperetin and eriodictyol (Harborne, 1994), which structures are shown in figure 1-9.

Biochemically, chalcones are the immediate precursors of flavanones, and some flavanones isomerize by ring opening into chalcones during isolation from plants or after chemical treatment with alkali (Tomás-Barberán & Clifford, 2000). In turn, flavanones are intermediates in the biosynthesis of most other flavonoid groups, including flavones, flavonols, and isoflavonoids (see figure 1-4).

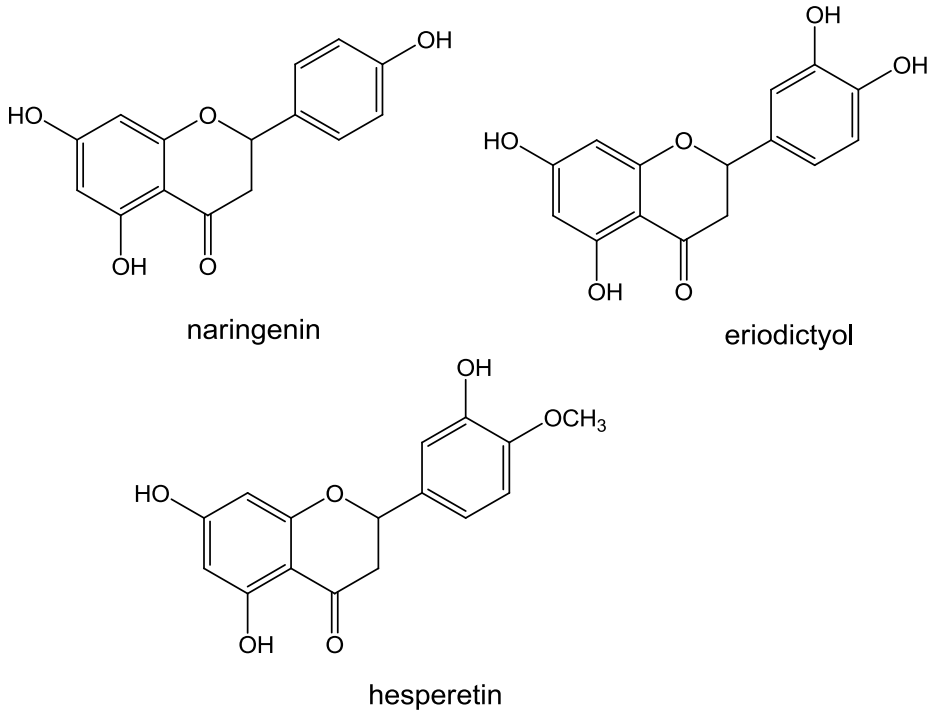


Figure 1-9 Structures of common flavanones

1.2.4. Isoflavones

Most of the flavonoids (flavanones, flavones, flavonols, flavanols and anthocyanins) bear ring B in position 2 of the heterocyclic ring. However, in isoflavonoids, ring B occupies position 3. It has been established that acetate gives rise to ring A and that phenylalanine, cinnamate and cinnamate derivatives are incorporated into ring B and C-2, C-3, and C-4 of the heterocyclic ring (Cassidy *et al.*, 2000).

Isoflavonoids include isoflavanones, isoflavones and isoflavonols, with isoflavones constituting the largest group of natural isoflavonoids. There are over 350 known isoflavones, making them the largest group of compounds in the class of isoflavonoids (Swinny & Markham, 2003). The best-known isoflavones are daidzein

(4′,7-dihydroxyisoflavone), genistein (4′,5,7-dihydroxyisoflavone), which occur mainly as the β-glycoside forms daidzin and genistin respectively, abundant in soybeans and consequently in a wide range of soy-derived foods and to a lesser extent in other legumes. Traditional soy foods are made from soy beans and include both fermented and non-fermented foods. Non-fermented soy foods contain isoflavones mostly present as the β-glycoside forms, while fermented soy foods such as miso and tempeh contain mostly unconjugated isoflavones (Wiseman, 2006). In addition, glycitein, formononetin, and biochanin A are also important isoflavones. These are commonly found to occur as the glucoside, glucoside malonate esters, or free aglycones. The structures of the 5 common isoflavones are shown in figure 1-10.

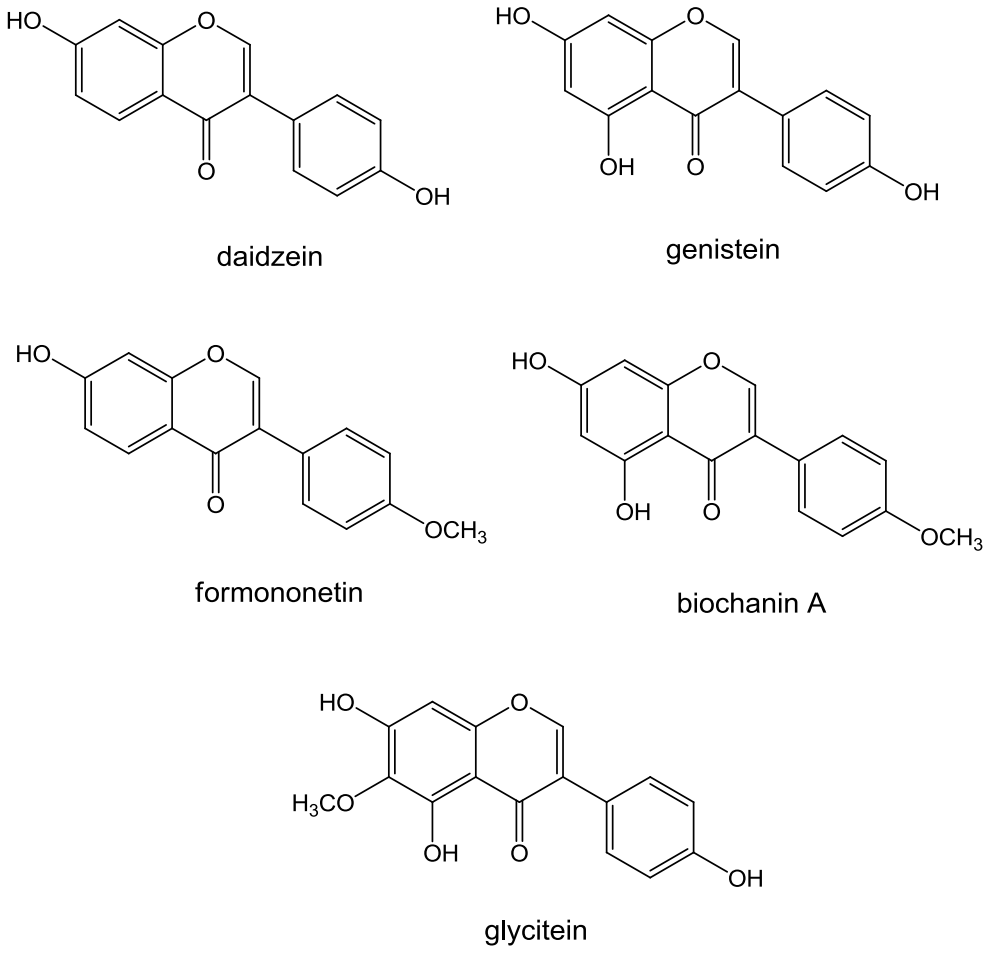


Figure 1-10 Structures of common isoflavones

Differing from flavonoids, the distribution of the isoflavones in the plant kingdom is largely restricted to the family Leguminoseae, with soybeans being the primary

human food rich in these compounds (Reinli & Block, 1996). Broad bean and red clover are also sources of isoflavones but at much lower levels.

Isoflavonoids are a distinct class of flavonoids best known for their estrogenic activity. Isoflavonoid phytoestrogens such as the soy isoflavones genistein and daidzein are plant-derived non-steroidal estrogen mimics, (other phytoestrogens include lignans such as secoisolariciresinol, coumestans such as coumestrol, and prenylflavonoids such as 8-prenylnaringenin), that are extensively investigated to determine their bioactive potential, particularly in the protection of human health via hormone-mediated mechanisms (Wiseman, 2006).

1.2.5. Flavanols

Flavanols are also referred to as proanthocyanidins, flavan-3-ols or simply catechins, which represent a large group of flavonoids with similar structures (see figure 1-5). However, the definition of this group is a little bit arbitrary. Generally speaking, flavans also include flavan-4-ols and flavan-3, 4-diols, of which the latter two are also named as leucocyanidins from which anthocyanidins can be produced, and flavan-3-ols which are also called catechins that are the best representatives of this flavonoid sub-class (Santos-Buelga & Scalbert, 2000). Much research on this sub-class has been carried out on catechins. Frequently, flavan itself is used to name that type of substance in this group without any hydroxyl group on the heterocyclic C ring. All four types are monomeric flavonoids. While proanthocyanidins are referred to as oligomers and polymers, most of the cases indicate flavan-3-ol oligomers excluding di- and tri- flavonoids (see figure 1-11) (Ferreira *et al.*, 2006).

The discriminating structural feature of flavanols, which they have in common with anthocyanidins only, is the lack of an oxygen group at the 4 position of the heterocyclic C ring (Ferreira *et al.*, 2006). The lack of a double bond between the 2 and 3 positions and the presence of a 3-hydroxyl group create two centres of asymmetry (carbons at positions 2 and 3). Thus, in principle, two stereoisomeric forms are possible. Nevertheless, only flavanols with a 2*R* configuration have been found so far in nature (Hollman & Arts, 2000). The predominating flavanols are (+)-

catechin (2*R*, 3*S* -3, 5, 7, 3′, 4′-pentahydroxyflavan), (–)-epicatechin (EC) (2*R*, 3*R* – 3, 5, 7, 3′, 4′-pentahydroxyflavan), (+)-gallocatechin (GC) (2*R*, 3*S* -3, 5, 7, 3′, 4′, 5′-hexahydroxyflavan) and (–)-epigallocatechin (EGC) (2*R*, 3*R* – 3, 5, 7, 3′, 4′, 5′-hexahydroxyflavan) and the following gallic acid esters: (–)-epicatechin gallate (ECG) and (–)-epigallocatechin gallate (EGCG), which structures are shown in figure 1-12.

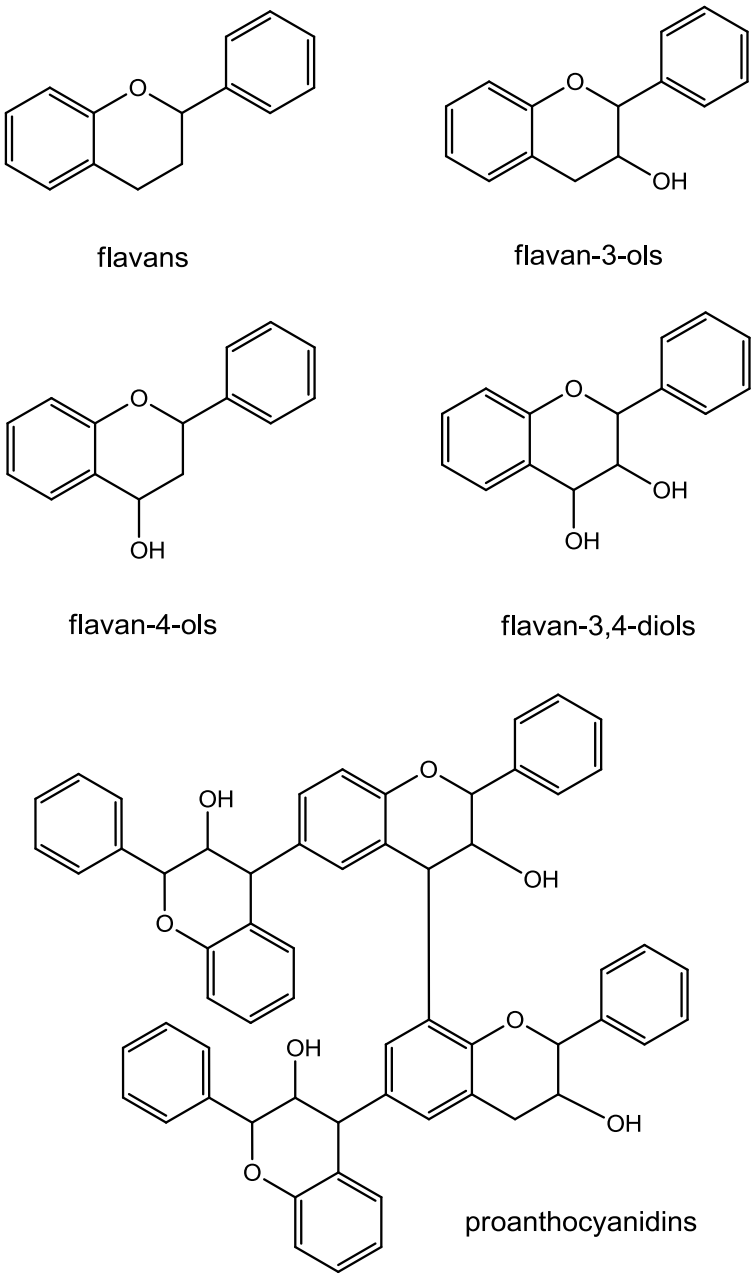


Figure 1-11 Structures of different flavanols

Flavanols are able to associate with each other to give rise to dimers, oligomers and polymers. Those molecules are known as proanthocyanidins, linked by bonds between carbons atoms of the rings of the monomers. The carbon atom at the 4 position on a monomer is bound to the next monomer at the 8 or 6 position (Manach *et al.*, 2004). Figure 1-13 shows the B-type proanthocyanidins, where the constituent flavanyl units are linked via only one bond. Analogs of the A-type possess an unusual second linkage, which also make the A-type much more complicated and therefore less discussed (Ferreira *et al.*, 2006). Catechin polymers contribute a bitter taste and brown pigments to foods such as tea and dark chocolate (Arts *et al.*, 1999; Harborne & Baxter, 1999b).

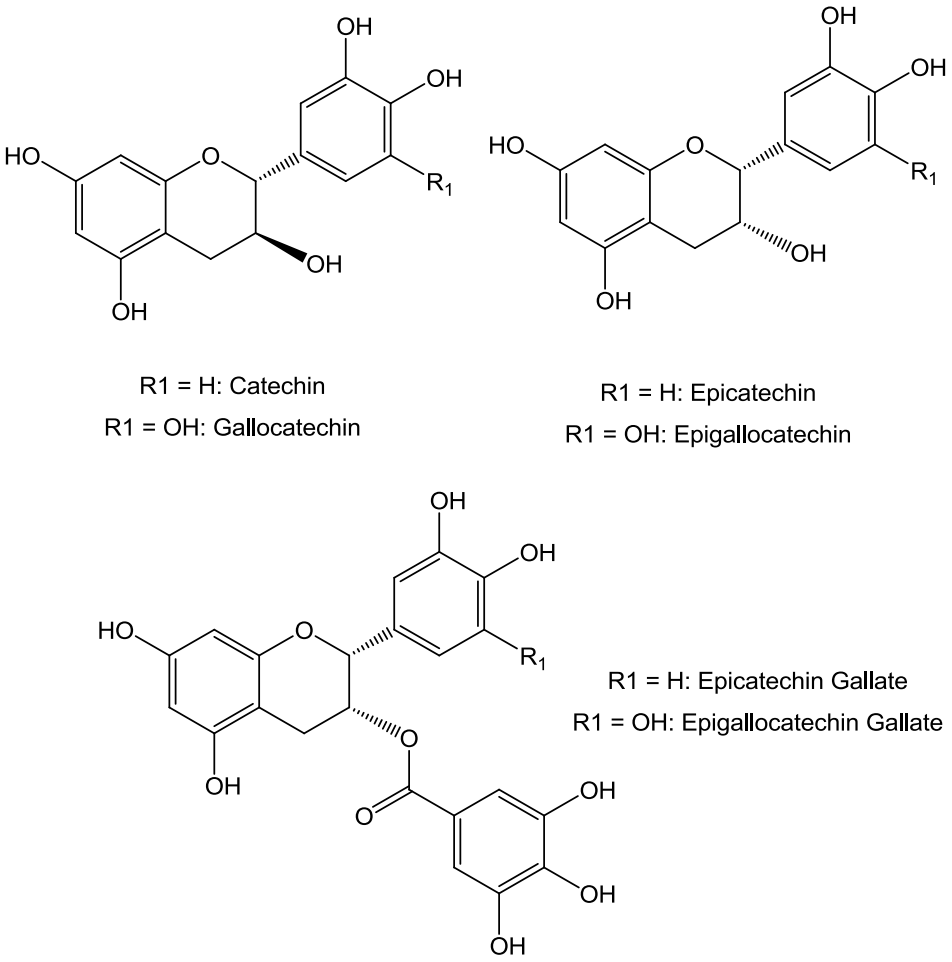


Figure 1-12 Stereoisomeric structures of flavanols

As opposed to other flavonoids which exist in nature as glycosides, flavanols exist in nature as aglycone forms while glycoside forms are rare (Hertog *et al.*, 1993c).

Flavanols are widespread in plant foods especially in woody and some herbaceous plants. They have been reported in tea, fruits and legumes. Tea is probably the most important flavanol source in many countries. It combines a high level of consumption with relatively high flavanol content. Furthermore, it is the only plant food for human consumption found so far which contains (–)-epigallocatechin gallate (EGCG) (Ferreira *et al.*, 2006). The most abundant monomeric flavanols of black tea are (–)-epicatechin gallate (ECG), (–) -epigallocatechin (EGC) and EGCG. Only a small part of the flavanol content of teas is constituted by (+)-catechin, (–)-epicatechin and (+)-gallocatechin. Flavanols are sometimes conveniently called tea flavonoids, and tea has been tested extensively for its biological actions in *in vitro* and *in vivo* animal experiments. Besides tea, flavanols have also been determined at high level in red wine, chocolate, black grape, cherry and other fruits (Harborne & Baxter, 1999b).

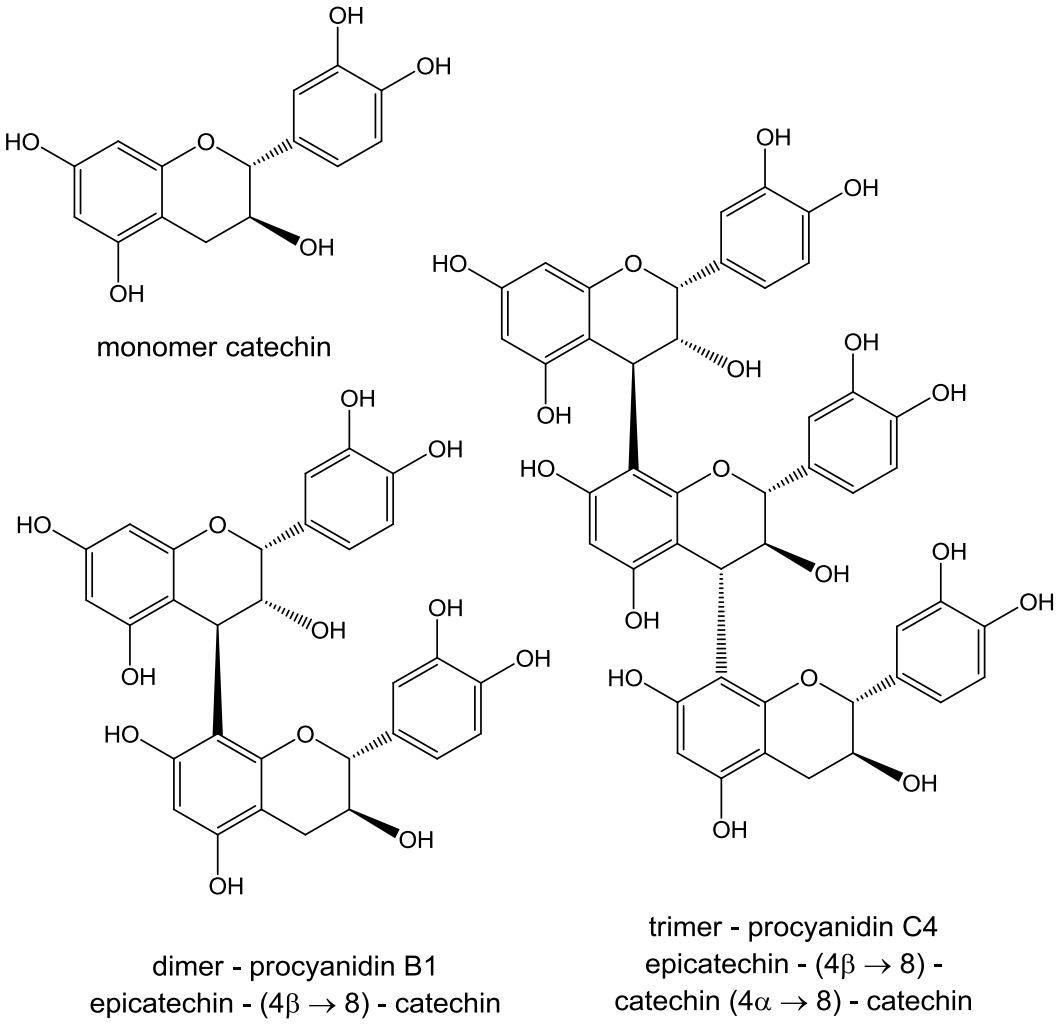


Figure 1-13 Flavanol polymers

1.2.6. Anthocyanins

The anthocyanins constitute a major flavonoid group that is responsible for cyanic colours ranging from salmon pink through red and violet to dark blue of most flowers, fruits, and leaves of angiosperms. Strong pigmentation usually indicates high anthocyanin content. They are sometimes present in other plant tissues such as roots, tubers, stems, bulbils, and are also found in various gymnosperms, ferns, and some bryophytes (Andersen & Jordheim, 2006). They are of great economic importance as fruit pigments and thus are used to colour fruit juices, wine and some beverages. The past twenty years has witnessed a renaissance in research activities on and general interests in these water-soluble pigments in several areas (Clifford, 2000).

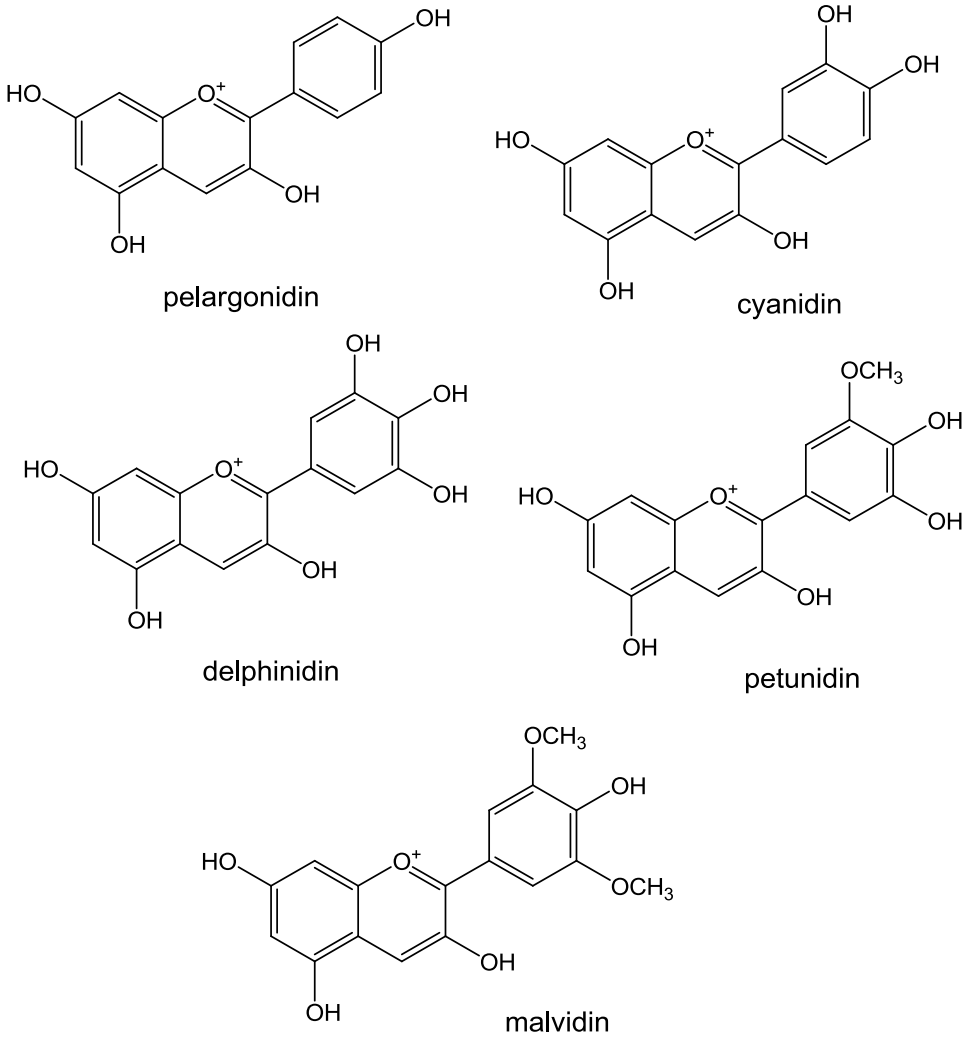


Figure 1-14 Structures of common anthocyanidins

The term anthocyanins refer to the glycosylated flavonoids whereas the aglycones are termed anthocyanidins. The most common anthocyanidins are cyanidin, pelargonidin, delphinidin, malvidin and petunidin (Harborne & Baxter, 1999b), which structures are shown in figure 1-14.

As for most types of flavonoids, anthocyanins are always glycosylated in nature. Most anthocyanins contain two, three, or just one monosaccharide unit. The most common forms are 3-glycosides and 3, 5-diglycoside (Clifford, 2000). However, as many as seven glycosyl units have been found. Altogether 240 and 24 anthocyanins have been reported to contain a disaccharide and a trisaccharide respectively, while no tetrasaccharide has been found yet. The sugar moieties commonly are connected to the anthocyanidins through *O*-linkages; however, 8-*C* -glucosylcyanidin 3-[6-(malonyl) glucoside] has been isolated by Saito *et al*. (2003). This was the first report of a natural *C*-glycosylanthocyanin although *C*-glycosylation is common in other flavonoids, especially flavones (Andersen & Jordheim, 2006).

Anthocyanins are possibly charged and exist in different chemical forms at different pH values, through alteration of the carbon backbone. Therefore colour is pH-dependent. They are usually red in acid conditions and blue in alkaline conditions and chelate with metal ions like Ca^{2+} and Mg^{2+} under alkaline conditions (Harborne *et al.*, 1975). The colour reaction is shown below.

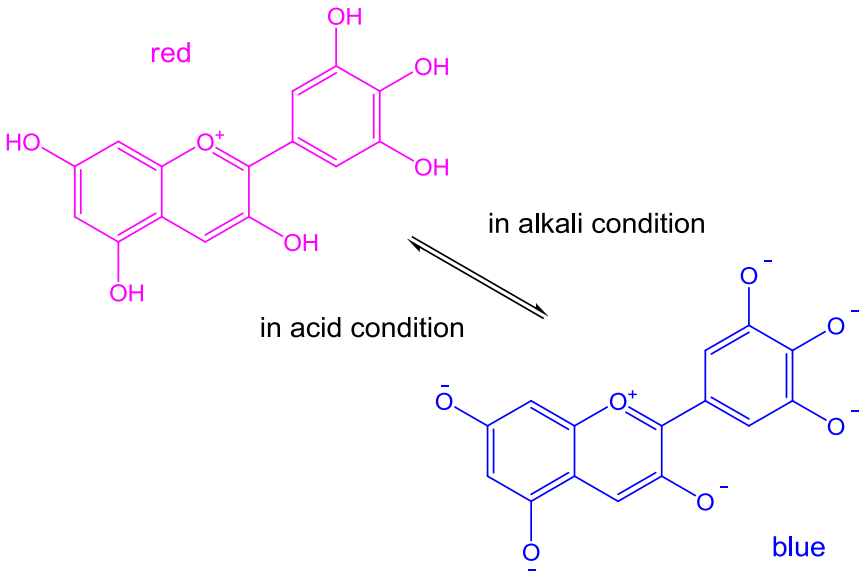


Figure 1-15 Colour reactions of anthocyanidins

1.2.7. Minor Sub-Classes

Besides the major sub-classes, there are other sub-classes of flavonoids, which have skeleton structures differing from the flavonoid basic structure (see figure 1-1). Few compounds have been found in these groups.

1.2.7.1. Neoflavonoids

Neoflavonoids are a group of chromane derivatives with ring B in position 4 (4-phenyl-coumarins = neoflavonoids). They are biogenetically derived by rearrangement of the flavonoid 2-pheylchroman system by means of 1, 2-aryl rearrangement (Harborne, 1994). The neoflavonoids can be considered to be formed from isoflavonoids following a further aryl rearrangement from the 3 position to the 4 position. The isoflavonoids and the neoflavonoids are regarded as abnormal flavonoids (Harborne *et al.*, 1975).

Neoflavonoids are glycosylated mostly as 5-*O*-forms though occasionally 7-*O*-glycosides may present. By contrast to the distribution of isoflavonoids restricted to the family Leguminoseae, neoflavonoids are concentrated in many plant families: the Guttiferae, Leguminosae, Rubiaceae, Passifloraceae, Polypodiaceae and Compositae. The notable activity and the reported presence of neoflavonoids in plants of traditional medicine have focused on their isolation and synthesis (Harborne, 1994). A typical neoflavonoid is melanin isolated from heartwood of *Dalbergia mwlanoxylon* (Leguminosae) the structure of which is shown below (Harborne & Baxter, 1999b).

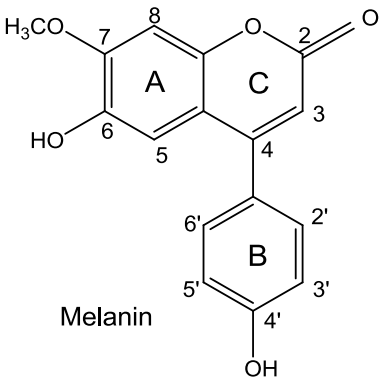


Figure 1-16 Structure of typical neoflavonoid

1.2.7.2. Chalcone

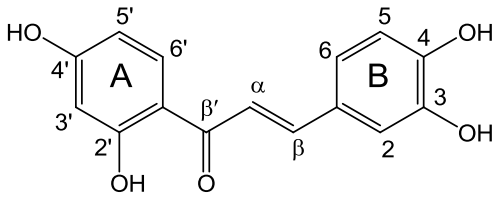
Chalcones, and the closely related dihydrochalcones, are unique in the flavonoid family in lacking a heterocyclic C ring (Harborne, 1994). They are open-chain C6-C3-C6 compounds which are the first C15 intermediates in flavonoid biosynthesis. The numbering system of chalcones differs from that of ring-closed flavonoids, which has been shown in figure 1-1 and figure 1-2. In chalcones and dihydrochalcones the 'prime' positions are on the A ring, as opposed to the B ring in all cyclic flavonoids.

The atom numbering of chalcones, dihydrochalcones, and aurones remains a potential source of confusion when compared to that of other classes of flavonoid. The A and B rings of all the flavonoids have the same origin in biosynthetic terms, with the A rings derived from the acetate pathway and the B rings from the shikimate pathway (Davies & Schwinn, 2006). The crucial difference is in the style of atom numbering, in which primed numbers are used to refer to the A ring of chalcones and dihydrochalcones, but to the B ring of other flavonoid classes, including the aurones. Similarly, the B rings of chalcones and dihydrochalcones carry the non-primed numbers instead of the A ring. The numbering scheme followed for chalcones and dihydrochalcones is also different, because the C3 unit linking the A and B rings is referred to only in terms of carbonyl (β'), α- and β-carbons, whereas the equivalent carbon atoms of the heterocyclic C rings of other flavonoids are numbered together with the rest of the molecule (Veitch & Grayer, 2006). For the B ring, the carbon can be numbered either clockwise or counterclockwise. The aurone numbering system is anomalous because of the five-membered C ring. The result is that the A ring positions equivalent to other flavonoids (excluding chalcones and dihydrochalcones) bear a number one less in value.

The common structures of chalcones and dihydrochalcones are all hydroxylated to varying degrees and many are also *O*-methylated as well. Chemically chalcones can be classified into two groups. The first are chalcones with varying hydroxylation. They may be partly *O*-methylated and some have prenyl substitution. They may occur as glycosides but glycosidic variation is limited with glucose being the common sugar.

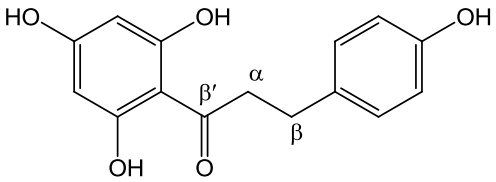
The second group are those with complex structures involving many cases extra furano or pyrano rings fused to either the A or B ring (Harborne & Baxter, 1999b).

Chalcones have a limited but scattered occurrence. They are abundant in the Leguminosae, for example being present in heartwood of trees or flowers of gorse, and in the Compositae, where they provide yellow flower colour in *Coreopsis* and related species. They are also present in the crystalline deposit of fronds of certain fern species. Chalcones can co-occur sometimes with the related flavanones and may be accompanied in flowers by related yellow aurone pigments (Veitch & Grayer, 2006). Apart from providing their yellow colour, no other clearcut functions have yet been elucidated in plants for the chalcones. However, they are of medicinal interest



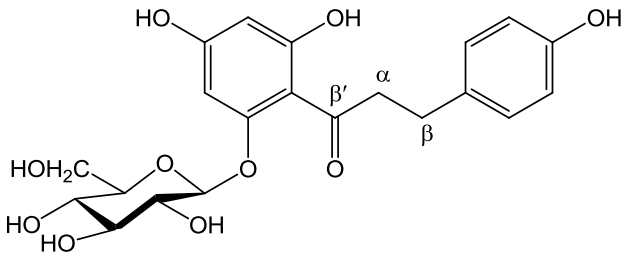
chalcones - butein

3,4,2',4' - Tetrahydroxychalcone



dihydrochalcone - phloretin

4,2',4',6' - Tetrahydroxydihydrochalcone



dihydrochalcone glycoside

phloridzin = phloretin 2' - glucoside

Figure 1-17 Chalcone and dihydrochalcone structures

and some structures have anti-peptic or anti-hepatotoxic activities (Harborne & Baxter, 1999b) and, more recently, chalcones have been found with anti-angiogenic effect (Mojzis *et al.*, 2008). A typical chalcone is butein found in many leguminous plants and in *Anacardiaceae* and *Compositae* as well.

Dihydrochalcones are directly related to the chalcones and are derived from them by reduction of the chalcone α, β-double bond. The best known dihydrochalcone is phloridzin, which occurs in the skin of apple (Harborne & Baxter, 1999b).

Most of the dihydrochalcones are hydroxylated naturally. A number are glycosidic, as in the case of phloridzin. Dihydrochalcones are a relatively small group of flavonoids and have a somewhat erratic distribution (Veitch & Grayer, 2006). They have been recorded in about 28 plant families, notably in species of *Ericaeae* and *Rosaceae*. They may also accompany chalcones in frond exudates of *Pityrogramma* and other fern species. A special property of some dihydrochalcones is their extremely sweet taste and there has been much interest in developing them as food sweeteners (Harborne & Baxter, 1999b). Figure 1-17 show the structures of butein, phloridzin and its glucoside.

1.2.7.3. Aurones

Aurones are highly coloured flavonoids based on the 2-benzylidenecoumaranone structure. Auronols which could be considered in the chemical sense as hydrated aurones, are 2-hydroxyl-2-benaylcoumaranones (Veitch & Grayer, 2006). The conventional numbering system for aurones and auronols has positions 4 to 7 on the A-ring, where position 4 is biosynthetically equivalent to position 5 in normal flavonoids and position 2' in chalcones (see figure 1-3).

The first aurone was isolated as a yellow pigment from *Coreopsis grandiflora* in 1943 named leptosidin. Since then a limited number of other aurones have been detected not only in flowers but also in bark, wood and leaves (Harborne & Baxter, 1999b). A well-known aurone is bracteatin which was discovered in snapdragon (*Antirrhinum majus*) (Harborne, 1963). A typical member of auronol is carpusin from *Pterocarpus*. The structures of bracteatin and carpusin are shown in figure 1-18.

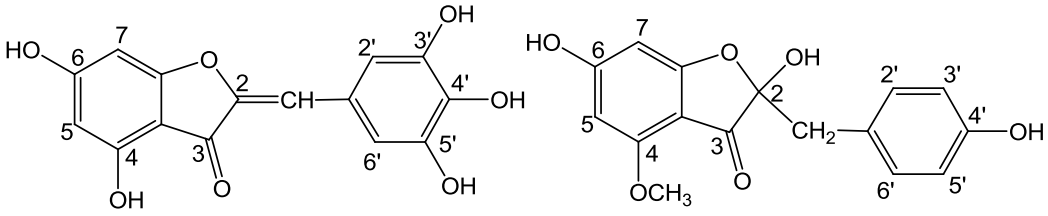


Figure 1-18 Typical aurone and auronol structures

Aurones are glycosylated in nature with the glucose as the most usual sugar but many aglycone forms are also been found. Aurones have a scattered occurrence in the flowering plants and provide yellow to brown colours (Harborne *et al.*, 1975).

Chalcones and aurones are best known as the yellow to orange coloured flower pigments of some species. The distribution of these compounds is not restricted to flowers, but can be found in many different plant tissues (Harborne *et al.*, 1975). The chalcones are structurally one of most diverse groups of flavonoids, as witnessed by the formation of a wide range of dimers, oligomers and conjugates of various kinds. At the same time, they are of great significance biosynthetically as the immediate precursors of all other classes of flavonoids (Davies & Schwinn, 2006). Underlying these important attributes is the unique feature that distinguishes chalcones and dihydrochalcones from other flavonoids, the open-chain three-carbon structure linking the A and B rings in place of a heterocyclic C ring. In plants, chalcones are converted to the corresponding (2S)-flavanones in a stereospecific reaction catalyzed by the enzyme chalcone isomerase. Compare the structural difference between naringenin chalcone (figure 1-2) and naringenin flavanone (figure 1-9). The close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products. It is also the reason why chalcones, dihydrochalcones, and aurones are sometimes described together with flavanones and dihydroflavonols (Veitch & Grayer, 2006).