PERGAMON

Phytochemistry 55 (2000) 481–504

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Review

Advances in flavonoid research since 1992

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Received 13 January 2000; received in revised form 17 April 2000

Abstract

Some of the recent advances in flavonoid research are reviewed. The role of anthocyanins and flavones in providing stable blue flower colours in the angiosperms is outlined. The contribution of leaf flavonoids to UV-B protection in plants is critically discussed. Advances in understanding the part played by flavonoids in warding off microbial infection and protecting plants from herbivory are described. The biological properties of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Angiosperms; Flavonoids; Blue flower colour; UV-B protection; Medicinal properties

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PII: S0031-9422(00)00235-1

1. Introduction

Advances in flavonoid research over recent decades have been reviewed in a series of four volumes, beginning with Harborne et al. (1975) and culminating in Harborne (1994). Since then, reviews of new structures in the anthocyanin and flavonoid field and with the isoflavones have appeared (Donnelly and Boland, 1995; Harborne and Williams, 1995, 1998) as well as accounts of isoprenylated flavonoids (Tahara and Ibrahim, 1995; Barron and Ibrahim, 1996). A volume of short research reports and reviews on flavonoids and bioflavonoids was published in 1995 (Antus et al., 1995). An introduction to flavonoids has been published (Bohm, 1999). The only other major work to appear recently has been "*The Handbook of Natural Flavonoids*" (Harborne and Baxter, 1999). This is essentially a listing of 6467 known flavonoid structures, with formulae, references and information on biological activities.

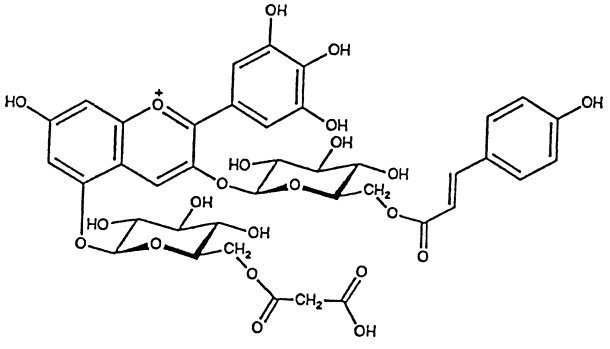
The purpose of the present review is to discuss recent developments in the biochemistry and medicinal aspects of the flavonoids. Much new information has accrued on the nature of the anthocyanin–flavone complexes that contribute to blue flower colour in several different plant families and it is appropriate to summarise these data here, since one of the best established functions of flavonoid pigments is in the production of flower colour and the provision of colours attractive to plant pollinators. By contrast with the very visible flavonoids in flower petals, the flavonoids present in leaves are completely hidden by the ubiquitous green of the chlorophylls. Nevertheless, there is increasing evidence that these flavonoids, particularly when they are located at the upper surface of the leaf or in the epidermal cells, have a role to play in the physiological survival of plants. Recent work on the UV-B protection provided by leaf flavonoids will be reviewed.

It is already well established that flavonoids make some contribution to disease resistance, either as constitutive antifungal agents or as phytoalexins. Some of the continuing research in this area will be mentioned. There is also increasing evidence that some flavonoids, and especially the flavolans or proanthocyanidins, provide defence against herbivory and some recent experiments in plant–animal interactions will also be mentioned.

Perhaps the most active area of flavonoid research at the present time is in the possible medicinal contribution that flavonoids make to human health. For example, the senior author contributed recently to a symposium entitled 'Wake Up to the Flavonoids' held in London, the proceedings of which are due to be published shortly. Recent research on the biological properties of flavonoids will therefore be a further subject of the present review.

2. Flavonoids and blue flower colour

Blue flower colour is usually due to the presence in the petals of an anthocyanin based on delphinidin. However, most delphinidin glycosides are mauve in colour and the shift to the blue region usually requires the presence of a flavone copigment, and occasionally of



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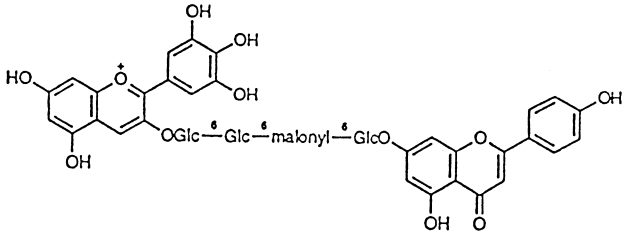

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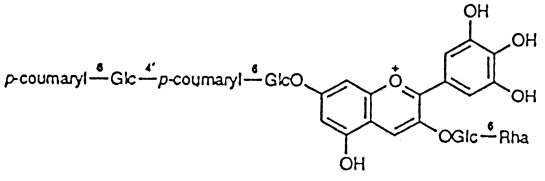

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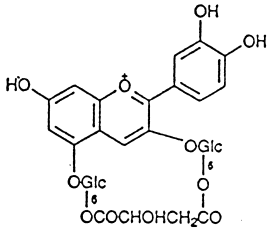

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one or more metal cations. Blue flower colour is the preferred attractant of bee pollinators, so that evolution towards blue colour is apparent in temperate floras where bee pollination is dominant. As Gottlieb (1982) has pointed out, blue flower colour is restricted to the more highly evolved angiosperm plant families. Thus, many more primitive families have floral anthocyanins based on cyanidin (in the red to magenta range) and this explains why families like the Rosaceae and genera like *Rosa* lack delphinidin-based blue flowers.

The chemical basis of blue flower colour was first extensively investigated in the case of *Commelina communis*. A blue complex called commelinin was shown to contain a delphinidin glycoside, malonylawobanin (**1**), a flavone copigment flavo-commelinin and two metals, iron and magnesium. X-ray crystallography showed unambiguously that the blue pigment, within the vacuole of the petal, consisted of a hydrogen-bonded complex of six molecules each of the anthocyanin and the flavone, together with one iron and one magnesium cation (Kondo et al., 1992). Since then, blue flowers of over 14 other plant species, drawn from a range of angiosperm families, have been investigated in detail (Table 1). We are now in a position to draw some

Table 1
Flavonoid pigment–copigment complexes in blue-flowered plants

Plant species	Pigment, copigment and metal[a]	Reference
Campanulaceae		
Campanula medium	Dp 3-rutinoside-7-(tri-*p*-hydroxybenzoyltriglucoside)	Brandt et al. (1993)
Compositae		
Centaurea cyanus	Succinylcyanin, apigenin 7-glucuronide-4′-malonylglucoside, Fe^{3+}, Mg^{2+} (6:6:1:1)	Bradley (1994)
Cichorium intybus	Dimalonyldelphin, unknown flavone copigment	Takeda et al. (1986)
Felicia amelloides	Dp 3-neohesperidoside-7-malonylglucoside, swertisin 2‴-rhamnoside-4′-glucoside (ratio 1:18)	Bloor (1999)
Senecio cruentus	Dp 3-malonylglucoside-7-dicaffeyldiglucoside-3′-caffeylglucoside	Goto et al. (1984)
Commelinaceae		
Commelina communis	Dp 3-(*p*-coumarylglucoside)-5(6-malonylglucoside), flavocommelinin, Fe^{3+}, Mg^{2+} (ratio 6:6:1:1)	Kondo et al. (1992)
Convolvulaceae		
Evolvolus pilosus	Dp 3-(dicaffeyltriglucoside)-5-malonylglucoside	Toki et al. (1994)
Pharbitis nil	Pn 3-(tricaffeylpentaglucoside)-5-glucoside	Goto (1987)
Hydrangeaceae		
Hydrangea macrophylla	Dp 3-glucoside, caffeylquinic acid, Al^{3+}	Takeda et al. (1985)
Labiatae		
Salvia patens	Dp 3-(*p*-coumarylglucoside)-5-malonylglucoside, apigenin 7,4′-diglucoside	Takeda et al. (1994)
Salvia uliginosa	Dp 3-(*p*-coumarylglucoside)-5-(4-acetyl-6-malonylglucoside), apigenin 7-cellobioside, apigenin 7-cellobioside-4′-glucoside	Veitch et al. (1998) Ishikawa et al. (1999)
Leguminosae		
Lupinus cv.	Dp 3-malonylglucoside, apigenin 7-malonylglucoside	Takeda et al. (1993)
Nymphaeaceae		
Nymphaea caerulea	Dp 3′-(galloylgalactoside), Dp 3′-(galloylacetylgalactoside) unknown flavone copigment	Fossen and Andersen (1999)
Papaveraceae		
Meconopsis betonicifolia	Cy 3-malonylsambubioside-7-glucoside, kaempferol 3-gentiobioside, kaempferol 3-xylosylgentiobioside (ratio 1:5:6)	Takeda et al. (1996)
Pontederiaceae		
Eichhornia crassipes	Dp 3-gentiobioside, apigenin 7-malonylglucoside	Taki et al. (1994)
Ranunculaceae		
Aconitum chinense	Dp 3-rutinoside-7-(di-*p*-coumaryldiglucoside)	Taki et al. (1994)
Delphinium hybridum	Dp 3-rutinoside-7-(tetra-*p*-hydroxybenzoylpentaglucoside)	Kondo et al. (1991)
Rhamnaceae		
Ceanothus papillosus	Dp 3-rutinoside-7-(*p*-coumaryl glucoside)-3′-glucoside, Dp 3-rutinoside, 7,3′-(di-*p*-coumarylglucoside), kaempferol 3-xylosyl (1→2) rhamnoside	Bloor (1997)

[a] Dp = delphinidin, Cy = cyanidin, Pn = peonidin. For simplicity, the location of acyl groups in the complex anthocyanins is omitted; normally *p*-coumaryl and malonyl residues are attached to relevant glucose units at the 6-position (see e.g. Harborne and Williams, 1998).

general conclusions about the role of flavonoids in the production of blue flower colour.

First of all, it is confirmed that delphinidin is the most common anthocyanidin in blue flowers (present in 15 of 18 species listed). This is in spite of the fact that two well known blue-flowered species, the cornflower *Centaurea cyanus* and the Morning Glory, *Pharbitis nil*, have cyanidin and peonidin glycosides respectively. These two exceptional species as well as the cyanidin-based blue flowered *Meconopsis betonicifolia* may be regarded as less effective in their production of blueness, as compared to the others. The spectral maxima of delphinidin glycosides of 535 nm (in MeOH) are nearer the blue region than are the maxima of cyanidin or peonidin glycosides (λ max 525 nm in MeOH). Hence, it requires less flavone copigment to be present to shift the spectrum to blue (λ max 580 nm) when delphinidin is the anthocyanidin present (Harborne, 1996). The regular identification of delphinidin derivatives in blue-flowered species (Table 1) agrees with the results of several earlier floral surveys, where delphinidin is associated with mauve and blue flower colour, cyanidin with magenta colour and pelargonidin with pink and orange colours (e.g. Saito and Harborne, 1992). The absence of methylated delphinidin derivatives (i.e. petunidin and malvidin) from all these blue flowers (Table 1) is noteworthy and agrees with earlier observations that malvidin in particular is chiefly associated with mauve to purple flower colour (Robinson and Robinson, 1934).

It is apparent from the data assembled in Table 1 that copigmentation is the most common mechanism for shifting the mauve colour of delphinidin glycosides towards blue. In fact, twelve of the eighteen species listed have copigments, and in eleven of these the copigment is a flavone or a flavonol. *Hydrangea* blue flowers are exceptional in having a simpler phenolic, caffeylquinic acid, as copigment (Takeda et al., 1985). While there may be several flavones accompanying a delphinidin glycoside in the petals of a blue-flowered species, there is usually only one specific flavone constituent which acts as a copigment. This is presumably related to the relative stabilities of the anthocyanin–flavone complexes that are formed. These anthocyanin–flavone complexes, where they exist (Table 1), have high flavone to anthocyanin ratios (e.g. 10:1), except when a metal cation is also present. One of the anthocyanin–flavone complexes, that in *Eichhornia crassipes*, is unique in that anthocyanin and flavone are covalently linked through a central malonic acid residue (**2**). A three dimensional structure, with the delphinidin and apigenin glycosides occupying a folding conformation as a binary complex, can be proposed, based on the observation of a negative Cotton effect at 535 nm (Toki et al., 1994).

The discovery that the blue pigment of *Commelina communis* has two metal cations, iron and magnesium, as essential components of the anthocyanin–flavone complex, suggested at the time that metal cations might be generally present in blue flowered species. This has, however, not been borne out by subsequent experimentation. In fact, the only comparable metal complex, closely similar to that of *Commelina*, is that of the blue cornflower, *Centaurea cyanus*, where the same two metals are present and where the same ratio (6:6:1:1) of anthocyanin to flavone to iron to magnesium occurs (Bradley, 1994). The only other clear example of a metal ion being required for blue flower colour is the case of the blue *Hydrangea macrophylla* where the metal is aluminium (Takeda et al., 1985).

Five of the 18 plant species listed in Table 1, namely *Campanula, Aconitum, Delphinium, Evolvulus* and *Pharbitis*, contain delphinidin or peonidin glycosides, with polyacyl substitution, and the shift to blue is simply achieved by 'intramolecular copigmentation'. Intramolecular stacking between anthocyanin and aromatic acyl groups is assumed to occur, thus stabilising the complex. In the case of *Aconitum* (**3**), *Campanula* and *Delphinium*, it may be significant that polyacylated glucose residues are present at the rarely substituted 7-hydroxyl group of delphinidin. The *Delphinium* pigment is remarkably stable in solution, remaining unchanged in neutral solution for more than one month (Kondo et al., 1991).

While the main emphasis of recent anthocyanin research has been the exploration of blue flower colour, some work has been carried out recently on the chemical basis of red–purple flower colour. Red–purple colours in the flowers of orchids have been shown to be derived from cyanidin and peonidin glycosides, with acylated sugars attached at both the 7- and 3′-positions. Intramolecular associations between these planar molecules provide stable colours without the need for any copigment or metal cation (Figueiredo et al., 1999). These authors have also explored the role of malonic acid residues, which are present in many anthocyanins. They appear to provide colour stabilisation, due to an increase in acidity in the vacuolar solution of the petal. The pKa of malonic acid is 2.83 and deprotonation of the malonyl group provides protection against alkanisation of the medium and hence loss of colour.

Finally, mention should be made of some experiments on red–purple and red flower colours in the carnation, *Dianthus caryophyllus*. Red–purple carnation flowers have yielded cyanidin 3,5-diglucoside-6″,6‴-malyl diester (**4**), the first macrocyclic anthocyanin ever to be characterised (Bloor, 1998). The corresponding pelargonidin 3,5-diglucoside-6″,6‴-malyl diester has been obtained from carnations with 'cyclamen red' colours, e.g. the cultivar Red Rox (Gonnet and Fennet, 2000). These two pigments are particularly unstable in acidic media and can only be extracted if neutral solvents are employed. In vivo, these two rare pigments appear to be stabilised by copigmentation with associated flavones,

as with the more familiar blue copigment complexes discussed above.

3. Flavonoids and UV-B protection in plants

Ultraviolet radiation is by convention divided into three bands, each with a different energy and with different ecological significance. Of these, UV-B (280–315 nm) is the band of lowest wavelength and highest energy. It can penetrate the ozone layer in the stratosphere and hence potentially cause damage to plant life. The concept of UV-B resistance in plants would explain the ability of plants to adapt to increasing amounts of UV-B that might reach the ground, e.g. from holes in the ozone layer. Resistance to UV-B may take many forms, but one type of resistance could lie in the flavonoid pigments, which are known to be almost universally present in green leaves. These flavonoids generally absorb in the 280–315 region and thus are capable of acting as UV filters, thereby protecting the underlying photosynthetic tissues from damage.

A number of early physiological experiments provided some circumstantial evidence that flavonoids, including anthocyanins, were involved in UV-protection. However, it is only within the last decade, that a series of experiments in different laboratories around the world have provided more convincing evidence that plants subjected artificially to UV-B radiation respond by changes in the pathway of flavonoid synthesis. Changes have been observed not only in the levels of flavonoids in epidermal cells of the adaxial leaf surface, but also in flavonoids in the leaf wax and in leaf hairs (Table 2).

It is clear that the response of individual plant species to harmful UV-B radiation can differ considerably in terms of flavonoid synthesis (see Table 2). At the same time, other detrimental effects may occur, including biomass reduction, decrease in pollen germination and reduction in photosynthetic activity (Murphy, 1997). Perhaps the most striking evidence supporting the idea that flavonoids are important in UV-B protection is that obtained in *Arabidopsis thaliana*, where mutants can be produced which lack the epidermal flavonoids of the wild type plant. These mutants become very sensitive to artificial UV-B radiation (Ormrod et al., 1995). It is interesting that *Arabidopsis* mutants which are blocked in the biosynthesis of related phenylpropanoids based on sinapic acid, are less affected by UV-B radiation (Chapple et al., 1992).

Although *Arabidopsis* is a specially favoured plant for genetic studies, mutant forms in other plants and especially in cereals can be obtained by appropriate experimentation. In maize for example, there are purple leaved (with anthocyanin) and green leaved cultivars. A study measuring the degree of DNA damage caused by UV-B radiation, showed that the purple strain did not suffer the induction of DNA damage produced in the green form (Stapleton and Walbot, 1994). Although the anthocyanin of maize leaves was not characterised in this work, it is possibly the same pigment, cyanidin 3-(6″-malonylglucoside), that has been identified in the seed coat (Harborne and Self, 1987). It presumably is able to provide UV-B protection in the same way that other flavonoids do, although the UV absorption of anthocyanins without aromatic acylation is around 280 nm.

Another cereal in which mutants affecting flavonoid synthesis exist is barley, *Hordeum vulgare*. Here, a mutant has been produced which contains only 7% of the flavonoids (mainly glycoflavones based on apigenin and luteolin) of the wild type. UV-B treatment of this mutant decreased the quantum yield of photosynthesis in

Table 2
Plant species in which UV-B protective flavonoids have been identified

Plant species	Flavonoid location	Protective flavonoids	Reference
Arabidopsis thaliana	Epidermal cells	Kaempferol 3-gentiobioside-7-rhamnoside and the 3,7-dirhamnoside	Ormrod et al. (1995)
Brassica napus (turnip)	Epidermal cells	Quercetin 3-sophoroside-7-glucoside, quercetin 3-sinapyl sophoroside-7-glucoside	Olsson et al. (1998)
Brassica oleracea (cabbage)	Epidermal cells	Cyanidin glycosides (and sinapyl esters)	Gitz et al. (1998)
Gnaphalium luteo-album	Leaf wax	Calycopterin and 3′-methoxycalycopterin	Cuadra et al. (1997)
Gnaphalium vira-vira	Leaf wax	7-O-methylaraneol	Cuadra and Harborne (1996)
Hordeum vulgare (barley)	Epidermal cells	Saponarin and lutonarin	Reuber et al. (1996)
Marchantia polymorpha	Thalli	Luteolin 7-glucuronide and luteolin 3,4′-diglucuronide	Markham et al. (1998a)
Oryza sativa (rice)	Epidermal cells	*Iso*-orientin acylated glucosides	Markham et al. (1998b)
Pinus sylvestris (Scots pine)	Epidermal cells	3″,6″-di-*p*-coumarylkaempferol 3-glucoside, 3″,6″-di-*p*-coumaryl-quercetin 3-glucoside	Schnitzler et al. (1996)
Quercus ilex	Leaf hairs	Acylated kaempferol glycosides	Skeltsa et al. (1996)
Sinapis alba (mustard)	Epidermal cells	Anthocyanin and quercetin glycosides	Buchholz et al. (1995)
Zea mays (corn)	Epidermal cells	Anthocyanin	Stapleton and Walbot (1994)

the plant. By contrast, the wild type plant photosynthesised normally and at the same time increased the amount of saponarin present by 30% and the amount of lutonarin produced by 500% (Reuber et al., 1996). Independent studies by Liu et al. (1995) confirmed that in normal barley leaves, large increases in glycoflavone synthesis occurs in both epidermal and mesophyll leaf tissue. Some increases in cell wall bound ferulic acid esters were also observed in lower epidermal tissues.

Another way of comparing plants which have and which lack flavonoid synthesis is to carry out similar experiments with plants treated with an inhibitor of phenylpropanoid production. This can be done with 2-amino-indan-2-phosphonic acid (AIP) at 50 μM. Treatment of red cabbage seedlings with AIP completely blocks anthocyanin synthesis but levels of sinapyl esters are unchanged. These treated plants were twice as sensitive as controls to UV-B damage, suggesting that the anthocyanins, and any co-occurring flavonol glycosides, serve as UV screens in young red cabbage plants (Gitz et al., 1998).

A more detailed analysis of the flavonol glycosides (Table 2) present in epidermal leaf cells of the related turnip *Brassica napus* has revealed a remarkably different response to UV-B treatment according to the structures of the flavonols present. The compounds present are the 3-sophoroside-7-glucosides and the related sinapyl esters of kaempferol and quercetin. UV-B treatment has no effect on levels of kaempferol glycosides, whereas 36- and 23-fold increases in the quercetin glycosides were recorded in the two cultivars Paroll and Stallion, respectively.

This shift in flavonol or flavone ratios has furthermore been noted in several other plants, including rice, *Oryza sativa, Petunia hybrida* and the liverwort *Marchantia polymorpha* (Table 2). In the case of rice, a UV-B tolerant cultivar produced increasing amounts of three iso-orientin glucosides on radiation, with only lesser amounts of isovitexin glycosides being formed. Significantly, a UV-susceptible cultivar failed to synthesis any of these flavonoids and also there was no enhancement in synthesis of other epidermal constituents. There is thus evidence in rice, as in *B. napus*, of a more subtle role for leaf flavonoids, over and above the simple UV-B screening. There is, in fact, an accumulation of 3′,4′-dihydroxyflavonoids, at the expense of 4′-hydroxyflavonoid synthesis. 3′,4′-Dihydroxyflavonoids (e.g. isoorientin, and quercetin glycoside) are capable of free radical scavenging and this may be the more important response to UV-damage in plants (Markham et al., 1998a,b).

How far the UV-B response in angiosperms is mirrored by increases in flavonoid synthesis in other plant groups is still uncertain. However, Markham et al. (1998a,b) have found that the liverwort, *Marchantia polymorpha*, responds in a similar way to rice and *B. napus*. The flavonoids present in the thalli are the 7-glucuronides and 7,4′-diglucuronides of apigenin (4′-hydroxyl) and luteolin (3′,4′-dihydroxy). On UV-treatments over 3 months, there was no significant increase in overall flavone production, but there was a large shift in the apigenin:luteolin ratio, with an increase in the proportion of luteolin glucuronides present.

The only gymnosperm to be examined so far is the Scots pine *Pinus sylvestris* (Schnitzler et al., 1996). Here the flavonols present are diacylated derivatives of kaempferol and quercetin 3-glucoside (Table 2). Both compounds increase in concentration after UV-B treatment, with the kaempferol derivative increasing in primary needles and the quercetin derivative increasing in cotyledonary needles. The kaempferol derivative is the major flavonol present and it accumulates to reach concentrations of 2.4 μmol g^{-1} f. wt. The quercetin derivative induced in cotyledonary needles reached a maximum of 0.8 to 0.9 μmol g^{-1}. Significantly, pulse labelling with L-[U-^{14}C] phenylalanine revealed that these flavonoids are formed de novo in the needles and cotyledons of young Scots pine seedlings.

While flavonoids are generally located in leaves as water soluble glycosides in the vacuoles of epidermal cells, they are also found less frequently on the upper leaf surface in the epicuticular wax. Such flavonoids are present in the free state (without glycosyl attachment), are very often *O*-methylated and are lipophilic. This *O*-methylation tends to shift the ultraviolet absorption properties to shorter wavelengths, so that they typically absorb significantly in the 250–320 nm region. Thus they are able to protect plant leaves from UV-B damage. In fact, studies in two *Gnaphalium* species of the family Compositae have suggested that they do indeed shield the leaf from damage by UV-B radiation. In *Gnaphalium vira-vira* plants there are two *O*-methylated flavones at the leaf surface: araneol (5,7-dihydroxy-3,6,8-trimethoxyflavone) and 7-*O*-methylaraneol. Twenty days of UV-B radiation increased the synthesis of the 7-methyl ether at the expense of araneol. Furthermore, UV-B radiation significantly increased the amount of the 7-methyl ether present from 0.42 to 0.52 μg 10 μg^{-1} surface extract (Cuadra and Harborne, 1996). In *Gnaphalium luteo-album*, the epicuticular flavonoids are gnaphaliin (5,7-dihydroxy-3,8-dimethoxyflavone), calycopterin (5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone) and 3′-methoxycalycopterin. Similar increases in surface flavonoids, as observed in *G. vira-vira*, also occurred in *G. luteo-album* after UV-B irradiation. In addition, increases in the internal UV-absorbing phenolics (chiefly caffeic acid esters) were also determined. After 21 days, there was a two-fold increase in these phenolics. This result indicates that resistance to UV-B damage can involve increases in the synthesis of flavonoids and/or related phenylpropanoids at both the leaf surface and in the epidermal cells.

One further site of flavonoid synthesis in leaves is in the leaf hairs or trichomes. Here again, the purpose of localising flavonoids at the leaf surface in the leaf hairs could be to provide resistance to damaging UV-B radiation. Skaltsa et al. (1994) claim that acylated flavonol glycosides present in the leaf hairs of *Quercus ilex* affords the plant useful protection against the damage of UV-B radiation. The key experiment here was to measure the photosynthetic efficiency of dehaired leaves. Indeed, there is a considerable reduction in photosystem II photochemical efficiency in treated leaves.

In summary then, it is possible to conclude that plant species vary in their ability to resist the damaging effects of UV-B radiation. Resistant genotypes in general all show significant increases in flavone or flavonol synthesis in epidermal cells and occasionally also in epicuticular waxes. In some cases, there is a striking shift in the pathway of synthesis so that 3′,4′-dihydroxyflavonoids accumulate at the expense of 4′-hydroxyflavonoids. The flavonoids most frequently cited as being UV-protective (Table 2) are flavone or flavonol glycosides having hydroxycinnamyl acylation linked through sugars. This, however, is not surprising because it is precisely such substituted flavonoids that absorb most strongly in the 280–320 nm region and thus are the most effective UV filters.

4. Antimicrobial flavonoids

One of the undisputed functions of flavonoids and related polyphenols is their role in protecting plants against microbial invasion. This not only involves their presence in plants as constitutive agents but also their accumulation as phytoalexins in response to microbial attack (Grayer and Harborne, 1994, Harborne, 1999b). Because of their widespread ability to inhibit spore germination of plant pathogens, they have been proposed also for use against fungal pathogens of Man. There is an ever increasing interest in plant flavonoids for treating human diseases and especially for controlling the immunodeficiency virus which is the causative agent of AIDS. Here, it is intended to review some recent work on plant–fungal interactions and then survey the literature on the characterisation of various plant flavonoids as antifungal, antibacterial or antiviral agents.

The isoflavonoid maackiain (3-hydroxy-8,9-methylenedioxypterocarpan) is well known as a constitutive antifungal agent in heartwood of legume trees and as an inducible phytoalexin in herbaceous legumes, such as *Pisum sativum* and *Trifolium* spp. Stevenson and Haware (1999) have now claimed it to be both constitutive and inducible in the plant *Cicer bijugum*, a wild relative of the chickpea *C. arietinum*. Thus, two strains of *C. bijugum*, resistant to *Botrytis cinerea* infection, contain 200–300 μg g^{-1} of foliage. By comparison, species of *Cicer* susceptible to *Botrytis* attack such as *C. arietinum* contain less than 70 μg g^{-1}. Moreover, the concentration of maackiain increased in *C. bijugum* to 400 μg g^{-1} after inoculation with *B. cinerea*. Such a concentration of maackiain severely inhibits spore germination in this fungus. These authors conclude that maackiain is an important component of fungal resistance in wild chickpea which is enhanced in the presence of the pathogen (Stevenson and Haware, 1999).

Another well known legume phytoalexin is mucronulatol (7,3′-dihydroxy-2′,4′-dimethoxyisoflavan) which is formed in *Astragalus* spp in response to fungal infection. Martin et al. (1994) have surveyed 41 populations of *Astragalus cicer* for the induction in leaves of mucronulatol, a related isoflavan, two isoflavones and the pterocarpan maackiain. All five compounds were generally produced, but the concentrations formed differed 12-fold. No relationship between isoflavonoid production and geographical origin could be established for this plant.

The majority of flavonoids recognised as constitutive antifungal agents in plants are either isoflavonoids, flavans or flavanones. The recognition that a flavone glycoside, namely luteolin 7-(2″-sulphatoglucoside), is an antifungal constituent of the marine angiosperm *Thalassia testudinum* is noteworthy (Jensen et al., 1998). This plant suffers periodic infections caused by zoosporic fungi such as *Schizochytrium aggregatum*. However, whole leaf concentrations of the flavone glycoside reach 4 mg ml^{-1} wet tissue, which is more than sufficient to reduce growth of the above fungus by 50%. The fact that the flavone is present in a water soluble form as a sulphate suggests that it may also be excreted from the plant to ward off fouling microorganisms in the marine environment.

The presence of a phenolic group in a natural flavonoid would be expected to provide antimicrobial activity and the addition of further phenolic groups might be expected to enhance this activity. Testing the effect of various flavonoids on mycelial growth in the fungus *Verticillium alba-atrum*, a pathogen of several serious wilt diseases, showed exactly the opposite was true. The most inhibitory compounds were the parent structures, flavone and flavanone, which were active at 1 and 5 ppm, respectively. Normal hydroxyflavonoids only inhibited growth in concentrations above 5 ppm and some were ineffective even at 200 ppm. In fact, increasing the number of hydroxyl, methoxyl or glycosyl substituents resulted in the steady loss of antifungal activity (Picman et al., 1995).

Experiments with other plant fungi suggest that *V. albo-atrum* may be exceptional in its response to hydroxy/methoxy substitution in the flavonoid series and there are many examples of antifungal flavonoids with such substituents. For example, the two chalcones present in leaves of *Myrica cerrata* inhibit the growth of

Cladosporium cucumerinum. They are 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethyl- and 2′,4′-dihydroxy-6′-methoxy-5′-methylchalcone (Gafner et al., 1996). Again, two new flavans characterised from the sedge, *Mariscus psilostachys*, are also inhibitory on *C. cucumerinum*. They are (2*S*)-4′-hydroxy-5,7,3′-trimethoxy- and (±)-5,4′-dihydroxy-7,3′-dimethoxyflavan (Garo et al., 1996).

Several recent papers report the regular presence of antibacterial activity among flavonoids. Thus, the retrochalcone licochalcone C (4,4′-dihydroxy-2′-methoxy-3′-prenyl) is active against *Staphylococcus aureus* with a minimum growth inhibitory concentration (MIC) of 6.25 μg ml^{-1} (Haraguchi et al., 1998). Also, the compound 5,7-dihydroxy-3,8-dimethoxyflavone has an MIC of 50 μg ml^{-1} towards *Staphylococcus epidermis* (Iniesta-Sanmartin et al., 1990). Again, the substance 5,7,2′,6′-tetrahydroxy-6-prenyl-8-lavandulyl-4′-methoxyflavanone completely inhibits the growth of *S. aureus* at concentrations between 1.56 and 6.25 μg ml^{-1} (Iinuma et al., 1994). The above flavanone is particularly active against antibiotic-resistant strains of *S. aureus* and could have value in treating patients, who inadvertently pick up this infection while in hospital.

Yet one further property of flavonoids that has been researched recently has been antiviral activity, most notably against the human immunodeficiency virus (HIV), the causative agent of AIDS. Some flavonoids appear to have direct inhibitory activity on the virus. This is apparently true of baicalin (5,6,7-trihydroxyflavone 7-glucuronide) from *Scutellaria baicalensis* (Li et al., 1997). Other flavonoids are inhibitory to enzymes required for viral replication. The two biflavones, robustaflavone and hinokiflavone, are active against HIV-1 reverse transcriptase with IC_{50} values of 65 μM (Lin et al., 1997b). Also, quercetin 3-(2″-galloylarabinopyranoside) isolated from *Acer okamatoanum*, is active against HIV-1 integrase with an IC_{50} value of 18.1 μg ml^{-1} (Kim et al., 1998).

It is not yet clear what range of flavonoids have anti-HIV activity. However, a study of the inhibition of tomato ringspot virus by flavonoids revealed that a range of common flavonols and an aurone were all strongly active. In fact, quercetin applied at a concentration of 5 μg ml^{-1} caused 70% inhibition of local lesion development of the virus on the test plant *Chenopodium quinoa*. Quercetin and the other flavonoids appear to interfere with an early event in the virus life cycle (Malhotra et al., 1996).

5. The role of flavonoids in plant–animal interactions

It is now generally accepted that flavonoids, along with other plant polyphenols, play a role in protecting plants from both insect and mammalian herbivory. In recent years, attention has been mainly centred on simple phenolic constituents or on the polymeric flavolans or proanthocyanidins (Harborne, 1995, 1999a), but some research has been concerned with low molecular weight flavones, flavonols and isoflavones. For example, three glycoflavones schaftoside, isoschaftoside and neoschaftoside have been identified in the phloem sap of rice plants, where they act as sucking deterrents to the pest insect, the brown plant hopper *Niloparvata lugens*. High levels of these glycoflavones are present in resistant cultivars of rice and when tested at these concentrations on plant hoppers, they exhibited an ingestion inhibiting activity (Grayer et al., 1994).

Another pest of the rice plant is the stem nematode *Ditylenchus angustus*, which is a particular problem on the crops growing in SE Asia. Again, a flavonoid and a related phenylpropanoid in the leaves have been recognised as providing resistance to nematode attack. Thus, the flavanone sakuranetin and the phenylpropanoid chlorogenic acid both increase in concentration in the leaves in response to nematode infection. After five days of inoculation of a resistant cultivar with the nematode, the concentrations of sakuranetin reached between 8 and 13 μg g^{-1} leaf. No changes in secondary chemistry occurred in a susceptible cultivar of rice (Plowright et al., 1996). It may be observed that the same flavanone, sakuranetin, is formed in rice in response to UV-irradiation or to fungal infection and hence is also involved, in part, in protecting rice plants from plant diseases (Dillon et al., 1997).

While most Lepidoptera feeding on green leaves are adapted to the flavonoids that are present in their food plants, there is evidence that several generalist feeders are sensitive to their dietary flavonoids. This is true of *Helicoverpa zea* and *Heliothis virescens* (see e.g. Harborne and Grayer, 1994). It has now been shown to be true also for the gypsy moth *Lymantria dispar* and for the cabbage looper, *Trichoplusia ni*. Experiments with the gypsy moth indicate that it is sensitive to flavonol glycosides in its diet, especially at the second instar larval stage. For this reason, it does not feed on pine needles, until later instars. Thus, a purified fraction of flavonol glycosides from *Pinus banksiana* significantly reduced growth and increasing mortality of gypsy moth larvae at the second instar stage. Similarly, when rutin and quercetin 3-glucoside were incorporated into an artificial diet, they significantly reduced growth of second instars (Beninger and Abou-Zaid, 1997). Again, with larvae of *Trichoplusia ni*, flavonoid extracts of soya bean leaves, *Glycine max*, affected survival, fresh larval and dry pupal weight, as well as feeding time. Comparative experiments with pure rutin indicated that the mixture of two flavonol glycosides (rutin and quercetin 3-glucosylgalactoside) with the isoflavone genistin present in soya bean acted synergistically in disrupting the consumption and assimilation of plant material by the insect (Hoffmann-Campo, 1995).

Under the right circumstances, flavonol glycosides can be phagostimulants to insects as well as feeding deterrents. There is evidence that quercetin 3-glucoside, which occurs in the pollen of sunflower, *Helianthus annuus*, is phagoactive for the western corn rootworm *Diabrotica virgifera*, which feeds on this pollen. However, it is only one of a number of phagostimulants present in sunflower pollen, and the lipid constituents of the pollen are considerably more active than the flavonol glucoside (Lin and Mullin, 1999).

Insects feeding on green plants are clearly sensitive to the flavonoids present, as has been well established by numerous feeding experiments (e.g. Bernays and Chapman, 1994) and by the experiments outlined above. A similar sensitivity to leaf flavonoids may be shown by the adult female butterfly or moth when choosing a suitable food plant for oviposition. Several swallowtail butterflies, feeding on Rutaceae or Umbelliferae host plants, have been found previously to require flavonol, flavone or flavanone glycosides as oviposition stimulants (Harborne, 1997). Now it has been demonstrated that the well known danaid butterfly *Danaus plexippus*, which has *Asclepias* spp. as the major food plants, is dependent on the flavonol glycosides present in the leaf for oviposition stimulation. A mixture of four flavonol glycosides — two 3-dirhamnosyl glycosides, the 3-rutinoside and the 3-rhamnosyl galactoside of quercetin — act together in *Asclepias curassavica* to attract the adult female butterfly to oviposit (Haribal and Renwick, 1996). This dependence on the flavonols, rather than the cardiac glycosides present which are actually taken up by the larvae during feeding, also extends to other host plants utilised in the Asclepiadaceae. There are three main classes of quercetin glycoside that may be encountered: (1) glycosides based on galactose, glucose and xylose; (2) glycosides based on galactose, glucose and rhamnose; and (3) glycosides based on all four sugars. The key feature for oviposition would appear to be a quercetin 3-galactoside with additional sugars attached to the 2″- and/or 6″-positions of the galactose (Haribal and Renwick, 1998).

Isoflavones are another class of flavonoid capable of interacting with phytophagous insects. Earlier investigations have shown that the isoflavones of clover are feeding deterrents to the beetle *Costelytra zealandica*, which attacks the roots of legumes (Sutherland et al., 1980). It has now been demonstrated that the isoflavones in leaves of *Trifolium subterraneum* provide resistance to feeding by the redlegged earth mite, *Halotydeus destructor* (Wang et al., 1998a). The free isoflavones genistein, formononetin and biochanin A, are active at concentrations between 0.05 and 0.15%, whereas the corresponding glucosides and malonylglucosides are less active and must be present at 0.5% to have any effect on feeding. Notably, genistein showed 93% deterrence at 0.08%, 68% deterrence at 0.045% but attractance to the mite at 0.01%. Thus, a feeding attractant becomes a deterrent as the concentration in the leaf increases. The active free isoflavones are not surprisingly located at the leaf surface, where they can interact immediately with the earth mite. Resistance to mite attack is directly correlated in subterranean clover cultivars with significant amounts of free isoflavones present on leaf surfaces (Wang et al., 1999a).

The role of the flavolans or proanthocyanidins in defending plants from herbivory has been reviewed extensively earlier (see e.g. Harborne, 1995, 1997, 1999a). Here, it is appropriate to mention three recent case studies, where proanthocyanidins are defensive, partly defensive or lack defensiveness. The first case refers to a study of procyanidin levels in leaf bud petioles of groundnut *Arachis hypogaea*. A strong negative correlation was established between the concentrations of procyanidin and the fecundity of the groundnut aphid, *Aphis craccivora*, feeding on the phloem of different genotypes. Thus resistant genotype EC 36892 contained the most procyanidin per weight of fresh petiole (*c*. 0.7%) and aphids feeding on it produced only half the offspring of aphids reared on genotypes with low procyanidin levels. It should also be noted that procyanidin is specifically located in the bud petioles of groundnut, where the aphids feed, and is essentially absent from the rest of the plant (Grayer et al., 1992).

The second case study refers to the amounts of condensed tannin (mainly procyanidins) and of sugars in the diet of chimpanzees living in the Budongo Forest of Uganda (Reynolds et al., 1998). Earlier studies of monkey feeding in Africa indicated a significant rejection of high tannin-containing plant species. The same is not true for chimpanzees, who appear to be able to tolerate much higher levels of tannin in their diet than monkeys or marmosets. Nevertheless, when eating the fruit of wild figs, chimpanzees rejected the seeds, which have high levels of tannin, and spit them out as a 'wadge' or oral boli. Also, when eating leaves, they tended to choose young leaves with lower tannin levels than mature leaves with higher levels. Otherwise, selection of plant foods depended more on the level of free sugars present than on the condensed tannin levels.

Examples of plants where proanthocyanidin levels might be expected to deter herbivory are the two *Eucalyptus* trees, *E. ovata* and *E. viminalis*. These are food plants of the ringtail possum and the koala bear in Australia. Ecological investigations reveal considerable variations in the amounts of leaf consumed of individual trees of the same species, caused apparently by some feeding deterrent. However, there were no correlations between feeding and nutritional quality or total tannin content. The problem was solved by bioassay, which showed that two simple phloroglucinol-based

phenolics, macrocarpol G and jensenone, are strongly antifeedant (Lawler et al., 1998). Subsequently, in feeding experiments with an artificial diet, it was found that a concentration of 2.1% macrocarpal G was sufficient to cause 90% reduction in voluntary food intake by the ringtail possum (Pass et al., 1998).

An important adaptation in mammals to a diet containing condensed tannin is the production of proline-rich proteins in the saliva. These proteins have a strong affinity for the dietary tannins and bind to them in the mouth and the hydrogen bonded complex passes through the stomach without causing any damage. This adaptation is variably present in herbivorous animals, but is absent from carnivores. Recent experiments have shown that the root vole *Microtus oeconomus* and the moose can be added to the list of mammals that secrete PR-proteins in the saliva.

The root vole, *Microtus oeconomus*, which lives in meadowland habitats in N. Finland has been shown to produce salivary tannin-binding protein. This is an adaptation to winter feeding, when it is forced to feed on the bark of birch and other deciduous trees. Incorporation of 0.1% birch tannin in vole diet did not affect the protein secretion, indicating that this adaptation is constitutive in this vole. By contrast with this European vole, it may be noted that two North American species *Microtus ochragaster* and *M. pennsylvanicus* are not able to produce the right salivary proteins for tannin binding (Juntheikki et al., 1996).

Related experiments on the moose from Scandinavia and from North America showed that both animals constitutively produce tannin-binding proteins in the saliva to allow them to feed on twigs and barks of a variety of trees and shrubs (Juntheikki, 1996). However, the tannin-binding capacity is restricted to condensed tannins and the proteins in the saliva do not complex with hydrolysable tannins. This means that they cannot eat tissue of *Rubus* and *Alnus*, which have both classes of tannin present. In fact, moose do avoid eating them. By contrast, the North American deer *Odocoileus hemianus* eats more widely than the moose because their salivary proteins bind both hydrolysable and condensed tannin (Hagerman and Robbins, 1993).

6. Medicinal properties of flavonoids

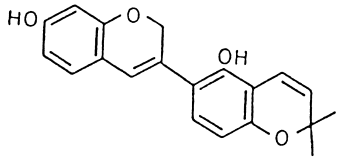
6.1. Antioxidant activity of flavonoids

Flavonoids have been shown to act as scavengers of various oxidising species i.e. superoxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals. They may also act as quenchers of singlet oxygen. Flavonoids do not react specifically with a single species and so a number of different evaluation methods have been developed which makes comparison of the various studies very difficult. Often an overall antioxidant effect has been measured. However, Tournaire et al. (1993) have developed an improved method to compare the antioxidant activity of 13 selected flavonoids from different classes by measuring the quantum yields of sensitised photo-oxidation of individual flavonoids. This was coupled with determination of photo-oxidation based on measuring the singlet oxygen luminescence. They concluded that the presence of a catechol moiety in the B-ring is the main factor controlling the efficiency of $^1O_2$ physical quenching (k_q) of flavonoids and the presence of a 3-hydroxyl largely determines the efficiency of their chemical reactivity with $^1O_2$(k_r). k_q is generally higher than k_r.

Previous workers (e.g. Das and Pereira, 1990) have shown that a carbonyl group at C-4 and a double bond between C-2 and C-3 are also important features for high antioxidant activity in flavonoids. Butein and other 3,4-dihydroxychalcones are more active than analogous flavones because of their ability to achieve greater electron delocalisation (Dziedzic and Hudson, 1983). Similarly, isoflavones are often more active than flavones because of the stabilising effects of the 4-carbonyl and 5-hydroxyl in the former (Dziedzic and Hudson, 1983). In the antioxidant action of o-dihydroxyflavonoids metal chelation is an important factor (Shahidi et al., 1991).

In most recent reports the antioxidant activity has been measured using a lipid peroxidation assay. Rios et al. (1992) found that hypolaetin 8-glucoside (8-hydroxyluteolin 8-glucoside) was the most potent inhibitor of non-enzymic lipid peroxidation amongst the flavone glycosides in the aerial parts of *Sideritis javalambrensis* (Labiatae). The root extract of another *Sideritis* species, *S. baicalensis*, showed high, concentration dependent, antioxidant activity in lecithin liposome membranes irradiated with UV light (Gabrielska et al., 1997). The three major flavonoid components: wogonin (5,7-dihydroxy-8-methoxyflavone), baicalein (5,6,7-trihydroxyflavone) and its 7-glucuronide (baicalin) were tested for their antioxidant activity. Baicalin was the most active compound with the highest (72%) inhibition of oxidation and represented 75% of the flavone fraction in the extract. Thus, the presence of a glucuronide moiety at C-7 seems to significantly increase antioxidant activity. In tart cherries the anthocyanidin, cyanidin and its 3-glucoside, 3-rutinoside and 3-(2^G-rutinoside) are the major antioxidant constituents with activities comparable to those of *tert*-butylhydroquinone and butylated hydroxytoluene and superior to vitamin E at 2-μM concentrations. In the USA, tart cherries are now incorporated into meat products to reduce the development of rancidity (Wang et al., 1999b). A number of different classes of flavonoid contribute to the antioxidant activity of licorice, *Glycyrrhiza glabra*. Lichochalcone A (4′,4-dihydroxy-2-methoxy-5-C-prenylchalcone) and lichochalcone B (4′,3,4-hydroxy-2-methoxychalcone) have an

antioxidant activity comparable to that of vitamin E, whereas the isoflav-3-ene, glabrene (**5**) is three times as active (Okuda et al., 1989). In a more recent study, 2′,4′,7-trihydroxy-3′-prenyl-3-arylcoumarin was found to have a protection factor of 2.7 compared with 6.2 for alpha-tocopherol (Gordon and An, 1995). These workers also suggested that synergistic effects of flavonoid mixtures may be responsible for the high activity observed in crude extracts. Another member of the Leguminosae, *Lespedeza homoloba*, is also very rich in isoflavonoids, a number of which have significant antioxidant activity. In a recent study Miyase et al. (1999a) have identified eight new phenolic compounds of which three had strong activity against lipid peroxidation in the rat brain homogenate test: the isoflav-3-enes, lespedozols A_2 (3,8,9-trihydroxy-10-geranylpterocarp-6a-en) and A_3 (**6**) and the 2-geranylbenzofuran, lespedezol B_2 (**7**). In a further analysis of the same plant Miyase et al. (1999b) tested 15 new isoflavonoids for their antioxidant activity and concluded that those compounds containing a catechol group showed the strongest activity against lipid peroxidation in the rat brain homogenate together with superoxide anion scavenging activity. However, the effects of a geranyl and isoprenyl side chain, present in these isoflavonoid structures, on these activities was not clear. In a different assay the flavonoids: quercetin, kaempferol, catechin and taxifolin, were shown to suppress the cytotoxicity of O_2^- and H_2O_2 on Chinese



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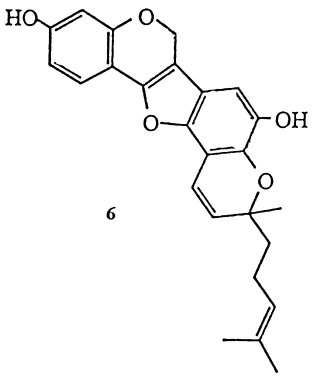

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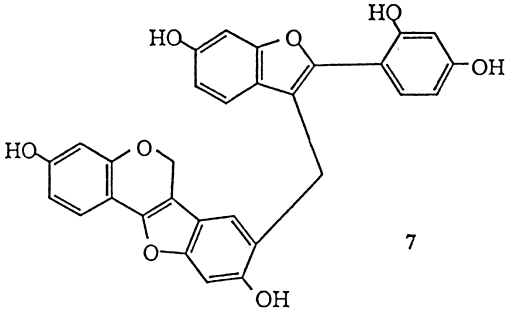

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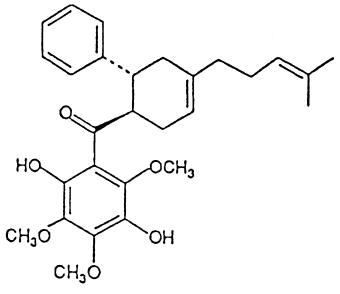

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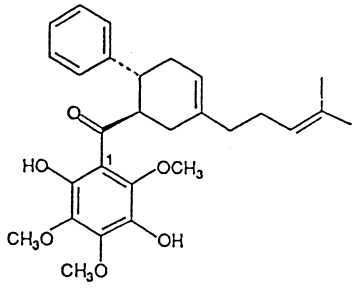

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hamster V79 cells in a protective manner i.e. by preventing the decrease in the number of colonies at concentrations at which the compounds themselves were not toxic (Nakayama et al., 1993). However there was a large difference in the dose dependency of the protective effects of quercetin and kaempferol compared with those brought about by catechin and taxifolin. Thus, the two flavonols showed protective effects at concentrations above 5 μM while much higher concentrations of both catechin and taxifolin were necessary to prevent the cytotoxicity of H_2O_2.

Another possible contributory mechanism to the antioxidant activity of flavonoids is their ability to stabilise membranes by decreasing membrane fluidity. Indeed, the results of recent study of this phenomenon showed that a series of representative flavonoids partition into the hydrophobic core of the membrane, causing a dramatic decrease in lipid fluidity in this region of the membrane (Arora et al., 2000).

6.2. Inhibition of enzymes by flavonoids

In a number of structure–activity studies, flavonoids have been tested for their ability to inhibit key enzymes in mitochondrial respiration. It was found that a C2,3-double bond, a C4-keto group and a 3′,4′,5′-trihydroxy B-ring are significant features of those flavonoids which show strong inhibition of NADH-oxidase. In a recent comparison of flavonoids with varied hydroxylation/methoxylation patterns (Hodnick et al., 1994) the order of potency for inhibition of NADH-oxidase activity was robinetin, rhamnetin, eupatorin, baicalein, 7,8-dihydroxyflavone and norwogonin with IC_{50} values of 19, 42, 43, 77, 277 and 340 nmol/mg protein, respectively. These workers also showed that flavonoids with adjacent trihydroxyl or *p*-dihydroxyl groups exhibited a substantial rate of auto-oxidation which was accelerated by the addition of cyanide.

Some flavonoids also inhibit the enzyme xanthine oxidase, which catalyses the oxidation of xanthine and hypoxanthine to uric acid. During the re-oxidation of xanthine oxidase both superoxide radicals and hydrogen peroxide are produced. In a structure–activity study, Cos et al. (1998) found that flavones showed higher inhibitory activity than flavonols and that hydroxyl groups at both C-3 and C-3′ were essential for high superoxide scavenging activity. The flavonoids could be classified into groups according to their ability to inhibit xanthine oxidase and/or scavenge for superoxide radicals or show no activity.

6.3. Dietary antioxidant flavonoids and coronary heart disease

Antioxidant flavonoids are naturally present in fruits, vegetables, tea and wine and have been found in vitro to inhibit oxidation of low-density protein (LDL). In such in vitro studies with the phenolic constituents of red wine Frankel et al. (1993a) found that red wine inhibits the copper-catalysed oxidation of LDL. Wine diluted 1000 times to contain 10 mol/l of phenolics had the same antioxidant activity as 10 mol/l of quercetin in inhibiting LDL oxidation whereas α-tocopherol only showed 60% of the activity of wine or quercetin. These authors concluded that it was the non-alcoholic components which were responsible for the activity of the wine. In most countries a high intake of saturated fats is strongly correlated with high mortality from coronary heart disease (CHD), but this is not the case in some regions of France; the so called ''French paradox''. This anomaly has been attributed to the regular intake of red wine in the diet. The concentration of phenols is higher in red than in white wines because they are present mainly in the grape skins which are removed in the production of white wine. The major flavonoid constituent of red wine is catechin with a concentration of *c*. 190 mg/l. Other phenolic constituents include: gallic acid (95 mg/l), epicatechin (82 mg/l), malvidin 3-glucoside (24 mg/l), rutin (9 mg/l), myricetin (8 mg/l), quercetin (8 mg/l), caffeic acid (7 mg/l), cyanidin (3 mg/l) and resveratrol (1.5 mg/l) (Frankel et al., 1993b). Two of the non-flavonoid constituents of red wine: resveratrol (3,4,5′-trihydroxystilbene) and its glucoside, have also been considered as LDL oxidation inhibitors because they have been reported to be the active components of *kojo-kon*, an oriental folk medicine (Kimura et al., 1985). Indeed addition of 10 mol/l of resveratrol to the dietary intake of two healthy volunteers did inhibit copper-catalysed oxidation of human LDL by 81 and 70%, respectively compared with 61 and 48% inhibition for 1000-fold diluted red wine (Frankel et al., 1993b). However, epicatechin and quercetin had *c*. twice the antioxidant potency of resveratrol. To put this in perspective, 10 mol/l of α-tocopherol,which has been linked to a reduction in CHD, gave only 40 and 19% inhibition, i.e. lower than for red wine, resveratrol, quercetin or epicatechin. The concentration of epicatechin and its isomers typically exceeds 15 mg/l in white wine and 150 mg/l in red wine whereas the concentration of resveratrol is usually below 1 mg/l. These authors concluded that epicatechin and quercetin are more important wine constituents than resveratrol in reducing CHD and support a previous suggestion that it is the combination of antioxidant phenolics in wine that may protect against atherogenesis with regular long term consumption.

In another dietary survey, the ''Zutphen Elderly Study'' the flavonoid intake of a sample of men (complete information on diet and risk factors for 805) aged 65–84 years old from Zutphen in the eastern Netherlands, was considered in relation to the incidence of CHD over a period of 25 years (Hertog et al., 1993a).

The mean baseline intake of flavonoids was 25.9 mg per day and the major sources of intake were tea (61%), onions (13%) and apples (10%). Flavonoid intake was found to be inversely associated with mortality from CHD. Intakes of tea, onions and apples were also inversely related to CHD but the associations were weaker. The authors concluded that regular consumption of flavonoid-rich foods may reduce the risk of death from CHD in elderly men. However, the absorption of catechins from tea or any other flavonoids in humans has only begun to be investigated. Marked differences in absorption rate and bio-availability have been found in pharmacokinetic studies with dietary quercetin glycosides. The bio-availability of quercetin 3-xyloside, rhamnoside, arabinoside and galactoside in apples and the pure 3-rutinoside was one third of that for the quercetin glycosides present in onions (Hollman et al., 1997a). Another study using pure quercetin glycosides indicated that the presence of a glucose moiety was important in increasing the rate and extent of absorption (Hollman et al., 1999). Hollman suggests that this could be explained by the absorption of quercetin in the small intestine and the absorption of rutin in the colon only after the removal of the rhamnose by bacterial hydrolysis of the sugar bond (Hollman et al., 1999). The peak levels for catechins in humans is reached after *c*. 2 h (Hollman et al., 1997b) with elimination half-lives of 3–5 h compared with 24 h for quercetin (Hollman et al., 1997a,b). The absorption of flavonoids could be affected by their ability to bind to proteins but the addition of milk to tea did not quantitatively affect the catechins or quercetin detected in plasma (Hollman et al., 1997b). The role of dietary antioxidant flavonoids in protecting against CHD has been more widely reviewed by Leake (1997).

6.4. Flavonoids with anti-inflammatory activity

Flavonoids may inhibit the cyclo-oxygenase and/or the 5-lipoxygenase pathways of arachidonate metabolism. Among the recent reports, Williams et al. (1995) found that the major surface flavonoid of feverfew (*Tanacetum parthenium*) inhibited both enzymes with similar potency when using rat leukocytes activated by the calcium ionophore A 23187. This active compound was first identified as 6-hydroxykaempferol 3,7,4′-trimethyl ether and named tanetin. However, after further NMR spectroscopic studies the structure was revised to santin, the known 3,6,4′ trimethyl ether isomer (Williams et al., 1999). Santin may contribute to the well known anti-inflammatory activity of this plant. In a later study the leaf surface flavonols of feverfew were compared with the leaf surface flavones of the related plant, tansy (*T. vulgare*). Two further flavonols were tested from feverfew: 6-hydroxykaempferol 3,6-dimethyl ether, which gave a similar enzyme profile to santin and quercetagetin 3,6,3′-trimethyl ether, which showed preferential activity against cyclo-oxygenase. Two of the tansy flavones: 6-hydroxyluteolin 6-methyl ether and its 6,3′-dimethyl ether, were found to inhibit both the cyclo-oxygenase and 5-lipoxygenase pathways but were less active as cyclo-oxygenase inhibitors than the corresponding flavonols. These results support previous findings (Moroney et al., 1988) that compounds containing vicinal diols make the most active 5-lipoxygenase inhibitors since none of the tested feverfew or tansy flavonoids had these groupings and none showed selective 5-lipoxygenase inhibition. A good example of a compound with a vicinal diol group which does selectively inhibit 5-lipoxygenase is hypolaetin (8-hydroxyluteolin) with an IC_{50} of *c*. 10 μM when applied systematically in rats (Alcarez et al., 1989). It is present as the 8-glucoside in several *Sideritis* species (Labiatae) but although the glycoside increased vascular permeability and neutrophil accumulation it showed only weak inhibition of 5-lipoxygenase. However, neither the aglycone nor the glycoside influenced skin edema when applied topically (Alcarez et al., 1989). In contrast, the topical application of some flavonol constituents of *Quercus ilex* (Fagaceae) leaves gave more positive results (Loggia et al., 1989). Thus, kaempferol showed good activity in Croton oil-induced dermatitis in the mouse ear but this was dramatically reduced by glucosylation at the 3-hydroxyl (astragalin). However, addition of a *p*-coumaroyl group to the sugar at 6″ increased the activity eight times, while addition of another *p*-coumaroyl group at 2″ gave an activity 30 times greater than that of astragalin. Astragalin 2″,4″ di-*p*-coumarate thus had a potency intermediate between indomethacin and hydrocortisone.

The anthocyanins of tart cherries were assayed for their anti-inflammatory efficacies because consumption of cherries had been reported to alleviate arthritic pain and gout. Three anthocyanins and their aglycone, cyanidin were tested for their ability to inhibit prostaglandin endoperoxide hydrogen synthase-1 and 2 (PGHS-1 and 2) (Wang et al., 1999b). The glycosides showed little or no activity at a concentration of 300 μM and higher concentrations actually increased the activity of the enzymes. However, the aglycone, cyanidin showed significant inhibitory activity against both enzymes with IC_{50} values of 90 and 60 μM, respectively compared with 1050 μM for aspirin in both tests. Ulcerogenic and adverse properties of non-steroidal anti-inflammatory drugs are attributable to the inhibition of PGHS-1, whereas the beneficial therapeutic effects result from the inhibition of PGHS-2. Thus, a strong preferential inhibition of PGHS-2, as exhibited by cyanidin, is desirable to reduce the adverse effects of PGHS-1.

Two flavonol glycosides, quercetin 3-xylosyl(1→2)-rhamnoside and quercetin 3-rhamnoside from the leaves

of *Erythrospermum monoticolum* (Flacourtiaceae), were shown to be active against acute inflammation in mice induced by TPA(12-0-tetradecanoylphorbol acetate) (Recio et al., 1995). They showed significant reductions in edema (71 and 62%, respectively) when compared with the reference drug, indomethacin. The flavonol aglycone, artemetin (5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone) from leaves of *Cordia verbenacea* (Boraginaceae) also showed marked anti-inflammatory activity. It significantly inhibited carrageenin-induced paw edema, in oral doses of 102.6 mg kg^{-1} and 153.9 mg kg^{-1} and was as effective as a reference dose of calcium phenylbutazone. It was also equally effective as the latter reference compound in inhibiting granuloma tissue formation and it markedly reduced vascular permeability (Sertie et al., 1990). The flavonol aglycone, kaempferol, has previously been shown to exhibit anti-inflammatory activity against carrageenin 5-hydroxytryptamine, to inhibit granulation tissue formation induced by croton oil and to protect against gastric ulcers induced by pyloric ligation and restraint stress in rats (Goel et al., 1988). In a further study Goel et al. (1996) have shown that kaempferol is also effective in reducing ethanol and cold resistant stress-induced gastric damage in rats. The flavanone, hesperitin also, has been shown to reduce carrageenin-induced paw edema in rats but in this case it was administered subcutaneously by injection (Emim et al., 1994). Pretreatment with hesperidin at 50 and 100 mg kg^{-1} s.c. reduced the paw edema by 47 and 63%, respectively, within 5 h. This is equivalent to the activity of indomethacin at 10 mg kg^{-1}, p.o. Selgardo and Green (1956) previously found that hesperidin was ineffective after oral administration but Emim et al. (1994) found that it remains active after repeated subcutaneous injections without harmful side effects. Hesperidin is a major byproduct of the citrus industry and therefore could be used as an inexpensive, mild anti-inflammatory agent. It also produced analgesia and exerted mild antipyresis (Emim et al., 1994).

Other simple flavonoids which have been shown to exhibit useful anti-inflammatory activity include apigenin and quercetin. Apigenin showed significant inhibition of fibroblast growth at all concentrations from 0.01 to 100 mg/ml (Koganov et al., 1999). During inflammation fibroblasts play an important part in granulation and scar tissue formation and interact with the immune system. Most of the delay in wound healing is due to insufficient or excessive fibroblast activity. Thus, inhibition of fibroblast growth by flavonoids such as apigenin could be beneficial for the treatment of any skin injury. Quercetin, together with the phenylpropanoid curcumin (diferuloylmethane), may be useful in healing after renal transplantation. Shoskes (1998) found that "serum creatinine levels were significantly improved after ischaemia-reperfusion injury following pretreatment with 1 mg of quercetin and at 7 days following treatment with quercetin, curcumin or both". The various antioxidant properties of these compound help to prevent the irreversible lipid peroxidation which occurs with reperfusion injury. The inflammatory chemokine response to the injury is also reduced. Strong antihistamine activity has been shown by thymonin (5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone) from *Mentha spicata* var. *crispa* (Labiatae) with an IC of 6.4 μM. 5,6-Dihydroxy-7,8,3′4′-tetramethoxyflavone from the same plant showed mild activity ($IC_{50} = 56$ μM) (Yamamura et al., 1998).

6.5. Vascular activity of flavonoids

Flavonoids may act in a number of different ways on the various components of blood such as platelets, monocytes, low density lipoprotein (LDL) and smooth muscles. Platelets are key participants in atherogenesis and pro-inflammatory mediators such as thromboxane A2, PAF and serotonin are produced from them. Flavonoids may inhibit platelet adhesion, aggregation and secretion. The subject has been reviewed in detail by Beretz and Cazenave (1988) and Middleton and Kandaswami (1994). Recent reports of flavonoids with antiplatelet activity include 2′,4′,4-trihydroxy-3′-prenyl chalcone (isobavachalcone) and 7,4′-dihydroxy-3′-prenyl isoflavone (neobavaisoflavone) isolated from the seeds of *Psoralea corylifolia* (Leguminosae) (Tsai et al., 1996). The former showed specific activity against arachidonic acid (AA)-induced aggregation with an IC_{50} of *c*. 0.5 μM and minimal inhibition of collagen PAF-induced aggregation. Neobavaisoflavone on the other hand inhibited both AA and PAF (platelet activating factor) aggregation of rabbit platelets although to different degrees. Thus the IC_{50} for AA-induced inhibition was 7.8 μM compared with 32.7 μM for aspirin but for PAF-induced aggregation its potency was less than the positive control CV-3988 (IC_{50}s of 2.5 and 1.1 μM, respectively. Other reports include the potent antiplatelet activity of luteolin, from *Gentiana arisanensis*, against AA- and collagen-induced aggregation and significant antiplatelet effects on thrombin- and PAF-induced aggregation (Lin et al., 1997a). Similarly, quercetin and kaempferol derivatives and apigenin have been demonstrated to inhibit the aggregation of rabbit platelets caused by various inducers (Chung et al., 1993). Luteolin also depressed the contractions induced in rat aorta by Ca^{2+} (1.9 μM) in high K^{+} (80 μM) medium with an IC_{50} of 156 μM and noradrenaline (NA) (3 μM) induced phasic and tonic contractions with an IC_{50} of 68 and 72 μM, respectively (Lin et al., 1997b). Luteolin and two other flavonoid constituents of the aerial parts of *Satureja obovata* subsp. *valentina* were tested for vasodilatory activity (Sánchez de Rojas et al., 1996). All three compounds relaxed the sustained

contraction induced by NA (10^{-6} M) and K^+ (80 μM) at a concentration of 5×10^5 M in isolated rat aorta but luteolin was the most effective with 98.7 and 40.3% relaxation compared with 12.41 and 3.05% for naringenin and 67.48 and 17.93% for eriodictyol, respectively.

In a survey of 65 flavonoids for procoagulant activity 18 were found to inhibit the interleukin 1-induced expression of tissue factor on human monocytes but the most active was the biflavonoid, hinokiflavone (Lale et al., 1996). Tissue factor is a glycoprotein that initiates blood coagulation but this activity is not normally expressed in monocytes and endothelial cells unless they are exposed to inflammatory mediators which cause them to acquire procoagulant properties. Hinokiflavone was found to inhibit endoxin- and interleukin-induced tissue factor expression within the same concentration range with IC_{50} values of 18 and 48 nM, respectively.

Flavonols such as kaempferol, quercetin and myricetin have been shown to inhibit adenosine deaminase activity in the endothelial cells of the aorta while flavones were found to be inactive (Melzig, 1996). The author indicates that "this supports the suggestion that many pharmacological actions of flavonoids are mediated by an amplification of the effect of endogenous adenosine via adenosine receptors because adenosine deaminase is responsible for the adenosine inactivation".

Flavonoids have been shown to be potent inhibitors of the oxidative modification of low density lipoproteins by macrophages (Whalley et al., 1990). In atherosclerotic lesions lipid-laden macrophages are a characteristic feature. The lipid is thought to come from LDL but uptake by the macrophages is normally slow in vitro and does not lead to significant lipid accumulation unless the LDL is in an oxidised form. LDL contains a number of endogenous antioxidants, including α- and γ-tocopherols, carotene, lycopene and retinyl stearate and it is only when these are all consumed that peroxidation can take place. Oxidised LDL is rapidly taken up by macrophages and may contribute to the formation of cholesterol-laden foam cells in atherosclerotic lesions. Whalley et al. (1990) showed that addition of flavonoids such as flavone itself, gossypetin, myricetin and hypolaetin 8-glucoside, to the macrophages, conserved the α-tocopherol content of the LDL and delayed the onset of lipid peroxidation. Flavonoids also inhibited the cell-free oxidation of LDL mediated by copper sulphate. In a further study Rankin et al. (1993) showed that myricetin and gossypetin are able to modify LDL themselves at a concentration of 100 μM to allow much faster uptake by macrophages. The lipid hydroperoxide content was not increased by myricetin nor was the amount of endogenous α-tocopherol in the LDL reduced. However, this modification did not occur at a concentration of 10 μM and it seems unlikely that normal levels of dietary myricetin would ever reach 100 μM. In any case the inhibitory effects of flavonoids in the circulation would predominate over any modification of LDL by myricetin or gossypetin.

6.6. Flavonoids with oestrogenic activity

The main group of flavonoids that are well known to possess oestrogenic activities are the isoflavones, such as genistein. This follows from the earlier recognition that a dietary disease of ewes in Australia was caused by isoflavone constituents of the clover plants present in their pasture. In a recent search for new phyto-oestrogens, Kitaoka et al. (1998) have isolated 8-isopentenylnaringenin from a Thai crude drug, derived from the heartwood of *Anaxagorea lutzonensis* (Annonaceae). In in vitro tests they found that this flavanone had an oestrogen agonist activity greater than that of genistein and that the presence of the 8-*iso*pentenyl group is an important factor for binding to the oestrogen receptor. Other flavones, flavanones and flavonols with an *iso*pentenyl group at C-8 also showed considerable affinity for the oestrogen receptor but 8-*iso*pentenylisoflavones were not active. Movement of the *iso*pentenyl group from C8 to C6 resulted in the loss of the activity but there was no difference in activity between the 2(*S*)- and 2(*R*)-enantiomers of 8-isopentenylnaringenin. In in vivo tests with rats both isopentenylnaringenin (30 mg/kg/day) and oestrogen (0.01 mg/kg/day) were found to suppress the increase in urinary excretion of bone resorption markers (hydroxyproline, pyridinoline and deoxypyridoline) and the decrease in bone mineral density caused by ovariectomy when administered subcutaneously for 2 weeks (Miyamoto et al., 1998). Miksicek (1993) has shown that a much larger number of flavonoid constituents possess oestrogenic activity than prevously thought. They may be less potent than 17 β-estradiol or oestrogenic stilbenes but appear to have a pharmacological efficacy, at optimal concentrations, which is equivalent to the natural hormone. The active flavonoids all have hydroxyls located at the 7 and 4′- positions of the flavone nucleus or 4,4′- of the chalcone molecule. Both apigenin and 4,4′-dihydroxychalcone were shown to mimic the activity of 17 β-estradiol when tested for their ability to stimulate the proliferation of MCF7 cells (an oestrogen-dependent human breast tumour cell line). In a normal human diet the presence of such active flavonoids is usually considered to be harmless because no single phyto-oestrogen is present in sufficient quantity to have physiological consequences. However, this may not be the case for vegetarians, especially those who eat a large percentage of legumes in their diet, which have a high isoflavonoid content, such as soya and pulses. Indeed prolonged supplementation of the diets of 25 asymptomatic postmenopausal women with soya, linseed and red clover sprouts was found to produce significant

oestrogenic effects on vaginal maturation and the concentration of follicle stimulating hormone (Wilcox et al., 1990).

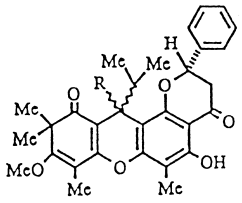
6.6.1. Cytotoxic antitumor activities of flavonoids

There have been many bioassay-guided searches for cytotoxic antitumour agents in plants especially those known to be used in folk medicine for this purpose. This has led to the isolation and identification of quite a large number of active constituents from all the different flavonoid classes, e.g. catechins, flavans, dihydrochalcones, chalcones, flavanones, dihydroflavonols, flavones, biflavonoids and flavonols. However, the choice and number of cell lines used in these bioassays has been very variable. Here we will give only comparatively recent examples from all the different flavonoid classes. The earlier literature and further recent findings have been reviewed by Wang et al. (1998b).

In a search for the cytotoxic constituents of the aerial parts of *Ononis natrix* ssp. *ramosissima* (Leguminosae) (Barrero et al., 1997) 4,2′,6′-trihydroxy-4′-methoxydihydrochalcone, 2′,6′-dihydroxy-4′-methoxydihydrochalcone and 2′,4′-diacetoxychalcone were identified as having moderate activity against P-388 (murine leukaemia), A-549 (human non-small cell lung cancer) and HT-29 (human colon cancer). However, the most potent compound was 2′,6′-diacetoxy-4,4′-dimethoxydihydrochalcone, which showed selective activity for the cell line P-388. The chalcone, pedicin (2′,5′-dihydroxy-3′,4′,6′-trimethoxychalcone), from leaves of *Fissistigma languinosum* (Annonaceae), was found to inhibit tubulin assembly into microtubules (IC_{50} value 300 μM) (Alias et al., 1995). From the same plant these workers discovered two new condensed chalcones, fissistin (**8**) and isofissistin (**9**), which showed cytotoxicity against KB cells. Twelve cytotoxic flavonoids: seven flavans, three flavones and two biflavans, were isolated from the roots of *Muntingia calabura* (Elaeocarpaceae) by Kaneda et al. (1991). For example, 8,3′-dihydroxy-7,4′,5′-trimethoxyflavone and its dimer were more active against P-388 cells than the corresponding flavones. Examples of cytotoxic flavanones are a series of unusual prenylated derivatives isolated from the leaves of *Monotes engleri* (Dipterocarpaceae) by Seo et al. (1997), which showed activity against a panel of human cell lines. 6,8-Diprenyleriodictyol and hiravanone (6,8-diprenyl-3′-methyleriodictyol), which both have two prenyl side chains, were found to be more active than the other compounds which have a 1,2-dimethylallyl substituent at C-6 (i.e. at C6 of naringenin, eriodictyol and 3′-methyleriodictyol). Two other pentacyclic flavanones (**10,11**) from the leaves of *Baeckea frutescens* (Myrtaceae) showed strong cytotoxic activity (IC_{50} of 0.25 μg/ml) against leukaemia cells (L1210) in tissue culture (Makino and Fujimoto, 1999). From the stem bark of *Cudrania tricuspidata* (Moraceae) three cytotoxic benzyl dihydroflavonols: 6,8-di-*p*-hydroxybenzyltaxifolin, 8-*p*-hydroxybenzyltaxifolin and 6-*p*-hydroxybenzyltaxifolin, have been isolated (Lee et al., 1996). These compounds were cytotoxic to human tumor cell lines such as CRL 1579 (skin), LOX-IMVI (skin), MOLT-4F (leukaemia), KM12 (colon) and UO-31 (renal) with ED_{50} values of 2.7–31.3 μg ml^{-1}.

A number of cytotoxic flavones have been isolated from *Scutellaria* species (Labiatae). One of the earliest discoveries was skullcapflavone II (5,2′-dihydroxy-6,7,8,6′-tetramethoxyflavone) from the roots of *S. baicalensis*, which showed activity with an ED_{50} of 1.5 μg/ml against L1210 cells in vitro (Ryu et al., 1985). In a more recent study of the root extract of *S. indica*, Bae et al. (1994) have identified two further flavones and three flavanones but only two exhibited significant cytotoxic activity: wogonin (5,7-dihydroxy-8-methoxyflavone) and 2(*S*)-5,2′5′-trihydroxy-7,8-dimethoxyflavanone. The latter compound showed the most potent activity against L1210 cells and expressed a potent and wide spectrum of activity against other cell lines (HL-60, K562 and SNU) greater than that of skullcapflavone II. Structurally these flavonoids are similar in that they both have a 2′-hydroxyl and this may account for their cytotoxicity. Another early discovery of a bioassay-directed search was the biflavonoid, hinokiflavone, which was identified from the drupes of *Rhus succedanea* (Anacardiaceae) by Lin et al. (1989). By comparison of the cytotoxicity of hinokiflavone with that of related biflavonoids, the authors suggested that a 4′-6-linkage between the two apigenin molecules may be important for high cytotoxic activity. However, amongst the three cytotoxic biflavonoids isolated from *Selaginella* species only one, isocryptomerin (**12**) had this linkage. The other compounds: 4′,7″-di-*O*-methylamentoflavone and 7″-*O*-methylrobustaflavone, were significantly cytotoxic against human cell lines including breast, lung, colon and prostate cancer, fibrosarcoma, glibostoma, oral epidermoid carcinoma and leukemia (Silva et al., 1995). Three biflavanones: calycopterone (**13**), isocalycopterone (**14**) and 4-demethylcalycopterone (**15**) and 5,5′-dihydroxy-3,6,7,3′-tetramethoxyflavone, isolated from the flowers of *Calycopteris floribunda* (Combretaceae) also showed a wide range of cytotoxic activity against a panel of human cell lines (Wall et al., 1994). Amongst the flavonols, quercetagetin 6,7,3′,4′-tetramethyl ether, a constituent of the aerial parts of *Artemisia annua* (Compositae) was found to show significant cytotoxicity against P-388, A549, HT-29, MCF-7 and KB tumour cells (Zheng, 1994). Similarly, a flavonol constituent of *Epimedium* species (Berberidaceae), baohuoside-1 (3,5,7-trihydroxy-4′-methoxy-8′-prenylflavone 3-rhamnoside) was shown to have both cytotoxic and cytostatic effects on six cancer lines (the solid tumours: Hela, MM96E, C180-13 S and the leukaemias: L-1210, MLA-144 and HL-60) (Li et al., 1990).

In the bud extract of *Platanus orientalis* (Platanaceae) the major cytotoxic component was thought to be a



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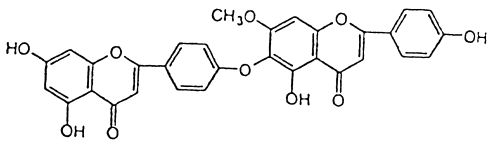

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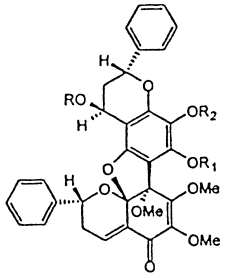

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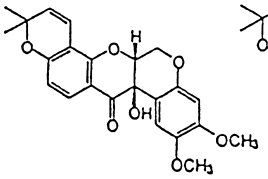

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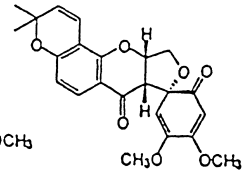

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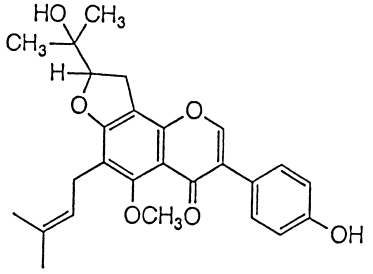

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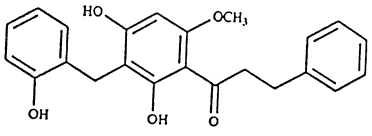

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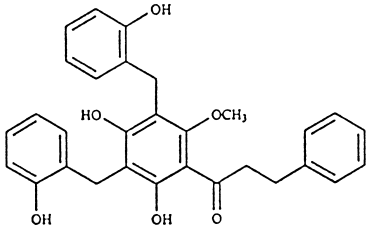

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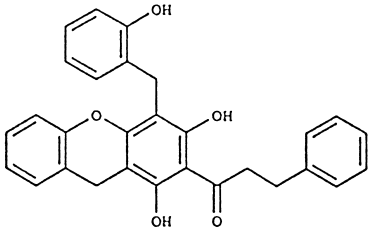

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flavonol glycoside, kaempferol 3-(2″,3″-di-*E*-*p*-coumaroylrhamnoside) because its presence significantly modulated the proliferation of HL60 (a promyelocytic cell line) and MOLT3 (a T-ALL with phenotypic characteristics of cortical thymocytes) (Mitrokotsa et al., 1993). Similarly, two highly methylated flavones, tangeretin (5,6,7,8,4′-pentamethoxyflavone) and nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) inhibited the proliferation of a squamous cell carcinoma (HTB43) and a gliosarcoma (9L) cell line at 2–8 μg/ml concentrations (Kandaswarmi et al., 1992). Another flavone, 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone (vitexicarpin) from fruits of *Vitex rotundifolia* (Verbenaceae), has been found to show inhibitory activity against T-lymphocyte proliferation but not against B-lymphocyte proliferation in vitro. This inhibitory action is reversible. Vitexicarpin also inhibited the growth of EL-4 and P815.9 cell lines at an IC_{50} of 0.25–0.3 μM (You et al., 1998). Other workers investigated the effect of the topical application of the simple flavone, apigenin, on chemically induced skin tumours in mice. They found that apigenin is a potent inhibitor of epidermal ornithine decarboxylase induction by TPA (12-*O*-tetadecanoylphorbol-13-acetate) and that it inhibited skin papillomas and showed a tendency to decrease conversion of papillomas to carcinomas (Wei et al., 1990). In a cytotoxicity test of 21 flavonoids from *Arnica* species (Compositae) against a human colorectal cancer line (COLO 320) and a human small lung carcinoma cell line (GLC4) the flavone, jaceosidin (5,7,4′-trihydroxy-6,3′-dimethoxyflavone) was the most toxic constituent (Woerdenbag et al., 1994). Two more flavones from *Scutellaria baicalensis*, 5,7,2′-trihydroxy- and 5,7,2′3′-tetrahydroxyflavone showed strong inhibition of the activation of the tumour promotor, EBV-EA (Epstein–Barr virus early antigen) by TPA (Konoshima et al., 1992). The rotenoids amorphispironone (**16**) and tephrosin (**17**), from *Amorpha fruticosa* (Leguminosae) also inhibited EBV-EA activation induced by TPA and inhibited mouse skin tumour promotion in vivo (Konoshima et al., 1993). 3,7,-Dimethoxyflavone showed reversible anti-invasive activity against the invasion of MCF-7/6 human mammary carcinoma cells into embryonic chick heart fragments at concentrations from 1 to 100 μM with no cytotoxic effects (Parmar et al., 1994). Two catechins with a pyrogalloyl B-ring were found to induce apoptosis in human histolytic lymphoma U937 cells (Saeki et al., 2000). Several recognised chemotherapeutic compounds have been reported to induce apoptosis, which may be a primary mechanism for their anti-cancer activity (Gunji et al., 1991).

6.7. Other biological activities of flavonoids

It is well known that some flavonoids can act as antispasmolytic agents by relaxing smooth muscles in various parts of the mammalian body (see previous review by Middleton and Kandaswarmi, 1994). In a recent screening of European medicinal plants used traditionally to treat respiratory complaints, four flavonols with spasmolytic activity were isolated from the aerial parts of *Artemisia abrotanum* (Labiatae) by Bergendorff and Sterner (1995). Quercetagetin 3,6,7,4′-tetramethyl ether and 3,6,4′ trimethyl ether and quercetin 3,4′-dimethyl ether showed a dose dependent relaxing effect on the carbacholine-induced contraction of guinea-pig trachea with EC_{50} values of 20–30 μmol/l, while quercetin 3,7-dimethyl ether was less active. Similarly, quercetin 3-glucoside and rutin isolated from the aerial parts of *Conzya filaginoides* (Compositae) induced a concentration-dependent inhibition of the spontaneous contractions of rat ileum (Mata et al., 1997). These glycosides were 18.75 and 15 times more potent than atropine and 8.76 and 7 times more potent than quercetin, respectively. Rutin has been reported to induce smooth muscle relaxation in various other in vitro preparations such as guinea-pig colon and rat duodenum at similar concentrations (Mata et al., 1997).

Flavonoids may also exhibit useful antibacterial activity. *Euphorbia hirta* (Euphorbiaceae) has been reported to be used to treat dysentery and other infectious diseases. Galvez et al. (1993) have demonstrated the anti-diarrhoetic activity in a lyophilised decoction of the whole plant against diarrhoea induced by castor oil, AA and prostaglandin E_2. They identified the active constituent as quercetin 3-rhamnoside (quercitrin). The use of *Microtea debilis* (Phytolaccaceae) in traditional medicine for the treatment of proteinuria has been explained by the adenosine A1 antagonistic action of cirsimarin (scutellarein 6,7-dimethyl ether 4′-glucoside), a major flavone constituent in the plant (Hasrat et al., 1997).

Several flavonoids have been shown to have potential as hepatoprotective agents (see Middleton and Kandaswarmi, 1994). In a more recent investigation the rhizome extract of *Smilax glabra* (Liliaceae) was found to significally improve a liver injury induced by a delayed-type hypersensitivity (DTH) reaction to picryl chloride when given in the effector phase but not during the induction phase. One of the active principles was identified as the flavanonol, dihydroquercetin 3-rhamnoside (Xu et al., 1997). Two further flavanonols, dihydrokaempferol 3-rhamnoside and 5,7,3′,5′-tetrahydroxyflavanonol 3-rhamnoside, isolated from the same plant, also showed some hepatoprotective activity but were not the most active phenolic components (Chen et al., 1999).

Three diprenylisoflavones, 6,8-diprenylgenistein, 6,3′-diprenylgenistein and derrisisoflavone (**18**) from *Derris scandens* (Leguminosae), were found to be active antifungal agents against the human pathogen, *Trichophyton mentagrophytes* TIMM1189 (Sekine et al., 1999).

There are a number of herbal remedies for calming an over anxious state of mind. Recently, the anxiolytic effects of *Passiflora coeulea* (Passifloraceae) has been explained by the presence of the simple flavone, chrysin (5,7-dihydroxyflavone) (Wolfman et al., 1994), which behaves as a competitive ligand of the benzodiazepine receptors with a K_I of 4 μM (Medina et al., 1990). Apigenin, a component of *Matricaria recutita* (Compositae) flowers has been reported to show similar activity in mice with only slight sedative effects (Viola et al., 1995).

Flavonoids such as gossypin (Viswanathan et al., 1984), epicatechin (Viswanathan, 1984), morin and rutin (Thirugnanasamambantham et al., 1985) have been found to show significant analgesic activity. In a later study, the analgesic activities of synthesised flavone, its 3,6,5,6,7, 2′, and 4′-monomethyl ethers and flavanone were compared (Thirugnanasamambantham et al., 1993). All the tested flavonoids except flavanone exhibited significant dose-dependent analgesic activity. Substitution at the 5-hydroxyl increased the analgesic potency almost eight-fold, while a methoxyl group at the 3-position greatly decreased the effect of flavone. Methoxylation at the 6- or 4′-postion produced a two-fold increase in activity but substitution at 7- or 2′ slightly decreased the potency.

In China, *Artemisia annua* (Compositae) is used traditionally for the prevention of malaria. The major active principle has been identified as the sesquiterpene lactone, artemisinin. However, Elford et al. (1987) have shown that the flavonol casticin (5,3′-dihydroxy-3,6,7,4′-tetamethoxyflavone), present in the whole plant and artemitin (5-hydroxy-3,6,7,3′,4′-pentahydroxy-flavone) present in cell cultures markedly enhanced the antimalarial activity of artemisinin. In later experiments Liu et al. (1989) showed a similar effect with 5 μM chrysosplenol-D (quercetagetin 3,6,7-trimethyl ether), a concentration at which it is not toxic. This suggests that there is a synergistic effect between at least some of the flavonoids and artemisinin in this plant. In some African medicinal plants the antimalarial constituents are the flavonoids themselves. Thus, in *Uvaria* species (Annonaceae) the active compounds are: uvaretin (**19**), a C-benzyldihydrochalcone and its derivatives, diuvaretin (**20**) and chamuvaritin (**21**) (Nkunka, 1992).

6.8. Flavonoids and human health

Flavonoids are ubiquitous in plant foods and drinks and therefore a significant quantity is consumed in our daily diet. These flavonoids are variously associated with the sensory and nutritional quality of our plant foods. The in vitro anti-oxidant activities (Section 6.1) have been recognised for decades, but it is still not clear whether there are in vivo beneficial effects. Many other biological activities for flavonoids have been described (see e.g. Section 6.4) but we are not certain how far flavonoids actually contribute to human health. However, some recent experiments do suggest that they may have value as anti-cancer agents.

In the 1970s, it was generally assumed that the average intake of dietary flavonoids is in the region of one gram a day (Kuhnau, 1976). This figure has been questioned by recent investigations of the flavonoid content of commonly consumed vegetables and fruits (Hertog et al., 1992). Measurements based on the acid hydrolysis of crude plant extracts were mainly of varying amounts of kaempferol and quercetin. For example, quercetin levels in edible vegetables were below 10 mg kg^{-1}, except for onions, kale, broccoli and beans (up to 486 mg kg^{-1}). In most fruits, quercetin averaged 15 mg kg^{-1}, except for apples which had between 21 and 72 mg kg^{-1}. On the whole, these values may be on the low side, since there may be loss of flavonol during acid hydrolysis. Our own experience suggests that quercetin in particular can undergo oxidative degradation in hot acid solution.

Related studies of the flavonoid content of common human beverages indicate that fruit juices have below 5 mg l^{-1}, except for lemon juice (7 mg l^{-1}) and tomato juice (with 13 mg l^{-1}). By contrast, tea infusions have up to 50 mg l^{-1} of the three common flavonols (Hertog et al., 1993b). The above data together confirm that significant concentrations of flavonols are present, mainly as glycoside, in vegetables such as onion, fruits such as apples and in drinks such as tea. However, they do not directly indicate the potential anti-carcinogenic effects of these food constituents. Nor do they indicate the relative content of other classes of flavonoid which may also be important (see below). Three recent studies of flavonoids in soya bean and tea do more directly implicate dietary flavonoids in the treatment of human cancers.

The first study centres on the isoflavone genistein, a plant oestrogen in soya bean, which has been shown to block the action of a transcription factor, known as CCAAT binding factor, neutralising it before the switch is tripped, so that the cancer cell starves, withers and dies. Thus genistein, commonly consumed as a component of soya bean, is a flavonoid capable of stopping cancer growth and angiogenesis. Crucially, it has no harmful effects on normal healthy cells (Coghlan, 1998).

The other two studies are concerned with the flavonoids of tea and the advantages of drinking many cups of this stimulating brew. Both studies draw attention to the relatively large concentrations of catechins (flavan-3-ols) and especially of epigallocatechin 3-gallate (EGCG) in tea. Human cancers need proteolytic enzymes to invade cells and form metastases. One of such enzymes is urokinase. Inhibition of urokinase in mice decreases tumour size and can even lead to complete cancer remission. EGCG acts by binding to urokinase blocking histidine 57 and serine 195 at the

catalytic site. Although it is a weaker urokinase inhibitor than the synthetic drug amiloride, EGCG is normally consumed by humans at a relatively high level. Thus, a single cup of tea contains 150 mg EGCG whereas the maximum tolerated dose of amiloride is 20 mg a day. Hence EGCG in tea through its inhibitory action on urokinase could be an important dietary constituent for reducing human cancers (Jankun et al., 1997).

The second study of EGCG in tea indicates that it is capable of suppressing angiogenesis, a key process of blood vessel growth required for tumour growth and metastasis. Since the growth of all solid tumours depends on angiogenesis, this finding may explain again why drinking tea is a useful preventative for avoiding the growth of many human cancers (Cao and Cao, 1999).

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