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COMMENTARY

FLAVONOIDS, A CLASS OF NATURAL PRODUCTS OF HIGH PHARMACOLOGICAL POTENCY

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Flavonoids are benzo-γ-pyrone derivatives which resemble coumarin and are ubiquitous in photosynthesizing cells. Their occurrence is therefore widespread in the plant kingdom. About 500 varieties of flavonoids are known. Through their food, all plant-eating animals are influenced by the flavonoids [1–3]. For centuries, preparations which contain flavonoids as the principal physiologically active constituents have been used by laymen and physicians in attempts to treat human diseases [4–7]. It is therefore surprising that such a large and important class of natural compounds has so far failed to attract the interest of any but a few biochemists and pharmacologists.

The structure of flavonoids and related natural products

Flavonoids occur as aglycons, glycosides and methylated derivatives [3, 8–11]. The flavonoid aglycons all consist of a benzene ring (A) condensed with a six-member ring (C) which in the 2-position carries a phenyl ring (B) as a substituent (see Fig. 1). The six-member ring condensed with the benzene ring is either a γ-pyrone (flavonols and flavonones) or its dihydroderivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavanones by a hydroxyl group in the 3-position and a C_2–C_3 double bond. Anthocyanidines are closely related to the flavonoids. They differ from the latter in the C-ring, which in anthocyanidines is open, but their biological properties are similar.

Flavonoids are often hydroxylated in positions 3, 5, 7, 3′, 4′ and 5′. Methyl ethers and acetyl esters of the alcohol groups are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose [3]. There are at least

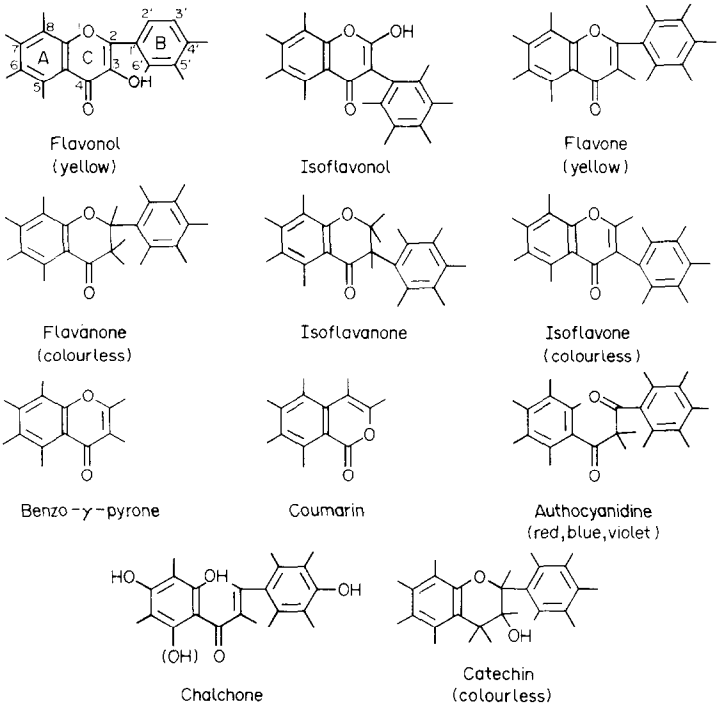

Fig. 1. The main classes of flavonoids and related compounds.

Table 1. Common, naturally occurring flavonoids

Name	Class	R_3	R_5	R_7	R_2	R_3'	R_4'	R_5'	Fluorescence*	Abbreviation
Quercetin	Flavonol	OH	OH	OH	H	OH	H	H	Yellow-orange	Q
Quercitrin	Flavonol	O-rh	OH	OH	H	OH	OH	H	Orange	Q-3-rh
Myricitrin	Flavonol	O-rh	OH	OH	H	OH	OH	H		
Luteolin	Flavone	H	OH	OH	H	OH	OH	H		
Kaempherol	Flavonol	OH	OH	OH	H	H	OH	H	Orange	K
Apigenin	Flavone	H	OH	OH	H	H	OH	H		
Rutin	Flavonol	O-rh	OH	OH	H	OH	OH	H	Yellow-orange	Q-3-rh-gluc
Hesperitin	Flavanol	H	OH	OH	H	OH	O-me	H		
Eriodictyol	Flavanol	H	OH	OH	H	OH	OH	H		
Hesperidin	Flavonol	H	OH	O-rut	H	OH	O-me	H		
Chrysin	Flavone	H	OH	OH	H	H	H	H	Orange	
Techtochrysin	Flavone	H	OH	O-me	H	H	H	H		
Silybin	Flavonol	OH	OH	OH	H	H	-O-lign-O			
Morin	Flavonol	OH	OH	OH	OH	H	OH	H		
Naringen	Flavonone	H	OH	OH	H	H	OH	H	Blue-grey	
Taxifolin	Flavonol	OH	OH	OH	H	OH	OH	H	Orange	
Pinocembrin	Flavanone	H	OH	OH	H	H	H	H		
Galangin	Flavonol	OH	OH	OH	H	H	H	H		
Robinin	Flavonol	O-gal-rh	OH	OH	H	H	OH	H	Yellow-grey	K-3-rh-gal-7-rh
Diosmetin	Flavone	H	OH	OH	H	OH	O-me	H		
Kaempferide	Flavonol	OH	OH	OH	H	H	O-me	H		
Fisetin	Flavonol	OH	H	OH	H	OH	OH	H		
Rhamnetin	Flavonol	OH	OH	O-me	H	OH	OH	H		

* The colour changes on methylation.
rh = rhamnoside, gal = galactose, lign = lignan, rut = rutoside, me = methyl, gluc = glucose.

$6 \cdot 2 \cdot 2 \cdot 6 \cdot 1 \cdot 2 \cdot 5 = 20{,}736{,}000$ possible members of the flavonoid class. The most common flavonoids are listed in Table 1.

Flavonoids structurally resemble nucleosides, isoalloxazine and folic acid and this similarity is the basis of many of the current hypotheses of their physiological action [1]. Flavonoids are easily oxidized at the B-ring which leads to opening of this ring at the oxygen atom [9].

The biosynthesis of flavonoids

The biosynthesis of flavonoids in plants has been elucidated. It commences with phenylalanine and proceeds through *trans*-cinnamic acid and *p*-cumaric acid. The details have been described elsewhere [3, 9, 12, 13].

The localisation and plant physiological action of flavonoids

Flavonoids are found in the thylakoid membrane. They probably participate in the light phase of photosynthesis as catalysts of the electron transport and/or as regulators of ion channels involved in photophosphorylation [14, 15]. The known interactions of flavonoids with proton and $(Na^+–K^+)$ATPase pumps in animal cells also suggest that flavonoids participate in such processess [16–18]. When the photosynthesizing cells die, flavonoids are released and appear in the plant juice, in honey and in the resin [2]. Bees gather fluids which are rich in flavonoids from the plants and manufacture from them a resinous material called Propolis with which they close openings in their hives [4]. The latter are thus not only rendered impervious to draught and moisture but also almost sterile [19]. Flavonoids and their decomposition products, e.g. anthocyanides, are to a large extent responsible for colourful autumn foliage.

Biochemical effects of flavonoids in animal systems

The high chemical reactivity of flavonoids is expressed in their binding affinity to biological polymers [1, 20–22] and heavy metal ions [23, 24] as well as in their ability to catalyse electron transport [14, 25–29] and to scavenge free radicals [30–34]. Each of these properties will be treated separately.

Binding of flavonoids to enzymes, hormone carriers and DNA. Numerous reports of a high standard have appeared on the inhibition by flavonoids of a perplexing number and variety of enzymes, e.g. hydrolases [9, 42] (such as β-glucuronidase [9], hyaluronidase [9], alkaline phosphatase [35, 36], arylsulphatase [9], H^+-ATPases of lysosomal and granular membranes, $(Na^+–K^+)$ATPase of the plasma membrane [16, 17, 37–41], β-galactosidase [9, 42], c-AMP phosphodiesterase [43–48] and lipases [9, 49], lyases (such as DOPA decarboxylase [9]) transferases (like catechol-*O*-methyltransferase [9]), hydroxylases (like aryl hydroxylase [50–54]), oxidoreductases [25–29, 55, 56] (like aldose reductase) and kinases (e.g. hexokinase [37]).

A single case of enzyme activation by flavonoids is also known, namely that of the proline hydroxylase [57]. However, the latter effect is probably due to the electrochemical potential of the flavonoid rather than to an allosteric mechanism, acid/base catalysis or stabilisation of the active site.

When one examines these enzymes, which are all influenced by a group of compounds of a rather homogeneous structure, one notices that they seem to have little in common. Therefore, they apparently interact with different parts of the flavonoid molecule, e.g. as suggested in Table 2.

Table 2.

Enzyme	Part of flavonoid molecule likely to interact with enzyme
β-Glucuronidase	Carbohydrate
β-Galacturonidase	Carbohydrate
Hyaluronidase	Carbohydrate
Alkaline phosphatase	Phenyl ring
Arylsulphatase	Phenyl ring or benzopyrone ring
DOPA decarboxylase	Phenyl ring
Lipases	Phenol (Me^{2+} chelator)
ATPases	Benzopyrone ring
c-AMP phosphodiesterase	Benzopyrone ring
Catechol-*O*-methyltransferase	Phenyl ring
Aryl hydroxylase	Benzopyrone ring
Aldose reductase	Benzopyrone ring
Proline hydroxylase	Benzopyrone ring

Flavonoids have a strong affinity for divalent ions of heavy metals [23, 24, 36], e.g. Cu^{2+} and Zn^{2+}. The position of the hydroxyl substituents and the electronic properties of the ring system suggest that flavonoids would make good ligands for the d_3-electrons of the transition elements of the fourth period.

The estrogenic effect of flavonoids was noticed when sheep ate a particularly flavonoid-rich vegetation [29, 58]. It may be rationalized by reference to the spatial relationship between the phenolic hydroxyl groups of estradiol and certain flavonoids. The close agreement between these configurations and between the associated chemical properties suggest that flavonoids after binding to the cytosolic estrogen receptor can derepress the same gene as true estrogens.

Some flavonoids have proven to be mutagenic [59–66] in the Ames test. However, this potency is less marked than the one found for similar substances. The mutagenic effect may be due to the resemblance between nucleosides and flavonoids which may lead to intercalation of the benzopyrone system between the bases. In addition, it seems possible that the Ames test is unable to distinguish between derepression of normally inactive genes and true mutagenesis since the observation which prompted the positive result was only the appearance of new traits which may have many consequences other than oncogenesis in animals. In fact, no serious side effects have been observed with the use of flavonoids at moderate doses (<1 g/day/adult patient [20]).

The electrochemical potential of flavonoids. Flavonoids are easily oxidised. This process is accompanied by opening of the γ-pyrone ring [9]. So far, the electrochemical potentials of flavonoids have not been measured but they can be estimated from the known potential of the reaction partners. The high propensity of flavonoids for electron transfer may explain their interference with the action of oxidoreductases [55], e.g. aldose reductase and proline hydroxylase. Besides, such properties would be expected from strong scavengers of free radicals, like flavonoids [30]. However, flavonoids may also be able to suppress the formation of free radicals by binding of heavy metal ions which are known to catalyze many processes leading to the appearance of free radicals [31–34, 6–6].

Therapeutic applications of flavonoids

The use of flavonoids in the treatment of diseases is to a large extent based on empirism since this praxis is much older than the science of chemistry. Until very recently, our knowledge of the biochemistry of flavonoids was not sufficient for rational medical application of these substances [70]. The controlled clinical experiments on the use of flavonoids by well-defined illnesses were hitherto few and the flavonoids used were those which were readily available as pure substances or in mixtures, like Propolis preparations [71–73]. Considering the number of flavonoids existing, the probability that the choice was less than optimal was high. Therefore, a negative result did not necessarily mean that no flavonoid could produce the desired effect.

Some of the diseases on which therapeutic attempts have been made with flavonoids are listed in Table 3.

Membrane permeability. Flavonoids are known to influence the permeability of both natural and synthetic membranes [74, 77]. This effect may well be the basis of the often mentioned bacteriocidal and antimycetic activity of flavonoids (and Propolis) [78–81]. At the natural membranes, the inhibition of enzymes like ATPases ([82–84], phospholipase A_2, prostaglandin cyclooxygenase [85] and lipooxygenase by flavonoids would suffice to make a plausible hypothesis which could explain the phenomenon. However, with synthetic membranes which do not contain any enzymes, other devices must be marshalled. One interesting possibility would be that flavonoids, like certain complement factors and ionophoric antibiotics, e.g. gramicidin, monatin and nonatin, pierce the membranes with a stack of oligomers. Some of the pore-forming antibiotics form dimers which, lodged in the membrane, protrude on both sides and leave an opening between the monomers of sufficient width to allow certain ions to pass. The polarity of flavonoids would permit such a mechanism. They also possess a structure which would be expected from an oligomerizing system.

Whereas the biochemical experiments were very

Table 3. Diseases treated with flavonoids

Disease	Target	Flavonoids	Result proven
Inflammation	PG synthesis	Quercetin etc.	Local pain relieved; body temp normalized
Diabetes mellitus	Aldose reductase	Quercetin etc.	Pressure in eye reduced
	Capillary wall (PG)	Rutin/citrin	Bleeding ceased
Allergy	H^+-ATPase of mast cell	Disodium chromoglycate Quercetin etc.	Secretion of histamine etc. prevented Symptoms disappeard
Headache	PG synthesis	Quercetin etc	Pain relief
Parodentosis	Capillary wall (PG)	Quercetin etc.	Bleeding ceased; gum tissue normalized
Cancer	$(Na^+–K^+)$ATPase	Quercetin etc.	Cells normalized (only tissue culture tested)
Virus infection	H^+-ATPase of lysosome membrane	Quercetin etc.	Coat removal prevented
Common cold	H^+-ATPase of lysosome membrane	Quercetin etc.	No scientific evidence
Chemical oncogenesis	Aryl hydroxylase Epoxide hydrolase	Quercetin etc.	Only laboratory experiment
Bee sting	PG synthesis	Quercetin etc.	Local pain relieved
Oral surgery	PG synthesis	Quercetin etc.	Local pain relieved
Stomach/duodenal ulcer	PG synthesis	Quercetin etc.	Bleeding stops; pain relief

PG = prostaglandins, thromboxanes and leucotrienes.

successful, the few controlled clinical experiments which have so far been performed yielded less encouraging, but still positive results. Such a discrepancy is also known from the investigation of other natural products, which for theoretical reasons should be effective and valuable. Examples are interferon and streptomycin. Flavonoids have, by the way, also been reported to induce the biosynthesis of lymphocyte interferon. In the case of streptomycin, the development of the drug from its discovery until its general acceptance and widespread use lasted many years. Such incidences are probably caused by our incomplete knowledge of biochemistry, cellular biology and pharmacology. Therefore, tenacity is often required to develop radically new drugs. The reward is not only successful, new, therapeutic methods but also, and this may be of equal importance, new and deep insight into the regulation of cell metabolism.

The biochemical explanation of the therapeutic effects which have been claimed or in some cases proven [71], and which are listed in Table 3, is the following.

Inflammation is known to be accompanied by the release of prostaglandins which by chemotaxis attract leucocytes to the point of invasion, create local pain and, after transport in the blood to the brain, raise the body temp by displacing the balance of the centre of thermal regulation. The known inhibition of prostaglandin cyclooxygenase and of lipoxidase would therefore lead to the observed local pain relief and antipyretic effect [78, 85–87].

With headache, the cause may be an inflammation or nervous strain. In the first case, the explanation above suffices and in the latter a plausible reason would be the relaxation of smooth muscles [88], which have been seized by cramps, through the action of prostaglandins and leucotrienes.

Aspirin, which, like flavonoids, relieves pain by inhibition of prostaglandin cyclooxygenase, has frequently been taken by patients suffering from a stomach or duodenal ulcer. A common side effect has been serious bleeding. Like aspirin, flavonoids relieve the pain but, in contrast to the salicylates and another common antiulcer drug, cimetidin, do not appear to cause side effects [72].

Similarly, inflamed joints are often treated with glucocorticoids to relieve the pain, but often at the cost of bleeding. The viscious alternatives, pain or bleeding, may be circumvented by the use of flavonoids, which do not decompose the connective tissue, but, on the contrary, fortify it [89, 90]. The mechanism may be a combination of the suppression of prostaglandin synthesis and the stimulation of proline hydroxylation, i.e. of collagen cross-linking [57, 91]. Prostaglandins are known to induce elastase and other catabolic hydrolases. Their suppression would therefore offer a plausible explanation for the fortification of the connective tissue.

Parodentosis is also a disease which involves inflammation and destruction of connective tissue. The beneficial effect of flavonoids on this ailment could therefore be explained in the same way as the previously mentioned condition.

The local anaesthetic action of flavonoids has been used with insect stings or bites and in oral surgery [92]. The potency of flavonoids in this respect has been found to be comparable to that of cocain. Many venoms contain phospholipase A_2 which releases arachidonic acid, which in turn is converted to prostaglandins. The pain relief may therefore be interpreted as a suppression of the prostaglandin synthesis by inhibition of both phospholipase A_2 and prostaglandin cyclooxygenase.

Diabetes mellitus raises, especially in the late stages, the lipid content of the blood and leads to narrowing of the blood vessels [93]. This can cause a considerable diminution of the draining from the eye [94] and the intestines. The back pressure, as well as the osmotic pressure of dulcitol which cannot be metabolized by human cells, can create visual disturbances and deficient intestinal absorption. The enzyme which reduces glucose to dulcitol is aldose reductase [25–29, 95]. This enzyme is inhibited by flavonoids by a yet unknown mechanism. Diabetes mellitus can also lead to microbleeding which can be stopped by flavonoids.

Allergy or delayed hypersensitivity and asthma have for a long time been treated with disodium chromoglycate [96]. This compound possesses, like flavonoids, the benzopyrone nucleus. Therefore, it is no surprise that they also share biochemical properties. The action of flavonoids on the symptom-producing mast cells has been identified as an inhibition of the proton ATPase in the membranes enclosing the granula in these cells [97–103]. Histamine and serotonin are trapped in their granula because the law of conservation of electroneutrality requires that the export of positively charged substances is balanced by a corresponding import, in this case of protons. Preparations of Propolis have been reported to cause allergy in especially predisposed patients although this resin contains many flavonoids. The reason is apparently that such preparations always contain pollen, unless the latter has been removed, e.g. by hyperfiltration of an extract.

Virus infection apparently remains completely harmless until the protein coat surrounding the nucleic acid has been removed by lysosomal digestion [133]. The latter process requires the fusion of the viral mantle with the lysosomal membrane which in turn must be aided by a proton ATPase [16, 104] and possibly also by phospholipase A_2 [36]. The former enzyme presumably activates the cathepsins by importing protons and the latter may weaken the lysosomal membrane. Both of these enzymes are inhibited by flavonoids and similar compounds [73, 105–109].

Attempts have been made to cure common colds with flavonoids but no scientific evidence of success has appeared. A possible reason is that the effect is preventive rather than curative.

Cancer cells of half a dozen different types, some of which were induced by viruses, like the Rous sarcoma virus, have been treated in tissue culture with flavonoids [110, 111]. Racker found that the plasma membrane (Na^+–K^+)ATPase pump of these cancer cells had a very low ion translocation efficiency, about 10% of the normal value before infection, and concluded that the deficient pump monopolized on the available supply of ATP, thus starving other cell functions and generating high concns of ADP and inorganic phosphate [12–115]. He also found that the ailing pump carried a phosphate residue on a tyrosine side-chain, whereas normal Na^+–K^+ pumps are only phosphorylated on serine and threonine side-chains. After incubation with flavonoids, the phosphate ester on tyrosine disappeared, the pump regained its normal efficiency and the cell assumed properties which, by all counts, were normal, i.e. it had been cured. The enzyme required for amino acid phosphorylation is a kinase and other kinases, e.g. hexokinase, have been reported to be inhibitable by flavonoids [37]. Therefore, there seems to be a good possibility that this kinase is also inhibited by flavonoids, presumably owing to the structural resemblance between adenosine and the hydroxylated and glycosylated benzo-γ-pyrone nucleus. This work, which has been checked by Professor Racker personally, was extended by his student M. Spector who claimed to have found a cascade of kinases starting with an enzyme prescribed by the virus. Only the latter work has been thrown in doubt because it could not be reproduced by other laboratories.

To my knowledge, so far, no controlled clinical experiments have been performed on the treatment of cancer with flavonoids. There may be some problems dispensing the desired flavonoids along a route of entry leading them to the target.

Chemical carcinogenesis may be induced in experimental animals with certain tars, e.g. from burned tabac. The oncogenic substances, especially condensed hydrocarbons like benzo-α-pyrene, are enzymatically hydroxylated in ring cleavage. Epoxides are formed as intermediates. The latter may add the amino group of nucleic acid bases, especially guanine, thus creating a covalent derivative in DNA which perturbs the replication and transcription and induces cancer [53, 54, 116–118]. Flavonoids induce both aryl hydroxylase and epoxide hydrolase [50–52, 119–124]. The former enzyme is necessary for detoxification of aromatic compounds which not only can interfere with polynucleotides but presumably also disturb various membrane processes [107, 125, 126]. However, its epoxide intermediate can become dangerous if it undergoes addition reactions instead of hydrolysis. The flavonoid induction of aryl hydroxylase can therefore be a mixed blessing. Ameliorating this, flavonoids also help to annihilate the epoxides by also inducing their hydrolase and by scavenging the free radicals which are intermediates in the addition reactions. Oral cancer in patients who have been heavy smokers probably belongs to this type of oncogenesis. It has been treated with flavonoids and success has been reported, but the circumstances were not controlled.

Pharmacodynamics of flavonoids

Flavonoids are metabolized by animal cells, especially those of the liver [127–129]. Labelled flavonoid metabolites, e.g. 3,5-dihydroxyphenylacetate and 3-hydroxyphenylacetate, have been detected in urine [130]. Therefore, no residuals of flavonoids are accumulated in the body.

The toxicity of flavonoids is very low in animals.

For rats, the LD_{50} is 2–10 g per animal for most flavonoids [20]. Similar doses in humans are quite unrealistic. As a precaution, doses less than 1 g per adult per day have been recommended for humans.

Pharmacokinetics of flavonoids

Only few data are available on the pharmacokinetics of flavonoids [92]. For this reason, and because of the wide range in the solubility of flavonoids, predictions of the rate of absorption of flavonoids from the intestine and penetration through the skin or mucous surfaces are difficult to make [131]. Ames has shown that bacterial glycosidases are capable of liberating flavonoid aglycons [59, 132]. Larger amounts of orally taken flavonoids must therefore be expected to be largely present as aglycons in the intestine and to become absorbed with micelles of bile acids into the epithelium and then into the blood. Through the portal vein, the major part of the flavonoids would probably be delivered more or less directly to the liver, which decomposes them. If the target of the flavonoids is not in the gastrointestinal tract or the liver, an i.v. or i.m. injection, or a local application, e.g. to the skin or through the nose, may be contemplated. The lack of success of a flavonoid treatment may often be ascribed to the failure of the compound to reach its target. So far, little is known about the affinity of flavonoids to blood plasma proteins but due to the low polarity of many aglycons, especially of those which are strongly methylated, they would probably be bound to serum albumin. Owing to the specific binding properties of flavonoids and to their chromophores in the visible range, the isolation of these compounds from tissue samples should not present many problems. Therefore, more pharmacokinetic experiments are overdue.

Clinical experiments with flavonoids

Clinical experiments with new drugs require the availability of large groups of patients suffering from the same well-defined disease. Few would embark upon such expensive and time-consuming projects without solid evidence from the fundamental biological sciences which gives strong hopes of success. The controlled clinical experiments on flavonoids which previously have been reported were, to my knowledge, all performed on small groups of patients, 10–20 in the group receiving flavonoids and a similar number receiving a placebo, and the nature of the diseases were not known, e.g. M. krohn and colitis ulcerosa. Besides, the flavonoids were given in the form of a variety of propolis which had not been qualitatively and quantitatively analyzed for flavonoids. Under such circumstances, the marginal results obtained cannot be surprising since the individualities in the sensitivity of a patient to almost any drug would be sufficient to sacrifice the experiment to the blur of chance.

Now, however, much more biochemical information on the properties of flavonoids in animal cells is available. Therefore, it appears that the prerequisites have been fulfilled for a serious investigation of the therapeutic possibilities which a new class of drugs, the flavonoids, can offer.

Summary

A review has been presented of the biochemistry and pharmacology of a class of natural products, the flavonoids. These substances which are widely distributed in the plant kingdom and present in considerable quantities in common food products, spices and beverages have in a concentrated form (Propolis) been used since ancient times by physicians and laymen to treat a great variety of human diseases but they have yet to pass the tests of modern, controlled, clinical experimentation. An attempt has been made to present the fundamental evidence from the basic biological sciences which is required to stimulate the interest of the clinicians in this new field. The few existing reports on the careful pharmacodynamic, pharmacokinetic and clinical studies which have been made have been summarized to provide a basis for a full-scale investigation of the therapeutic potential of flavonoids.

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