Flavonoids

1. INTRODUCTION

Flavonoids in the broad sense of the term are virtually universal plant pigments. Almost always water-soluble, they are responsible for the color of flowers, fruits, and sometimes leaves. Examples are yellow flavonoids (chalcones, aurones, and yellow flavonols) and red, blue, or purple anthocyanins. When they are not directly visible, they contribute to the color by acting as copigments: for example, colorless flavone and flavonol copigments protect anthocyanins. In some cases, the molecule absorbs near-UV radiation: this "color" is only perceived by insects, who are thus efficiently attracted and guided to the nectar, and therefore compelled to ensure pollen transport, a necessary condition for the survival of the plant species. Flavonoids are also ubiquitous in the leaf cuticle and epidermal cells where they ensure tissue protection against the damaging effects of UV radiation.

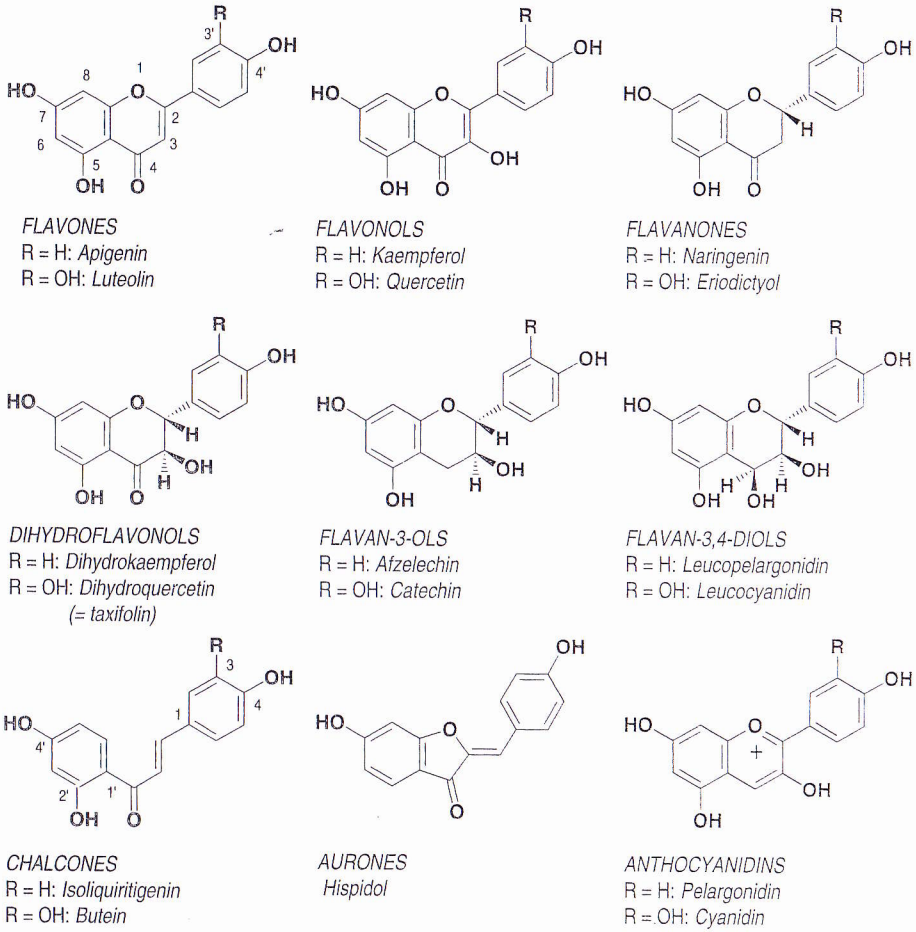
All flavonoids—approximately 4000—have a common biosynthetic origin, and therefore possess the same basic structural element, namely the 2-phenylchromane * skeleton. They fall into about a dozen classes depending on the degree of oxidation of the central pyran ring, which can be opened and recyclized into a furan ring (dihydrofuranone):

- 2-phenylbenzopyriliums, i.e., anthocyanins;
- 2-phenylchromones;
  - flavones, flavonols, and their dimers,
  - flavanones and dihydroflavonols (2,3-dihydrogenated derivatives),
  - isoflavones, isoflavanones... *,
- 2-phenylchromanes;
  - flavans,
  - flavan-3-ols, flavan-3,4-diols **,
- chalcones and dihydrochalcones (the pyran ring opens);
- 2-benzylidene coumaranones (= aurones).

Some authors apply the term flavonoid to all of these compounds. Although it is permissible, considering their structural homogeneity, to speak of flavonoids in the broad sense of the term for this extensive group, in this text, in order to reflect their particular behavior and properties, we choose to separate flavan derivatives, anthocyanins, and isoflavonoids, and to reserve the term flavonoids in the strict

* Phenyl migration yields 3-phenylchromane, the basic structure of isoflavonoids, which in this text are purposely detached from the group and covered separately.

** Oligomers and polymers, i.e. proanthocyanins, together with gallic polyesters of glucose and their derivatives, belong in the group of tannins.



sense for flavones, flavonols, their 2,3-dihydrogenated derivatives, their dimers, and "yellow" flavonoids, in other words aurones and chalcones.

2. OCCURRENCE

Distribution. To date no flavonoids have been found in algae. Flavonoids are common in Bryophytes (mosses and hepaticas), always in the strict sense, and mainly represented by *O*- and *C*-glycosides of flavones and of *O*-uronic derivatives. In Pteridophytes, the structural variety of flavonoids is not much greater, in that Psylotales and Selaginellales are characterized by their biflavonoids, and Equisetales by their proanthocyanins. *O*-glycosides of flavonols dominate in Filices, some of which also elaborate chalcones or proanthocyanins. In Gymnosperms, pro-anthocyanins are remarkably ubiquitous, and the occurrence in the Cycadales and

noteworthy; the distribution of these compounds, alongside flavone and flavonol glycosides, varies greatly as a function of the organ (wood, bark, or leaf). It is in the Angiosperms that the structural diversity of flavonoids is maximal: indeed about thirty flavonoid types have been identified in the Asteraceae.

Many authors have strived to link the distribution of these molecules to the different taxonomic systems proposed by contemporary systematists*: along the main lines there is no disagreement with the evolutionary tendencies highlighted by these systems. It even allows, especially for the Dicotyledons, to develop a rather coherent phylogenic scheme between groups that have conserved a large number of ancestral characteristics and those that have evolved the most.

Location. The glycosidic forms of flavonoids are water-soluble, accumulate in vacuoles, and depending on the species, either concentrate in the epiderm of the leaves or spread in both the epiderm and the mesophyll (although these two tissues can specifically accumulate different structures, as demonstrated in some cereals). In flowers, they are concentrated in epidermal cells.

Whenever flavonoids are found in the leaf cuticle, it is almost always as free aglycones, made even more lipophilic by the partial or total methylation of their hydroxyl groups. This is especially true for plants growing in arid or semi-arid regions, which often have secretory elements.

3. CHEMICAL STRUCTURE AND CLASSIFICATION

In all of the classes of flavonoids mentioned so far, biosynthesis frequently introduces at least three phenolic hydroxyl groups in the 5-, 7-, and 4'-positions of the aglycone; however, one of these may also be absent.

A. Flavones, Flavonols

In these molecules, which represent the majority of known flavonoids in the strict sense, ring A, in over 90% of cases, is substituted by two phenolic hydroxyl groups at C-5 and C-7. These hydroxyl groups are either free or etherified, and one of them may be engaged in a glycosidic linkage. A third hydroxyl group, free in chalcones, provides the oxygen atom of the pyran ring in other flavonoids or that of the furan ring in aurones.

Other substitutions are possible, with variable frequency: free or etherified hydroxyl groups at C-6 or C-8 or both, isoprenylation or methylation at C-6 or C-8, or involvement of C-6, C-8, or both in a carbon-carbon bond with a saccharide (see below).

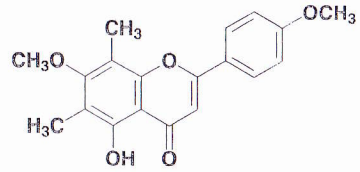
* Among others: (1) Cronquist, A. (1988). An Integrated System of Classification of Flowering Plants, 2nd Ed., New York Botanical Garden. (2) Thorne, R.F. (1992). Classification and Geography of Flowering Plants, Bot. Rev., 58, 225-348. For a bibliography and on-line access to the major classification systems, see http://www.inform.umd.edu/PBIO/nb250

The B ring, substituted at C-4' in 80% of cases, may be 3',4'-di-substituted or, less frequently, 3',4',5'-trisubstituted; the substituents are -OH or -OCH_3 groups. Finding substituents on the other positions (2' and 6') is exceptional.

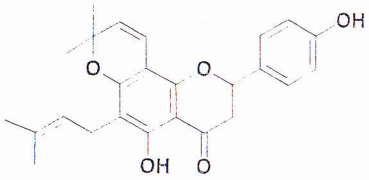
These flavones, flavonols, and their glycosides are universally distributed, but some of the substitution patterns are restricted to some families or groups of families, hence their interest to chemotaxonomists: thus, 6-*O*-substituted flavonoids are common in the Lamiaceae, Rutaceae, and Asteraceae, 5-deoxyflavones in the Fabaceae and Myrtales, and 2'-*O*-substituted flavonols in the Lamiaceae, Solanaceae, and in the mealy exudate that coats the leaves and inflorescences of the primrose (Primulaceae).

B. Flavanones and Dihydroflavonols

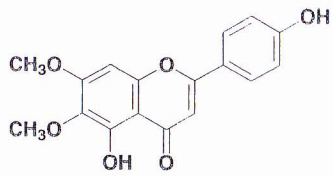
These compounds are characterized by the absence of a 2,3-double bond, and by the presence of at least one asymmetric center. In natural flavanones C-2 is normally in the 2*S* configuration. In theory, for dihydroflavonols, four isomers are possible, however the quasi totality of the compounds known to date in this series have the



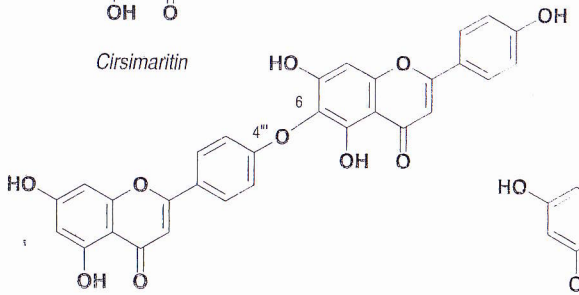
Eucalyptin



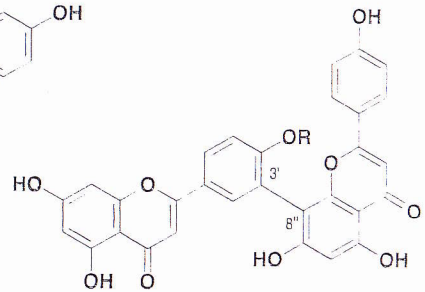
Cajaflavanone



Cirsimaritin



Hinokiflavone



R = H: *Amentoflavone*
R = CH_3: *Bilobetol*

2*R*,3*R* configuration, with the phenyl and hydroxyl groups in *trans*. Structural variations are the same as those described above for flavones and flavonols. These flavanoids are somewhat less common than their unsaturated homologs, and it is noteworthy that some families tend to accumulate their *C*-alkylated derivatives (Asteraceae, Fabaceae).

C. Biflavonoids

Flavonoids can also bond to one another, particularly through their very reactive C-6 or C-8. The result is a dimer known as a biflavonoid. The majority of natural biflavonoids are dimers of flavones and flavanones, are generally 5,7,4'-trisubstituted, and the interflavanic linkage can be of the carbon-carbon-type (3',8", e.g., in amentoflavone; 6,8", e.g., in agathisflavone; 8,8", e.g., in cupressiflavone) or of the carbon-oxygen-carbon-type (6-*O*-4''', e.g., in hinokiflavone). The two consecutive units of the biflavonoid may or may not be of the same type (biflavone, biflavanone, flavone-flavanone, flavanone-chalcone *). The hydroxyl groups may be free or (frequently) methylated. In this group, few glycosides are known. Biflavonoids are characteristic of the Gymnosperms (see above), and are sporadic in the Angiosperms (e.g., *Hypericum, Semecarpus, Schinus, Garcinia*).

D. Chalcones, Aurones

Chalcones do not have a central heterocyclic nucleus and are characterized by a three-carbon chain with a ketone function and an α,β-unsaturation. Substitutions on the A ring are most often identical to those of other flavonoids (2',4',6') **, whereas the B ring is fairly often unsubstituted. Isoprenyl- and pyranochalcones seem rather common, especially in the Fabaceae. Aurones are characterized by a 2-benzylidene-coumaranone structure.

E. Glycosylflavonoids

The sugar moiety may be a mono-, di-, or trisaccharide. Monosaccharides include D-glucose, D-galactose or D-allose, pentoses (D-apiose, L-arabinose, L-rhamnose, or D-xylose), or D-glucuronic or D-galacturonic acid. Structural variability expands in the glycosides formed with a disaccharide (about thirty had been described by 1988)

* Oligomerization and polymerization are possible among flavanic derivatives: the condensation of flavan-3-ols and flavan-3,4-diols yields proanthocyanidins; see p. 378.

** This is equivalent to the 5- and 7-positions and to the oxygen of the pyran ring. Caution! The numbering is reversed, so that the benzophenone carbons are identified by digits followed by the prime sign (').

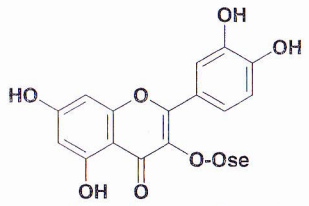
structure	usual name
O-β-D-xylosyl-(1—>2)-glucose	sambubiose
O-α-L-rhamnosyl-(1—>2)-glucose	neohesperidose
O-α-L-rhamnosyl-(1—>6)-glucose	rutinose
O-β-D-glucosyl-(1—>2)-glucose	sophorose
O-β-D-glucosyl-(1—>6)-glucose	gentiobiose
O-β-glucosyl-(1—>2)-*O*-β-glucosyl-(1—>2)-glucose	sophorotriose
O-α-rhamnosyl-(1—>2)-*O*-β-glucosyl-(1—>3)-glucose	2'-rhamnosyl-laminaribiose
O-α-rhamnosyl-(1—>4)-*O*-[α-rhamnosyl-(1—>6)-galactose]	4^{Gal}-rhamnosylrobinobiose

Examples of di- and trisaccharides found in glycosylflavonoids

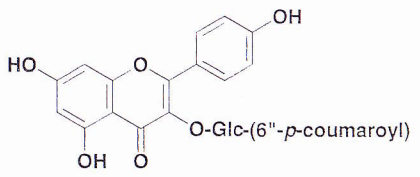
or with a trisaccharide, which may be linear or branched, for added complexity (about twenty were known by the same year; see examples below).

The bond between the aglycone and the saccharide may be established through any of the phenolic hydroxyl groups on the aglycone, but as a general rule, the hydroxyl groups in the 7-position of the flavones and in the 3-position of the flavonols are most often involved.

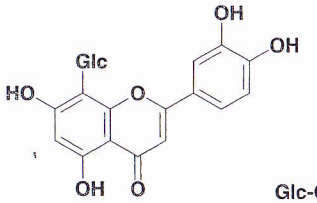
Advances in analytical technology, especially in the field of *mass spectrometry* or MS (*fast atom bombardment*-MS or FAB-MS, *desorption chemical ionization*-MS or DCI-MS), make possible the characterization of an increasing number of acylated



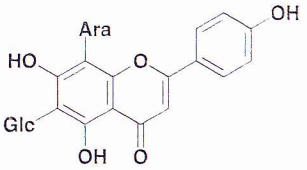
Ose = rhamnose: Quercitrin
Ose = galactose: Hyperoside
Ose = glucose: Isoquercitrin



Tiliroside



Orientin



Shaftoside

Saponarin (7-O-glucosyl-isovitexin)

structures, in which a hydroxyl group of the sugar moiety is esterified by an aliphatic acid (acetic, malonic, tiglic, and others) or an aromatic acid (gallic, benzoic, 4-coumaric, and other cinnamic derivatives). Over 80 sulfated flavonoids are also known.

F. Special Case: *C*-Glycosylflavonoids

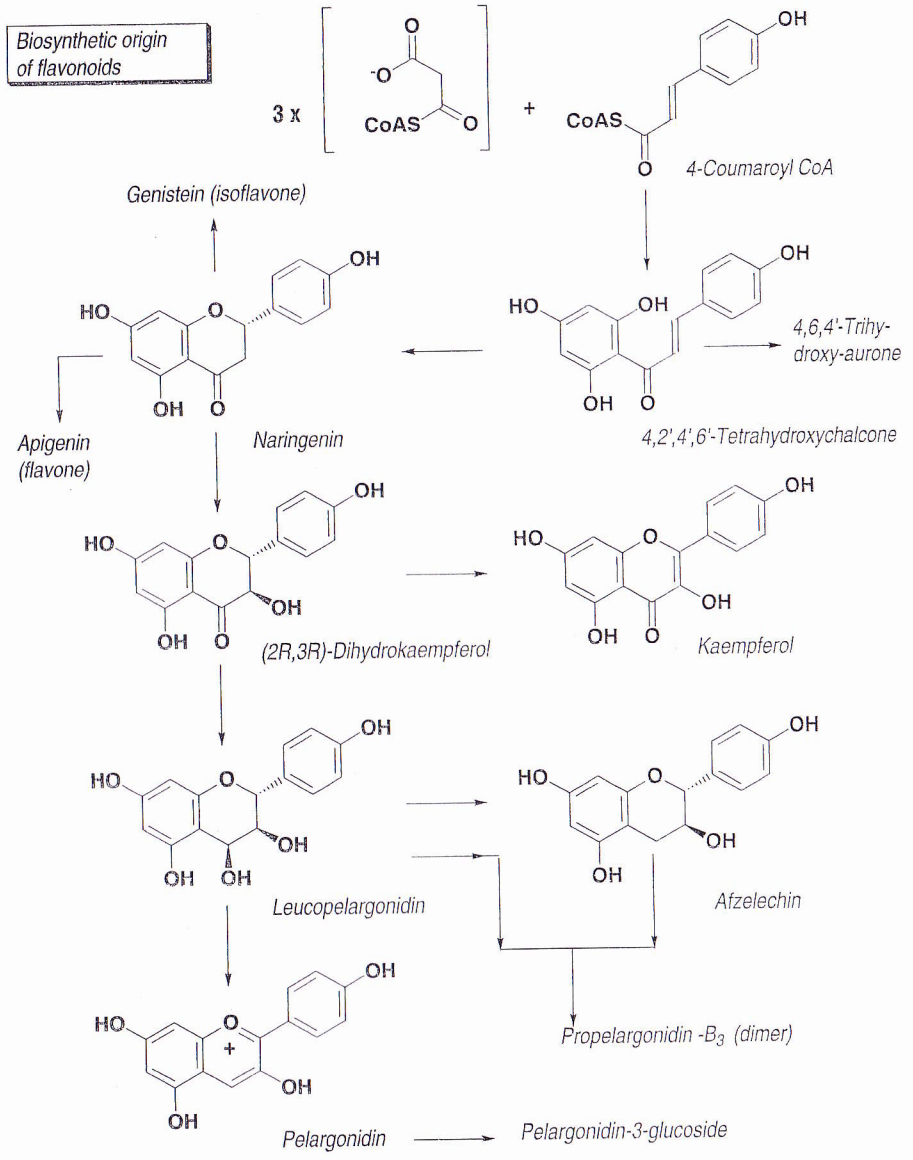
C-Glycosylflavonoids * are not rare: more than 300 are known. The bond is established between the asymmetric carbon on the sugar (often glucose, also galactose or a pentose) and the C-6 or C-8 of the aglycone, which is often a flavone, but can also very well be of another type, including a flavonol or a chalcone. Several types of structures can be distinguished: 1. mono-*C*-glycosylflavonoids (e.g., scoparoside in Scotch broom); 2. di-*C*-glycosyl-flavonoids (e.g., isoschaftoside in tea); 3. *C*-glycosyl-*O*-glycosylflavonoids (e.g., saponarin [= 7-*O*-glucosylisovitexin] in passion flower); 4. acyl-*C*-glycosyl-flavonoids (e.g., 4'''-*O*-acetyl-2''-rhamosyl-vitexin in hawthorn). The heterocycle in derivatives of the 5-hydroxy-*C*-glycosyl-flavone type opens readily in acidic conditions, which explains their facile isomerization (6 <–> 8, 8 <–> 6). This so-called Wessely-Moser isomerization remains of interest for the structure elucidation of these compounds.

4. BIOSYNTHETIC ORIGIN

The origin of flavonoids is as if watermarked in their very structure. It is transparent in chalcones: condensation of a "triacetate" (A ring) with cinnamic acid (B ring), and cyclization forming the central pyran ring. This hypothesis has been confirmed by experiments with radiolabeled precursors, and with enzymes in tissue culture, as well as in whole plants (petals in particular).

The key step in the formation of flavonoids is the condensation, catalyzed by chalcone synthase, of three molecules of malonyl-CoA with an ester of coenzyme A and a hydroxycinnamic acid, as a general rule 4-coumaryl-CoA (the incorporation of caffeoyl-CoA seems limited to a few species, as the extra hydroxylation of the B ring occurs late in the process). The reaction product is a chalcone, 4,2',4',6'-tetra-hydroxychalcone, or, if the condensation takes place in the presence of an NADPH-dependent polyacetate-reductase, a 6'-deoxychalcone, 4,2',4'-trihydroxychalcone. Under normal physiological conditions, chalcone tends to isomerize spontaneously to racemic flavanone. In fact, the cyclization of chalcone is catalyzed by an enzyme, chalcone isomerase, which induces a stereospecific closure of the ring (*syn* addition onto the *E* double bond) leading to a (2-*S*)-flavanone only: naringenin and liquiritigenin in the two pathways above, in other words the immediate precursors of flavonoids and 5-deoxyflavonoids, respectively.

* Be careful to distinguish glucosyl derivatives (of glucose) from glycosyl derivatives (of any saccharide).



A dioxygenase, flavanone 3-hydroxylase, has been isolated. It catalyzes hydroxylation at C-3 only on (2-*S*)-flavanones; it specifically induces the hydroxylation of (2-*S*)-naringenin to (2-*R*,3-*R*)-dihydrokaempferol, and that of (2-*S*)-eriodictyol to (2-*R*,3-*R*)-dihydroquercetin. These are then the substrates of flavonol-synthase: this dioxygenase functions, like the previous one, in the presence of oxoglutarate. It introduces the double bond between C-2 and C-3.

Closely related enzymes, flavone-synthase I and II—the former is known to occur in Apiaceae, the latter is widely distributed—convert flavanones into flavones

by introducing, as before, a double bond between C-2 and C-3. In contrast with what had long been postulated, these desaturations do not go through an intermediate hydroxylation at C-2; they are more likely to involve the direct elimination of protons at C-2 and C-3. Thus the mechanism of formation of flavones and flavonols from their dihydrogenated precursors would be similar to that which leads from flavanones to isoflavones.

The other flavonoids (flavanols, proanthocyanidins, anthocyanins) no longer have a carbonyl group at C-4. They arise from a (2*R*,3*R*,4*S*)-*trans*-2,3-flavan-*cis*-3,4-diol (= *cis*-flavan-3,4-diol = "leucoanthocyanidin"), itself a reduction product of a (2*R*,3*R*)-dihydroflavonol (by a NADPH- or NAD-dependent dihydroflavonol 4-reductase). The subsequent formation of flavan-2,3-*trans*-3-ols (flavan-3-ols) is induced by a reductase. The same reaction sequence on a (2*R*,3*S*)-dihydroflavonol leads to the epi series, but nothing is known of the mechanisms involved in the biosynthesis of flavan-2,3-*cis*-3-ols (*ent*). The enzymology behind the condensation reaction leading to proanthocyanidins (*via* a carbocation or a methide-quinone) is not known. The mechanism of formation of anthocyanins also remains hypothetical (glucosylation of a flav-3-en-3,4-diol intermediate followed by dehydration?).

C_9 Acids other than 4-coumaric acid can be incorporated *in vitro* to yield 3',4'-di- or 3',4',5'-trisubstituted flavonoids, but the additional hydroxyl and methoxyl groups on the B ring actually arise from substitutions on C_{15} molecules mediated by hydroxylases or *O*-methyltransferases, with the latter being, most often, highly selective.

The formation of the glycosidic linkage(s) is catalyzed by transferases which are also highly specific, as far as the substrate and the glycosylation position are concerned; it requires the presence of uridine diphospho-saccharides (UDP-saccharides). A specificity of the same type has been observed for the acyl-transferases which induce the acylation of some glycosides, especially anthocyanins.

5. PHYSICO-CHEMICAL PROPERTIES, EXTRACTION, CHARACTERIZATION, AND QUANTITATION

A. Solubilities and extraction

Although, as a general rule, glycosides are water-soluble and soluble in alcohols, a fair number are sparingly soluble (rutin, hesperidin). Aglycones are, for the most part, soluble in apolar organic solvents: when they have at least one free phenolic group, they dissolve in alkaline hydroxide solutions.

Lipophilic flavonoids of the superficial leaf (or frond) tissues are directly extracted by solvents of medium polarity (e.g., dichloromethane); next they must be separated from the waxes and fats extracted simultaneously (of course, a preliminary hexane wash is possible, but the selectivity of this solvent is not absolute).

The glycosides can be extracted, most often at high temperature, by acetone or by alcohols (ethanol, methanol) mixed with water (20 to 50% depending on whether the drug is fresh or dried). Solvent evaporation under vacuum can be next followed, when only the aqueous phase is left, by a series of liquid-liquid extractions by nonmiscible solvents: petroleum ether which eliminates chlorophyll and lipids; diethyl ether which extracts free aglycones; and ethyl acetate which dissolves the majority of glycosides. The free saccharides remain in the aqueous phase with the most polar glycosides when these are present.

The separation and purification of the different flavonoids is based on the usual chromatographic techniques (on polyamide, cellulose, or Sephadex® gel). As in the case of most other secondary plant metabolites, in the last few years HPLC has taken a place of choice in the battery of isolation techniques for glycosylflavonoids (C_8 or C_{18} reverse phase with solvents such as water [or acetonitrile, or *tetrahydrofuran* = THF] + methanol + acetic acid).

B. Characterization

Although several color reactions allow the characterization of aglycones and glycosides in crude extracts, preliminary work on these extracts is conventionally dominated by TLC analysis (but paper chromatography has not been abandoned). The chromatograms can be studied:

- directly, since chalcones and aurones are usually visible, and turn orange and red, respectively, in the presence of ammonia vapors;
- by examination under UV light before and after spraying with aluminum trichloride, or before and after exposure to ammonia vapors (the nature of and changes in the observed fluorescent spots provide information on the flavonoid type);
- after spraying with a 1% solution of the ester of 2-aminoethanol and diphenylboric acid, in other words the "*Naturstoff Reagenz A*", by examination under UV light then under visible light; the sensitivity can be improved by overspraying with a 5% methanolic solution of polyethylene glycol 400 (= macrogol 400);
- after spraying with ferric chloride, anisaldehyde, diazotized sulfanilic acid or other general reagents for phenols;
- by utilizing more or less specific reactions or properties, such as:

– reaction, known as the cyanidin reaction, with magnesium powder (for flavanones and dihydroflavanols) or with zinc (for flavonoids in the strict sense), both in the presence of hydrochloric acid,

– reaction of dihydrochalcones, first with sodium borohydride, then with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Structure elucidation and hydrolysis methods for glycosides will not be covered here. Let us emphasize, however, that the considerable advances in mass spectrometric, as well as NMR techniques (proton, ^{13}C, homo- and heteronuclear correlations) should not lead one to neglect, for this group of compounds, the very useful information provided by UV spectroscopy: the spectra of these molecules, acquired under neutral conditions (in methanol), and in the presence of bases (sodium acetate or hydroxide), Lewis acids (aluminum trichloride), or boric acid, provide reliable indications of the structure type, nature, and location of substituents

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The usefulness of UV data extends to the use, in routine HPLC analysis, of diode array detectors.

C. Quantitation

The classic quantitation methods are mostly colorimetric or spectrophotometric (e.g., measuring the absorbance after reaction with $AlCl_3$). HPLC is widely used because it allows a rapid and precise estimate of the total flavonoids.

6. BIOLOGICAL PROPERTIES

The main property that is recognized for flavonoids is "venoactivity", in other words their ability to decrease capillary permeability and fragility. In animal models, they can decrease the signs of experimental vitamin C deficiency. Because of this property, they were first referred to as "vitamin P". Since they are not vitamins (flavonoid deficiency does not cause any particular syndrome), they were later referred to as "vitamin P factors" or, better yet, "P factors". Nevertheless, these terms were ambiguous and they are practically no longer used: instead these natural products and their derivatives are now referred to as "venoactive" and reference books on pharmaceuticals list them under "vascular protective and venous tonic agents". The interest in flavonoids is far from unanimous: the US *Food and Drug Administration* (= FDA) does not recognize any activity for them; furthermore, a review of pharmacology textbooks leaves little doubt that their therapeutic value is generally minimized. Nevertheless, flavonoids and flavonoid-based preparations are—in France, Germany, Spain, Italy—widely prescribed, often recommended by pharmacists, and commonly self-prescribed to treat minor circulatory disorders. In addition, a few compounds in this class have proven efficacy, at least at high doses. At this time, there is a lot of interest in the interaction between flavonoids and free radicals, and its potential applications in preventive therapy. There is active research to try to define, *in vitro*, the activity of these compounds on the cells and systems involved in immune responses and inflammation.

Flavonoids and Capillary Fragility and Permeability

Historically, the concept of P factor is linked to the following observation: certain scurvy symptoms, cured by the administration of lemon juice, are not cured by the administration of ascorbic acid (= vitamin C) alone. Hence the postulate that ascorbic acid is active only in combination with a "C2" or "P" factor, first identified as flavonoids in the strict sense, then, more globally, as anthocyanins and flavanolic oligomers.

Indeed, it is possible to show that all these molecules are capable of decreasing capillary fragility and permeability. The most classic method of evaluation of capillary fragility consists of measuring the vacuum required to rupture them. The

vacuum is applied by placing a cupping glass on the skin, and capillary lesions appears as petechiae. To evaluate the effect on capillary permeability, it is possible to measure, in animals, the time it takes for a dye injected systemically to appear in a patch of irritated abdomen skin. Many other methods may be used: retardation of the diffusion of radiolabeled proteins across capillaries, induction of venous stasis, tests on isolated veins... The increase in vascular tone in man can be shown by various techniques: plethysmography, ^{133}Xe clearance... the decrease in capillary fragility can also be estimated (with radiolabeled albumin).

Flavonoids and Free Radicals

Many properties, shown *in vitro*, could explain the actions of flavonoids. Initially, it was postulated that they act on the reduction of dehydroascorbic acid *via* glutathione by acting as hydrogen donors. The more reducing the flavonoid, the greater the ascorbic acid sparing.

It is now more generally accepted that the phenols that flavonoids scavenge free radicals formed under different circumstances:

- anoxia, which blocks the flow of electrons upstream from cytochrome oxidases and results in the production of the superoxide radical anion ($O_2^{\bullet -}$); the superoxide radical reacts with protons by dismutation to oxygen (O_2) and hydrogen peroxide (H_2O_2);
- inflammation, which corresponds, among other things, to the production of superoxide radical anions ($O_2^{\bullet -}$) by the membrane NADPH-oxidase of activated leucocytes; it also corresponds, by dismutation, to the production of oxygen peroxide, which, in the presence of ferrous ions, gives rise to the very reactive hydroxyl radical ($OH^{\bullet}$, Fenton reaction; it is also produced by electromagnetic radiation) and to other reactive species (HOCl, chloramines). These species are normally involved in the phagocytosis process, can be released into the external medium by exocytosis, and can cause serious biochemical damage;
- lipidic autoxidation, which is generally initiated by a hydroxyl radical (or by $NO^{\bullet}$) which removes one hydrogen from the lateral chain of a fatty acid, leaving behind a radical ($R^{\bullet}$). This radical reacts with oxygen to form cyclic peroxides and hydroperoxide radicals ($ROO^{\bullet}$), which propagate the chain reaction. Lipophilic alkoxyl radicals ($RO^{\bullet}$) are also formed.

Normally, the cascade of reactions resulting from the pairing of one of the lone pair electrons of oxygen is interrupted by enzymatic systems: (mitochondrial and cytoplasmic) superoxide dismutases which convert the superoxide radical anion ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2); catalase and glutathione peroxidase which reduce peroxides to water, and later on, to hydroperoxides (ROOH + 2GSH —> R-OH + H_2O + GS-SG).

Biochemically, free radicals are thought to be responsible for nucleic acid alterations, mutations, initiation and promotion of carcinogenesis, and cellular damage, because of their ability to react with membrane phospholipids, among other reasons. Despite the lack of absolute proof, and despite the fact that their

physiological role is not yet completely elucidated, free radicals are believed to be in part responsible for the genesis of atheromatous lesions, the beginning of some cancers, and neurological degeneracy. This has spurred research, including epidemiologic studies, on the potential role of antioxidants (i.e., free radical scavengers) such as flavonoids, some lignans, and other metabolites found in the daily diet, in preventive therapy.

The antagonist effect towards free radical production can be studied experimentally. This is possible because radicals can be produced *in vitro* by radiolysis (hydroxyl radical) or chemically (diphenylpicrylhydrazyl radical), and detected respectively by *electron spin resonance* (= ESR, also called *electron paramagnetic resonance* = EPR) or colorimetrically. Using experimental free radicals, radical scavenging capability can be measured *in vitro* on a lipid peroxidation model, or else the activity can be evaluated *in vivo* by comparison with that of a reference antioxidant. Many flavonoids in the broad sense and many other phenols (especially tocopherols [= vitamin E]) react with free radicals to prevent the degradations linked to their intense reactivity. It appears that the antioxidant capability of a flavonoid depends on its affinity for free radicals, therefore it depends on its structure (*in vitro*, flavanols are more active than flavonols, which are themselves more active than flavanones, etc. *).

No matter how exciting these models and research are **, an activity shown *in vitro* cannot be extrapolated to preventive or curative therapy. During the late 1990s, two large-scale studies have shown, in the case of flavonoids *stricto sensu* (quercetin, kaempferol, apigenin, luteolin, myricetin), that :

- there is no relationship between the incidence of cancer and flavonoid intake , or between cancer mortality and flavonoid intake ***;

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* On first approximation. The substituents must be taken into account: kaempferol (mono-hydroxylated on the B ring) is less antioxidant than taxifolin, a flavanone dihydroxylated at C-3' and C-4'.

** Data interpretation is often difficult. For example, quercetin is shown, in multiple studies, to be a carcinogen, a cocarcinogen, an anticancer agent, or an inhibitor of cancer growth. Its *in vitro* mutagenicity and teratogenicity, however, seem subject to controversy (but it inhibits the mutagenicity of benzopyrene). See Suschetet, M. (1997). Microconstituants végétaux présumés protecteurs, *in* "Alimentation et cancer", (Riboli, E., Decloître, F. and Collet-Ribbing, C., Eds.), chap. 24, pp. 458-506, Tec & Doc, Paris.

*** This in no way diminishes the current international consensus: a diet rich in fruits and vegetables protects against cancers of the lung, upper respiratory and upper gastrointestinal tracts, and gastrointestinal tract. Practically all fruits and vegetables contain flavonoids, but in different quantities: small (<10 mg/kg) in cabbage, carrot, pea, spinach, and peach; medium (<50 mg/kg) in lettuce, tomato, strawberry, apple, and grape; large (>50 mg/kg) in onion, green bean, Belgian endive, brocoli, and celery. Fruit juices, wines (especially red wines), and tea also contribute to the flavonoid intake. According to a late 1990s study, the average consumption is 15-34 mg/day of flavones and flavonols in three countries of the European Union, and 68 mg/day in Japan.

- there is an inverse correlation between flavonoid intake and cardiovascular disease mortality. Although some reviewers pointed out bias in the relevant study, the recent publication of Finnish data confirmed this finding. It is postulated that flavonoids protect the LDLs from oxidation.

Flavonoids: Enzyme Inhibitors

As a general rule, flavonoids are enzyme inhibitors *in vitro*:

- histidine decarboxylase inhibition by quercetin or naringenin;
- elastase inhibition;
- hyaluronidase inhibition, by flavones and especially by proanthocyanidins (see p. 378 *sqq.*), which would maintain the intercellular ground substance in the perivascular sheath;
- non specific inhibition of catechol-*O*-methyltransferase, which would increase the amount of available catecholamines and result in a decrease in vascular fragility;
- inhibition of cAMP phosphodiesterase which might explain, among other things, their anti-platelet aggregation activity;
- inhibition of aldose reductase— known to be involved in cataract pathogenesis—by quercitrin and by methoxyflavones (rodents, *per os*);
- inhibition of protein-kinase *in vitro*, for example by luteolin;
- several flavonoids—cirsiliol, hypolaetin—are potent inhibitors of 5-lipoxygenase, therefore they inhibit the production of the leukotrienes that mediate inflammation and allergic reactions. Several flavonoids (luteolin, apigenin, chrysin) inhibit cyclo-oxygenase and platelet aggregation. These properties which have been demonstrated *in vitro*, may explain, in part, the anti-inflammatory and antiallergic properties commonly attributed to various drugs known to contain flavonoids.

In rare cases, flavonoids may stimulate enzymatic activity: such is the case of proline hydroxylase. This stimulation would favor the formation of cross-links between collagen fibers, reinforce their strength and stability, and prevent their denaturation. This activity on collagen seems to be due mainly to flavanol oligomers (proanthocyanidins, see the chapter on "tannins"). Note also that the superoxide anion radical appears to be involved in the non-enzymatic proteolysis of collagen, and that *in vitro*, anthocyanins inhibit this degradation process.

Other Properties. Flavonoids are often promoted to be anti-inflammatory agents—which is compatible with what is known of their (*in vitro*) interactions with polymorphonuclear leucocytes, thrombocytes, or the metabolism of arachidonic acid. They can be antiallergic, hepatoprotective (e.g., isobutrin, hispidulin, flavanolignans), or antispasmodic (flavonoids of thyme and other Lamiaceae) on the Guinea pig ileum, stimulated by various agonists; they can decrease blood cholesterol levels, be diuretic, antibacterial, or antiviral *in vitro* (non-glycosidic 3-hydroxy- and 3-methoxyflavones). A few of them have an anticancer effect and inhibit the growth of tumor cells *in vitro*: they interact with the enzymes of the metabolism of xenobiotic compounds; they have effects against initiation, or promotion, or both; or they are cytostatic or even cytotoxic. Most flavonoids are antimutagenic *in vitro*; in contrast, some flavonols are mutagenic on the same models. It is not possible to generalize the changes in activity as a function of the structural characteristics.

The extrapolation of all of these data requires the greatest caution: the bioavailability of these molecules in humans is generally low (when it is even known) and the activities described *in vitro* are only rarely correlated with effects *in vivo*. Furthermore, some of the results are obtained with glycosides, when in fact these are probably hydrolyzed by bacterial glycosidases in the digestive tract. Despite the large number of publications on the pharmacological potential of these molecules, no clear rules have emerged in terms of structure-activity relationships. With a few exceptions, no relevant study has shown any clinical benefit whatsoever.

7. USES OF FLAVONOID-CONTAINING DRUGS

Some crude drugs are used for the industrial extraction of flavonoids, for example total citroflavonoids, diosmin, hesperidin, rutin (diosmin occurs in *Citrus* but is obtained by semisynthesis).. Others, the activity of which is due to several active principles, are used as titrated extracts (maidenhair tree). In the case of the drugs used in phytotherapy, it would be improper, outside of rare exceptions, to speak of "flavonoid-containing drugs", because although these phenols probably contribute to the activity of the drugs, they only rarely do so alone: essential oils, other phenolic compounds, mineral salts, saponins, and other substances are sometimes the basis of part of the reported activity.

8. THERAPEUTIC USES*

Beyond the partial results provided by biochemical tests or by pharmacology experiments in animals, the truth on the clinical efficacy of most flavonoids—let alone of drugs containing them—has rarely been established correctly. Trials in man are often mere "observations", and have not always been conducted according to current standards.

Only a small number of compounds or titrated extracts have proved moderately efficacious, in spite of the subjectivity of the symptoms and the importance of the placebo effect in the principal type of pathology, namely chronic venous insufficiency in the legs (about 50% of patients improve with the placebo). French regulatory texts allow these products to claim a "full indication" worded as "for the treatment of".

* Not all flavonoids are used in therapeutics. Neohesperidin dihydrochalcone (E959) is an intense sweetener synthesized from neohesperidin, a bitter natural compound. It is used in most food products (e.g., 30 mg/kg in non-alcoholic beverages, 20 mg/kg in one cider, 50 mg/kg in fruit juices [1994 European directive]), and at low doses (<5 mg/kg) it is a flavor enhancer. Acceptable daily dose in humans: 0-5 mg/kg.

About one hundred flavonoid-containing medicines, including plant-based medicines, are currently available*. For the vast majority of them, experts feel that their efficacy has not been demonstrated according to current methodological standards. Therefore the medicines are "proposed for" (wording equivalent to "to improve"), or "used for" (wording equivalent to "for the adjunctive treatment of") certain indications. For plant-based medicines, the 1998 French Explanatory Note allows the wording: "traditionally used for" (codes 15-18 of Annex 1). These comments, as well as the uses listed below, apply to anthocyanins, proanthocyanidins, their derivatives, and the drugs that contain any of those.

Flavonoids are essentially used to treat capillary and venous disorders: alone or in combination with other drugs, they are common ingredients of vascular protective agents, venous tonics, and topical agents used in phlebology.

The proprietary drugs currently on the market have the following indications or proposed uses:

- treatment of the symptoms of venous and lymphatic vessel insufficiency: tiredness or fullness in the legs, paresthesias, restless legs syndrome (or "to improve" or "used for the functional manifestations of");
- treatment of dysfunctions linked to the acute attack of piles (or "used for the functional manifestations of").

A few proprietary drugs claim additional indications or "proposed" uses:

- to improve capillary fragility disorders of the skin (petechiae); as an adjunctive treatment of the functional symptoms of capillary fragility (or used in the symptomatic treatment of);
- to treat metrorrhagias due to minipill use or to intra-uterine contraceptive devices after the etiology has been established;
- proposed for symptoms involving circulatory disorders of the retina, choroid (or used for loss of visual acuity and alterations of the field of vision presumably of vascular origin);
- treatment of the lymphedema of the arm subsequent to breast cancer radiation therapy and chemotherapy.

Experts agree to emphasize that "venoactive" agents have no demonstrated benefit for the prevention of trophic symptoms in patients with varicose veins on the legs or to promote the healing of leg ulcers. For venous insufficiency, they are not indicated in the absence of functional symptoms; using them is no dispensation from treating the true cause of the symptoms.

* In France. Some European countries do not use "venoactive" drugs (northern Europe), or else only to a small extent (United Kingdom), because of insufficient proof of activity in

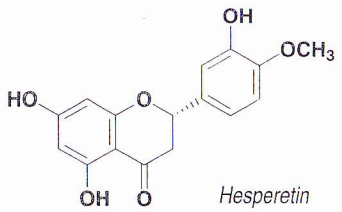
9. CHIEF FLAVONOIDS ON THE MARKET

A. Citroflavonoids: Flavonoids from the Fruits of Various *Citrus*

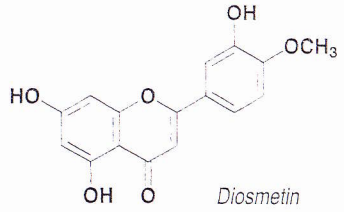
Citrus (Rutaceae) are trees of oriental origin, of which many species, varieties and hybrids are cultivated for their fruits and their edible endocarp. Widely used for their essential oils (see p. 563), they are also a source of pectins and flavonoids. These are very abundant in the pericarp, and are mainly flavonone glycosides (hesperidin or hesperetin 7-*O*-rutinoside, neohesperidin, naringin, eriodyctin, eriocitrin). Structurally, these glycosides comprise two rhamnoglycosides that differ by their linkage type—rutinose and hesperidose, 1–>6 and 1–>2, respectively—and 4',5,7-trisubstituted aglycones (naringenin, isosakuranetin) or 3',4',5,7-tetrasubstituted aglycones (eriodictyol, hesperetin) to which they are bonded through their hydroxyl group at C-7. The pericarps also contain flavone glycosides (diosmin). The composition varies depending on the species, among other factors: neohesperidin and naringin are found in bitter orange, hesperidin (0.12-0.25 g/kg) in sweet orange, and the grapefruit is rich in naringin (up to 0.4 g/kg). Citroflavonoids are extracted from pericarps and pulps with water, and isolated using different procedures (as calcium and magnesium derivatives, by adsorption onto XAD resin).

Currently, the pharmaceutical industry uses:

- a mixture of total citroflavonoids, sometimes titrated for one particular flavonoid;
- glycosides of pure flavanones: hesperidin, naringin;
- semisynthetic derivatives such as hesperidin methyl chalcone (the opening of the pyran heterocycle markedly increases solubility);
- a flavone glycoside obtained by semisynthesis, diosmin.



Hesperetin
7-Rutinosyl: Hesp eridin



Diosmetin
7-Rutinosyl: Diosmin

All of these flavonoids are used pure (diosmin, naringin) or in combinations (with one another and/or with ascorbic acid, aesculetin, ruscosides, methylaesculetin, and more). The accepted indications for preparations containing high doses of citroflavonoids (especially diosmin, 0.3-0.6 g/unit dose) are to improve the symptoms of venous and lymphatic vessel insufficiency, for the adjunctive treatment of the functional signs of capillary fragility, and to treat the functional symptoms of the acute attack of piles (1.2-1.8 g/day). The efficacy of high doses is significantly, although only slightly greater than that of a placebo, even though, in venous and

adopting elementary elements of a healthy lifestyle. Lower doses are proposed or used for the same indications.

B. Rutin: quercetin 3-*O*-rutinoside

Sources of rutin. Although rutin is relatively abundant in plants, only a small number of drugs contain quantities sufficient for industrial extraction.

- **Japanese Pagoda Tree,**
  Sophora japonica L., Fabaceae

This is a tall tree from central and northern China. It is cultivated in temperate climates as an ornamental species and the pharmaceutical industry uses its flower buds. These contain, just before blossoming, 15 to 20% rutin. Traditionally used in the Orient to dye silk, they have been replaced by synthetic dyes.

- **Buckwheat,**
  Fagopyrum esculentum Moench., *F. tataricum* (L.) Gaertn., Polygonaceae

This annual pseudocereal originated in China. It is cultivated in Europe for its edible starch-containing akenes. Rutin can be extracted from the leaves which contain between 2-3 and 5-8% in improved varieties.

- Other sources. Rutin can be extracted from the leaves of *Eucalyptus macrorrhyncha* F. Muell., (Myrtaceae) and from the fruits of Brazilian Caesalpiniaceae of the genus *Dimorphandra*.

Rutin extraction from the Japanese pagoda tree flower buds does not present any special difficulties: extraction by boiling water and crystallization upon cooling, recrystallization from ethanol. In the case of buckwheat, the presence of leaf pigments and the need to eliminate photosensitizing substances (fagopyrins) complicate the extraction.

Rutin, alone or in combination (with aesculin, citroflavonoids, or ascorbic acid, see sweet clover, p. 269), is promoted for the symptoms of venous and lymphatic vessel insufficiency, for the symptomatic treatment of the functional signs of capillary fragility, to treat the functional symptoms of the acute attack of piles, and for loss of visual acuity and alterations of the field of vision presumably of vascular origin. Its very low solubility has led to the optimization of more soluble derivatives: morpholinylethylrutin (ethoxazorutin, INN), 3',4',7-tris-(hydroxy-ethyl)-rutin (troxerutin, INN), and sodium rutosylpropylsulfonate. The uses of these derivatives

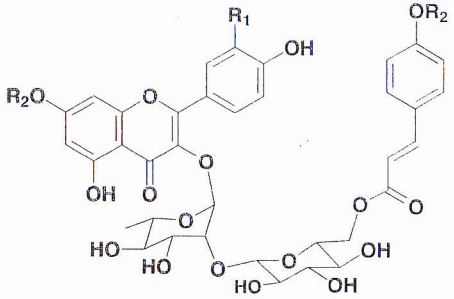
are identical to those of rutin. Rutin and its derivatives are sometimes combined with alkaloids (e.g., vincamine) in proprietary drugs promoted for symptomatic treatment for senile cerebral insufficiency.

10. DRUGS FOR WHICH PART OF THE ACTIVITY MAY BE DUE TO FLAVONOIDS

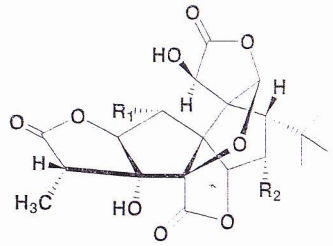
- **Maidenhair Tree,**
  Ginkgo biloba L., Ginkgoaceae

The Plant, the Drug. The maidenhair tree or ginkgo, also called in French the forty-coin tree or *arbre aux quarante écus*, is a dioecious species with deciduous leaves which originated in the Orient, the only survivor of an order widely represented until the end of the tertiary era. It is characterized by specific reproductive organs and by a "fruit" with an unpleasant odor (in reality a fertilized ovule with pulpous aril). The tree is cultivated (in Korea, the southeast of France, and the United States [South Carolina]) to supply the pharmaceutical market with leaves. These are commonly bilobate, although they can also be almost entire or very divided. The petiole contains two bundles of conducting tissue which divide by dichotomy into the blade, giving it a very characteristic striated look.

Chemical Composition. Next to sterols, aliphatic alcohols and ketones, 2-hexenal, organic acids, cyclitols, mono- and polysaccharides, the ginkgo leaf contains two groups of compounds with interesting pharmacological properties: flavonoids (0.5-1%) and terpenes—diterpenes (up to 0.5%, wide range depending on individual trees, season), and sesquiterpenes (bilobalide, 0.%).



R_1 = H or OH ; R_2 = H or Glc:
Examples of complex flavonoids from Ginkgo biloba leaf



R_1 = R_2 = H: *Ginkgolide A*
R_1 = OH, R_2 = H: *Ginkgolide B*
R_1 = R_2 = OH: *Ginkgolide C*

The flavonoids are represented by about twenty flavonol glycosides, namely *O*-glucosides, quercetin and kaempferol 3-*O*-rhamnosides and 3-*O*-rutinosides, their 4-coumaric esters at C-6''' (with several of them characterized by a 1''' → 2'' bond

between sugars). The ginkgo leaf also contains flavan-3-ols, proanthocyanidins, and biflavonoids which are all 3'—> 8" biflavones (amentoflavone, bilobetol and 5-methoxybilobetol, ginkgetin, isoginkgetin, sciadopitysin). The buds are the organs with the highest acylated flavonoid content; the biflavonoid level is three times higher in the fall than in the spring, which is the season in which the monomer level is the highest.

Known as ginkgolides A, B, C, J (and M in the roots), ginkgo diterpenes have a very specific hexacyclic structure, characterized by a spiro-[4,4]-nonanic sequence, a *tert*-butyl group, and three lactone rings.

The fertilized ovules have a foul smell because they contain medium-chain fatty acids (C_4-C_8). The fleshy part contains alkenylphenols, which can be oxidized to quinones, which are capable of forming adducts with proteins and inducing cutaneous allergies. The central almond contains 4'-*O*-methylpyridoxine (= ginkgotoxin), which may be toxic*.

Pharmacological Activity. Ginkgolide B is an inhibitor of the *platelet activating factor* (= PAF), a phospholipid intercellular mediator secreted by platelets, leucocytes, macrophages, and vascular endothelial cells. This mediator is involved in various processes: platelet aggregation, thrombosis, inflammatory reaction, allergy, and bronchoconstriction (this explains the trials conducted in the late 1990s, particularly for the treatment of asthma). This anti-PAF activity and the activities of flavonoids, particularly as free radical scavengers, may explain the numerous properties of ginkgo extract that have been observed in animals and detailed in hundreds of research papers, reviews, and books. This extract is said to be a vasoregulating agent (an arterial vasodilator and a venous vasoconstrictor able to decrease capillary fragility), an inhibitor of cyclo-oxygenase and lipoxygenase, and an inhibitor of platelet and erythrocyte aggregation. It decreases capillary hyper-permeability, improves tissue irrigation, and activates cell metabolism, particularly in the cortex (by increasing glucose and oxygen uptake). The terpene-containing fractions prolong the survival of hypoxic rats; they protect neurons and astrocytes from damage by transient ischemia.

Uses. Ginkgo leaves are used to produce an extract titrated to contain 24% flavonoids and 6% ginkgolides-bilobalide. This extract has undergone several dozen human clinical trials, especially to assess its efficacy for "cerebral insufficiency". A 1992 review analyzed eight of those studies, which the authors of the review found to

* In Japan, where the cooked almonds are traditionally eaten, several case reports of poisoning have been published, particularly in children. The main symptoms are the loss of consciousness and convulsions. They are explained by the antagonist effect of 4'-*O*-methyl-pyridoxine on vitamin B_6. Since the same causes produce the same effects, this syndrome is reminiscent of that which is observed in the south of Africa, in animals intoxicated by the pods of *Albizia versicolor* Welw. and other species in the genus that contain the same toxin. The toxin is also found in the leaves, but the quantity used in medicines (<10 µg) is harmless (11 mg/kg are necessary to induce convulsions in the guinea pig, 50 mg/kg to cause its death).

be "well-conducted trials". Seven trials showed the beneficial effect of ginkgo extract (120-160 mg/day x 12 weeks, subjects' age 59-82) on the symptoms of "cerebral insufficiency" in the elderly (difficulties concentrating, memory loss, confusion, mood problems, lack of energy, headaches). Two other trials, despite the fact that some reviewers fault them for minor bias, indicated a positive effect on intermittent claudication; further research seems necessary. These and other trials were not unanimously well received, and this is apparent in the wording approved by French regulatory texts for the indications of ginkgo extract-containing medicines: "proposed" to correct the symptoms of senile cerebral insufficiency; "proposed" for some types of vertigo or tinnitus or both, for some types of loss of hearing or loss of visual acuity (thought to be of ischemic origin); and "proposed" for retinal insufficiency likely to be of ischemic origin. Only one indication is not preceded by the keyword "proposed": the symptomatic treatment of the intermittent claudication due to chronic occlusive arterial disease of the lower limbs. For this indication, ginkgo extract is more efficacious than merely adopting a healthy lifestyle and diet. The side effects of the oral use of the extract are rare and minor (headaches, digestive symptoms). In contrast, parenteral administration has caused serious accidents. The parenteral route is not used in France, but it was recently selected to test the potential benefit of ginkgo extract in the treatment of mild dementia (Alzheimer's* and others). Note, however, that subdural hematomas were reported in 1996: a woman experienced headaches and nausea after using 2 x 60 mg/day of ginkgo for 2 years (form not specified). The bleeding time was greatly lengthened and was returning to normal one month after discontinuing ginkgo. This accident might have been the consequence of the anti-platelet-aggregating activity of ginkgo **. Combinations (e.g., with heptaminol and trihydroxyethylrutin [orally], or butoforme [topically]) are indicated to treat the symptoms related to venous and lymphatic vessel insufficiency, and the functional symptoms of the acute attack of piles.

● Passion Flower, *Passiflora incarnata* L., Passifloraceae

The dried aerial parts of the passion flower are listed in the 10th edition of the French Pharmacopoeia. Since the constituents responsible for the sedative activity of the drug are not known with certainty, the preparations in use systematically

* The published results of a clinical trial indicate that long term oral administration of ginkgo extract may stabilize or even modestly improve the patients' cognitive performance and behavior. See Le Bars, P.L., Katz, M.M., Berman, N., Itil, T.M., Freedman, A.M. and Schatzberg, A.F. (1997). A Placebo-controlled, Double-blind, Randomized Trial of an Extract of *Ginkgo biloba* for Dementia, *JAMA*, **278**, 1327-1332.

** Since then, at least two more case reports of incidents apparently linked to ginkgo administration have been published: 1° Rosenblatt, M. and Mindel, J. (1997). Spontaneous Hyphema Associated with Ingestion of *Ginkgo biloba* Extract, *New Engl. J. Med.* 336, 1108; 2°

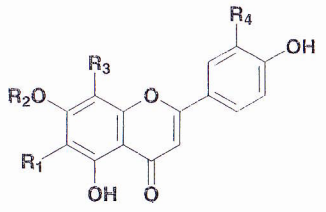
contain the constituents as a group (see the French "Herbal Remedies, Notice to Applicants for Marketing Authorization" 90/22 *bis*, chapter I., 2.1.1., fifth paragraph).

The Plant, the Drug. The official passion flower grows wild in the bushes of the south of the United States and Mexico. It is a creeping plant with alternate leaves with a finely serrate margin and long petioles. Axillary tendrils allow the plant to climb onto supports. The large solitary flowers (5-9 cm in diameter) are characterized by five thick sepals which are white on the underside, five white petals, a double crown of petaloid appendices, crimson red on the edge, stamens with orangy anthers, and a unilocular ovary with three stigmas. The fruit is ovoid and resembles a small, flattened, greenish to brownish apple with yellow flesh.

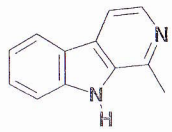
The drug includes fragments of lignified, hollow, grayish stem, with slender and smooth tendrils. The leaf has a long petiole and is deeply divided into three lobes with the middle one being most developed. The drug may be falsified with the stems and leaves of *P. edulis* Sims, whose fruit, the passion fruit, is edible—*f. edulis* with purple fruits and *f. flavicarpa* Degener with yellow fruits are cultivated— and whose leaf blade is dentate. There is another falsification which is easier to detect: that by *P. cærulea* L., a species cultivated for its ornamental blue flower crowns, whose leaves are pentalobate.

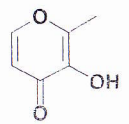
Chemical Composition. Next to phenolic acids, coumarins, phytosterols, 1 mL/kg essential oil, and cyanogenic glycosides (gynocardin), the drug contains 0.05% maltol (or 2-methyl-3-hydroxypyrone, maybe an artefact), and traces of indole alkaloids: harman, harmol, harmine). All of the later studies, except two, characterized only harman, and only at very low concentrations. In fact, the most recent work (HPLC) showed that harman was not detectable in the majority of commercial specimens. In one sample, its concentration was 0.1 ppm, far from the 0.01-0.09% published in the late 1950s.

The drug can contain up to 2.5% flavonoids. The major ones are flavone di-*C*-glycosides: shaftoside and isoshaftoside (apigenin *C*-glucosyl-*C*-arabinosides, 8,6



	R_1	R_2	R_3	R_4
vitexin	*H*	*H*	*glc*	*H*
isovitexin	*glc*	*H*	*H*	*H*
orientin	*H*	*H*	*glc*	*OH*
iso-orientin	*glc*	*H*	*H*	*OH*
saponarin	*glc*	*glc*	*H*	*H*
schaftoside	*glc*	*H*	*ara*	*H*
isoshaftoside	*ara*	*H*	*glc*	*H*
vicenin-2	*glc*	*H*	*glc*	*H*





and 6,8 isomers) and 2''-*O*-glycosides of isovitexin and iso-orientin (i.e., apigenin and luteolin *C*-sophorosides). Other compounds have been characterized: isovitexin, iso-orientin, vicenin-2 (apigenin di-*C*-glucoside), the 2''-*O*-glucoside of isoscoparin, swertisin, and lucenin-2 (luteolin di-*C*-glucoside). Saponarin (isovitexin 7-*O*-glucoside) which had been described in the drug in the late 1960s, was not found again in recent analyses: it is thought that 2''-*O*-glucosyl-isovitexin was mistaken for it. The qualitative composition can vary widely. In general, isovitexin and its glucosyl derivative are dominant.

Tests. The drug is identified by the macro- and microscopic characteristics of the leaf section: salient vein on the underside, covering trichomes generally unicellular, prominent conducting system composed of three large cribriform vascular bundles surmounted by a fourth, inverted one. The assay *per se* includes TLC analysis of flavonoids and alkaloids. A study of the flavone glycoside content allows identification of the official species and rejection of the non-official ones (*P. cærulea* and *P. edulis*), by TLC of a methanol extract and characterization of orientin, vitexin (actually these flavonoids occur in very small quantities), iso-orientin, and *saponarin* (?). After a specific extraction of alkaloids, the chromatogram of the residue *may have* a spot identical to that obtained with harman. The French official drug must contain not less than 0.8% total flavone derivatives, expressed as vitexin (by measuring the absorbance after reaction with $AlCl_3$).

Pharmacological Activity. Tradition attributes to the passion flower sedative, antispasmodic, and "tranquilizing" properties partially confirmed by animal experiments (IP route). In the absence of clinical trials conducted according to current methodological standards, a body of observations supports the usefulness of "neurosedative" preparations of this drug. What are the substances responsible such an activity? Maltol? It is a depressant, but its concentration in the drug is insignificant. Alkaloids? In fact, like most β-carbolines, they are CNS stimulants, MAO inhibitors, and some of them are hallucinogenic. In any event, their concentration, when they are even found, is minute. Flavonoids? Recently, an Argentinean group discussed the possible anxiolytic effect of the 5,7-dihydroxy-flavone isolated from *P. cærulea* L. and showed that it is a ligand for benzodiazepine receptors (but this flavone was not identified in the official species). Work by other authors on *P. alata* Aiton suggests a synergy instead. Other experiments confirm the activity of the passion flower extract on the CNS of the rat and point to the existence of two active compounds not yet identified, one of which is lipophilic and the other very polar, and neither of which corresponds to any alkaloid or flavonoid structure described to date in the drug.

Uses. The drug (in infusions), its galenical preparations (powders, extract, tincture, nebulisate) and the phytopharmaceuticals containing it are traditionally used by the oral route to treat abnormalities of the cardiac rhythm in the adult (normal heart), and to treat the symptoms of nervousness in adults and children, particularly minor sleeplessness [French Food Nat. 1998]. This use is doubtful or unjustified

hawthorn * with which it is frequently combined; other common combinations are with valerian and other sedative plants. The drug is reputed to be harmless **.

In Germany (Commission E), the indications are similar: nervous restlessness, "a motility-inhibiting effect repeatedly observed in animals". Package inserts are to also mention mild sleeping difficulties and gastrointestinal signs of nervous origin.

• THYME, *Thymus vulgaris* L., *T. zygis* L., Lamiaceae

This Mediterranean Lamiaceae is an antibacterial and spasmolytic, but above all it is an "essential oil-containing drug", and it will be discussed as such in this book (see p. 545). It is unlikely that the constituents of the essential oil alone are responsible for the spasmolytic activities recognized for the aqueous preparations of the flower and flowering tops. In fact, Lemli and Van den Broucke have shown that the concentration of the volatile essential oil phenols in these preparations is insufficient to account for the spasmolytic activity, which is due to polymethoxyflavones and di-, tri-, and tetramethoxylated flavones, all substituted at C-6.

• ROMAN CAMOMILE, *Chamaemelum nobile* (L.) All., Asteraceae.

"Roman camomile flower consists of the dried flower-heads of the cultivated double variety of *Chamaemelum nobile* (L.) All. [...]" (Eur. Ph., 3rd Ed.). Like in the previous case, the activity attributed to this drug is in part due to flavonoids.

The Plant, the Drug. Roman camomile is a perennial plant with ramified stems and pinnatisect pubescent leaves of a whitish-green color. The capitulums of the cultivated variety have a diameter ranging from 8 to 20 mm. They practically only have ligulate flowers which are white, sterile, and inserted onto a solid receptacle which bears, between the flowers, elongated and translucent paleas. The capitulum involucre is reduced to two to three rows of tight and imbricate bracts which are scarious on the edges. The ligules are lanceolate with three veins and five teeth.

The pharmaceutical market is supplied by culture (in France and Belgium among other countries).

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* Hawthorn contains, alongside proanthocyanidins that are active on the heart, flavone mono *C*- and di-*C*-glycosides closely related to those of the passion flower.

** Recently, five case reports in which altered mental status was observed were published i Norway, following the use of a passion flower-based product (Solbakken, A.M., Rørbakken G. and Gundersen, T. (1997). Naturmedisin som rusmiddel [A Herbal Product Used fo Intoxication]. *Tidsskr. Nor. Laegeforen.* **117**, 1140-1141).