

## Phenolic compounds

The term phenolic compound embraces a wide range of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic substances tend to be water-soluble, since they most frequently occur combined with sugar as glycosides and they are usually located in the cell vacuole. Among the natural phenolic compounds, of which several thousand structures are known, the flavonoids form the largest group but simple monocyclic phenols, phenylpropanoids and phenolic quinones all exist in considerable numbers. Several important groups of polymeric materials in plants - the lignins, melanins and tannins - are polyphenolic and occasional phenolic units are encountered in proteins, alkaloids and among the terpenoids.

While the function of some classes of phenolic compound are well established (e.g. the lignins as structural material of the cell wall; the anthocyanins as flower pigments), the purpose of other classes is still a matter of speculation. Flavonols, for example, appear to be important in regulating control of growth in the pea plant (Galston, 1969) and their adverse effects on insect feeding (Isman and Duffey, 1981) have indicated that they may be natural resistance factors; neither of these functions, however, has yet been firmly established.

To the plant biochemist, plant phenols can be a considerable nuisance, because of their ability to complex with protein by hydrogen bonding. When plant cell constituents come together and the membranes are destroyed during isolation procedures, the phenols rapidly complex with protein and as a result, there is often inhibition of enzyme activity in crude plant extracts. On the other hand, phenols are themselves very susceptible to enzymic oxidation and phenolic material may be lost during isolation procedures, due to the action of specific 'phenolase' enzymes present in all plants. Extraction of the phenols from plants with boiling alcohol normally prevents enzymic oxidation occurring and this procedure should be adopted routinely.

The classic procedure for detecting simple phenols is by means of the intense green, purple, blue or black colors many of them give in solution when 1% aqueous or alcoholic ferric chloride is added. This procedure, modified by using a fresh aqueous mixture of 1% ferric chloride and 1% potassium ferricyanide, is still used as a general means of detecting phenolic compounds on paper chromatograms. However, the majority of phenolic compounds (and especially the flavonoids) can be detected on chromatograms by their colors or fluorescences in UV light, the colors being intensified or changed by fuming the papers with ammonia vapour. The phenolic pigments are visibly colored and they are thus particularly easily monitored during their isolation and purification. Phenolic compounds are all aromatic, so that they all show intense absorption in the UV region of the spectrum.

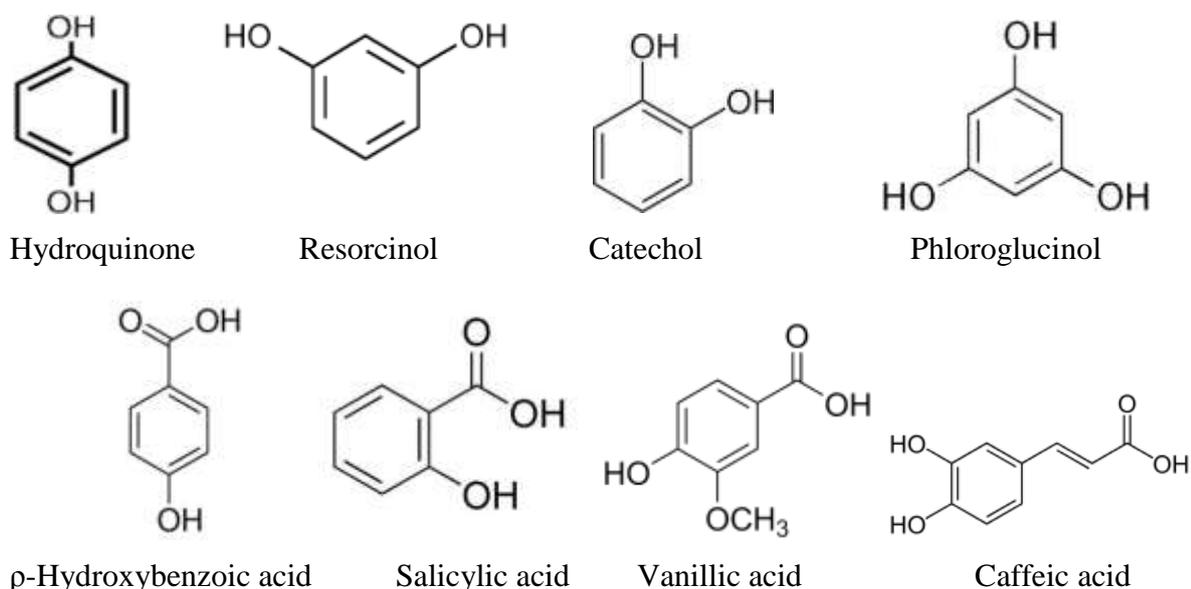
## PHENOLS AND PHENOLIC ACIDS

The free phenols and phenolic acids (for formulae, see Fig. 1) are best considered together, since they are usually identified together during plant analysis. Acid hydrolysis of plant tissues releases a number of ether soluble phenolic acids, some of which are universal in their distribution. These acids are either associated with lignin combined as ester groups or present in the alcohol-insoluble fraction of the leaf; alternatively they may be present in the alcohol-soluble fraction bound as simple glycosides. Universal among the angiosperms are p-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid. Gentisic acid is also widespread; less common are salicylic and  $\alpha$ -protocatechuic, two acids found characteristically in the Ericaceae. Finally, there is gallic acid, reported as a natural inhibitor of flowering, in leaves of *Kalanchoe* (Pryce, 1972). Gallic acid is found in many woody plants, bound as gallotannin, but it is a very reactive substance. More common in acid-hydrolysed plant extracts is its dimeric condensation product, ellagic acid, formed from ellagitannins (Seikel and Hillis, 1970) present in direct extracts.

By contrast with the above acids, free phenols are relatively rare in plants. Hydroquinone is probably the most widely distributed; others, such as catechol, orcinol, phloroglucinol and pyrogallol, have been reported from only a few sources. Alkaline degradation or reductive cleavage of flavonoids and other complex phenols yields one or more of the simple phenols and phenolic acids shown in Fig. 1.

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural color pigments responsible for the color of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Puupponen-Pimiä *et al.*, 2008). They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolics.

Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin and chlorogenic (Fig. 1).



**Figure 1** Structures of phenols and phenolic acids

## FLAVONOIDS

The flavonoids are all structurally derived from the parent substance flavone, which occurs as a white mealy farina on *Primula* plants, and all share a number of properties in common. Some ten classes of flavonoid are recognized (Table 2.7) and these different classes will be considered in more detail in subsequent sections. In this section, general procedures of identification are discussed and methods for distinguishing the different classes are outlined.

Flavonoids are mainly water-soluble compounds. They can be extracted with 70% ethanol and remain in the aqueous layer, following partition of this extract with petroleum ether. Flavonoids are phenolic and hence change in color when treated with base or with ammonia; thus they are easily detected on chromatograms or in solution. Flavonoids contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible regions of the spectrum. Finally, flavonoids are generally present in plants bound to sugar as glycosides and anyone flavonoid aglycone may occur in a single plant in several glycosidic combinations. For this reason, when analysing flavonoids, it is usually better to examine the aglycones present in hydrolysed plant extracts before considering the complexity of glycosides that may be present in the original extract.

Flavonoids are present in all vascular plants but some classes are more widely distributed than other; while flavones and flavonols are universal, isoflavones and biflavonyls are found in only a few plant families.

### Preliminary classification

Flavonoids are present in plants as mixtures and it is very rare to find only a single flavonoid component in a plant tissue. In addition, there are often mixtures of different flavonoid

classes. The colored anthocyanins in flower petals are almost invariably accompanied by colorless flavones or flavonols and recent research has established that the flavones are important co-pigments, being essential for the full expression of anthocyanin color in floral tissues. Mixtures of anthocyanins are also the rule, particularly in the flowers of ornamental plants, and any one flower tissue may contain up to ten different pigments.

### **FLAVONOLS AND FLAVONES**

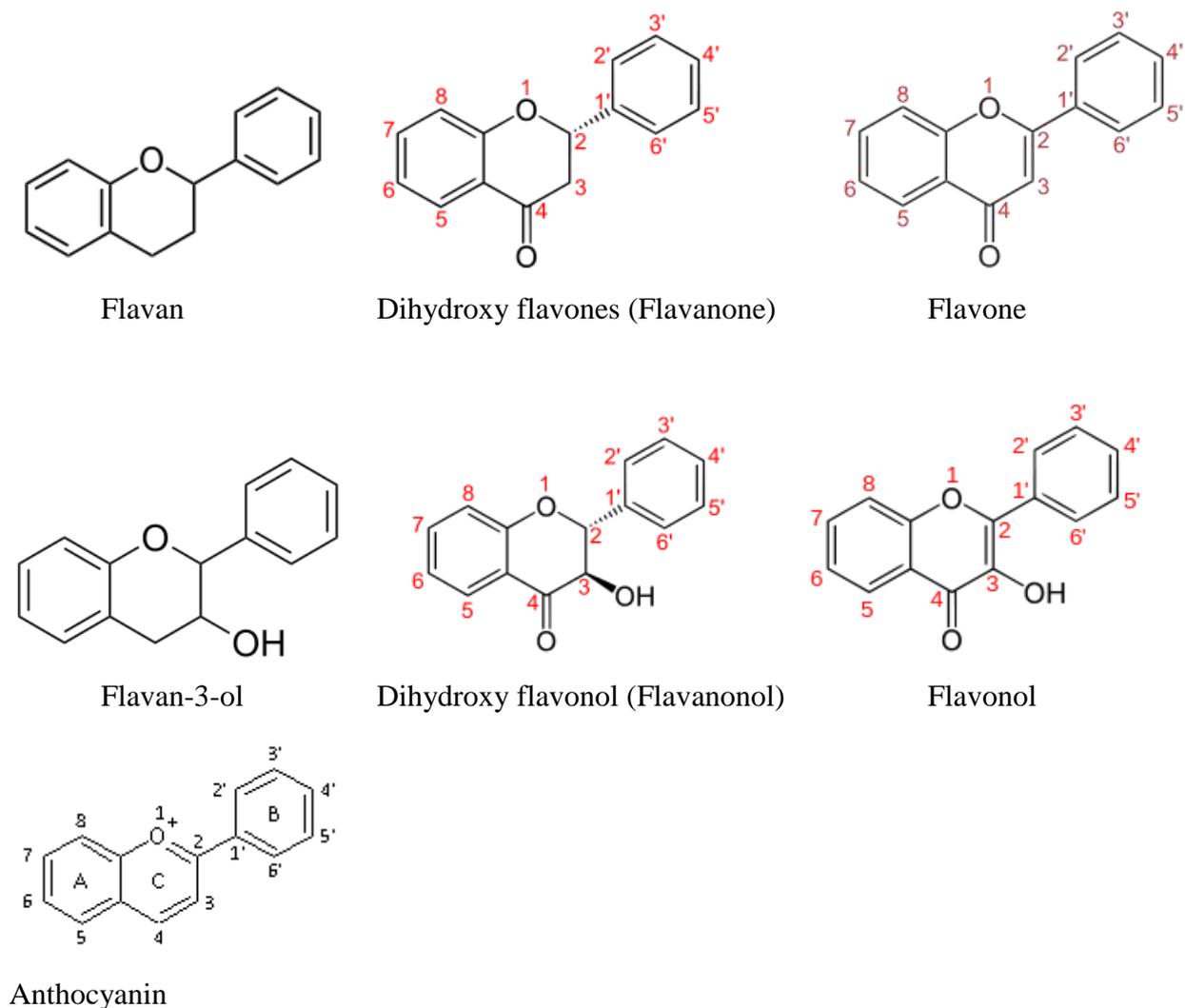
Flavonols are very widely distributed in plants, both as co-pigments to anthocyanins in petals and also in leaves of higher plants. Like the anthocyanins, they occur most frequently in glycosidic combination. Although two or three hundred flavonol aglycones are known, only three are at all common: kaempferol (corresponding in hydroxylation pattern to the anthocyanidin pelargonidin); quercetin (d. cyanidin) and myricetin (d. delphinidin). The other known flavonols are mostly simple structural variants on the common flavonols and are of limited natural occurrence. In the case of quercetin, a number of O-methylated derivatives are known, the 3'-methyl ether (isorhamnetin) and the 5-methyl ether (azaleatin) being but two examples. Addition of a hydroxyl in the 8-position to the structure of quercetin gives gossypetin, one of the few flavonols which are pigments in their own right, providing yellow flower color in the primrose, and in the cotton plant.

There is a considerable range of flavonol glycosides present in plants. More than two hundred different glycosides of quercetin alone have been described. By far the commonest is quercetin 3-rutinoside, known as rutin, which is of pharmaceutical interest in relation to the treatment of capillary fragility in man. Flavones only differ from flavonols in lacking a 3-hydroxyl substitution; this affects their UV absorption, chromatographic mobility and color reactions and simple flavones can be distinguished from flavonols on these bases. There are only two common flavones apigenin and luteolin, corresponding in hydroxylation pattern to kaempferol and quercetin. The flavone tricetin, corresponding to myricetin, is known but it is of very rare occurrence. More common are two methyl ethers: chrysoeriol, the 3'-methyl ether of luteolin, and tricetin, the 3',5'-dimethyl ether of tricetin.

Flavones occur as glycosides but the range of different glycosides is less than in the case of the flavonols. A common type is the 7-glucoside, exemplified by luteolin 7-glucoside. Flavones, unlike flavonols, also occur, remarkably, with sugar bound by a carbon-carbon bond. A series of such glycosyl flavones have been described, one example being orientin, the 8-C-glucoside of luteolin. The carbon-carbon bond is very resistant to acid hydrolysis, so that it is relatively easy to distinguish these C-glycosides from O-glycosides, which are more readily hydrolysed.

One other structural variant in the flavone series must be mentioned; the biflavonyls. These dimeric compounds are formed by carbon-carbon or carbon-oxygen coupling between two flavone (usually apigenin) units. Most also carry O-methyl substituents, a typical example being kayaflavone. Biflavonyls occur almost exclusively in the gymnosperms, but they have been found occasionally in angiosperms and methods for their identification are of general importance.

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers (Kar, 2007). The compounds are derived from parent compounds known as flavans. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other group of flavonoids include: flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones and catechin and leucoanthocyanidins.



**Figure 2** Structures of flavonoid classes