Vaccinium

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Tannins

1. GENERALITIES

Historically, the importance of tannin-containing drugs is linked to their tanning properties, in other words their ability to transform fresh hides into an imputrescible material: leather. In this day and age, tanning is achieved with mineral compounds, but for several millennia, it had required exclusively the use of plant products. These included, in Europe, chestnut tree tannin (*Castanea sativa* L.), oak tannin (*Quercus robur* L., *Q. petræa* [Mattuschka] Liebl.), but also, in other parts of the world, tannins from Anacardiaceae (quebracho [*Schinopsis* spp.], sumacs [*Rhus* spp.]), Legumes (acacias [*Acacia* spp.], dividivi and tara [*Caesalpinia* spp.], algarobilla [*Balsamocarpon* sp. ≈ *Caesalpinia* sp.]), or Combretaceae (myrobalans [*Terminalia* spp.]). Some of these tannins are still prized for leathers designed for specific uses (fine leather products).

The consequence of tanning is the formation of bonds between the collagen fibers in the hide, which imparts resistance to water, heat, and abrasion. This capability of tannins to combine with macromolecules explains why they precipitate cellulose, pectins, and proteins; it also explains their characteristic astringency and tartness: by precipitating the glycoproteins contained in saliva, tannins make the latter lose its lubricating power.

The combination between tannins and macromolecules is established by hydrophobic interactions and hydrogen bonds between the phenolic groups of tannins and the proteins or other polymers. Other types of linkages (irreversible) must also be involved to ensure the lasting stability of the combination between tannins and collagen structures. They include the covalent bonds established after oxidation of the phenols to quinones. There is a necessary condition to the formation of these linkages: the tannin molecular weight must fall within a well-defined range. If it is too high, the molecule cannot insert itself into the interfibrillar spaces of the macromolecule; if it is too low, the molecule can insert itself but cannot form enough bonds to stabilize the combination.

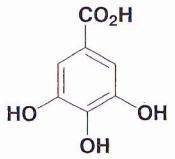
These properties are the basis of the classic definition of tannins: "water-soluble phenolics of molecular weight between 500 and 3,000, which, in addition to displaying the classic reactions of phenols, can precipitate alkaloids, gelatin, and other proteins" (Bate-Smith and Swain, 1962). Although this definition remains valid, it began to lose its interest when clear ideas developed about the exact chemical structure of the complex *polyphenols* that *proanthocyanidins* and *galloyl polyesters* are (these three terms tend to replace that, less precise, of tannin). More recently, Mole and Waterman (1987) defined tannins as "phenolic natural products that precipitate proteins from their aqueous solutions".

Since the late 1970s, the rapid developments in structural investigation methods have made possible considerable advances in the knowledge of the structure of tannins. This is especially true for fast-atom bombardment mass spectrometry (FAB-MS, in the positive $[M+H]^+$ or negative $[M-H]^-$ mode) which allows, in spite of the polarity and thermal instability of these large molecules, the determination of their molecular weight, and to observe significant fragmentations, particularly by adding salts to the matrix (KCl, NaCl) to observe peaks at $(M+K)^+$ or $(M+Na)^+$. NMR (^1H, ^{13}C) helps to characterize the monomeric building blocks and linkages. The structural complexity of tannins still makes it necessary to resort to more or less sophisticated chemical analyses (e.g., hydrolysis of oligomers, degradations, rearrangements, correlations, DP [degree of polymerization] determination).

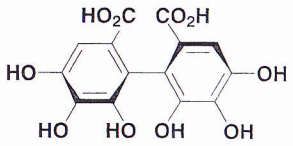
2. CLASSIFICATION OF TANNINS

In higher plants, two groups of tannins are generally distinguished, which differ by their structure, as well as their biogenetic origin: hydrolyzable tannins and condensed tannins.

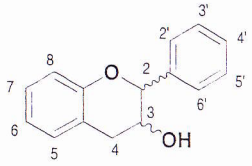
Hydrolyzable tannins. Hydrolyzable tannins are esters of a sugar (or related polyol) and of a variable number of phenolic acid molecules. The sugar is most generally glucose. The phenolic acid is either gallic acid, in the case of *gallotannins*, or else *hexahydroxydiphenic* acid (= HHDP) and its oxidized derivatives (*dehydrohexahydroxydiphenic* acid [= DHHDP]; chebulic acid), in the case of the tannins conventionally (but improperly) referred to as *ellagitannins* *. Since 1985, several representatives of a new category of tannins have been isolated. These, the *complex tannins*, are modified ellagitannins resulting from the addition of a phenylchromane derivative onto a molecule of HHDP ester of glucose: flavanol (flavano-ellagitannin), procyanidin (procyanidino-ellagitannin), or flavonol



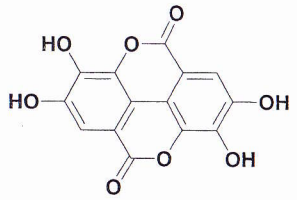
Gallic acid



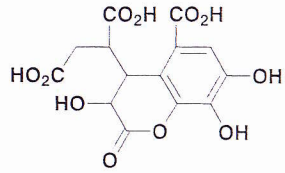
(S)-Hexahydroxydiphenic acid



Favan-3-ol



Ellagic acid



Chebulic acid

* Ellagitannin, nut*GALL* tannin (spelled backwards as e*LLAG*!); proanthocyanidin: the term is reminiscent of the fact that these molecules yield (in acidic medium and at high temperature) anthocyanins

(flavono-ellagitannin). Gallotannins, ellagitannins, and dehydroellagitannins (simple or complex) are characteristic of Dicotyledon Angiosperms (especially Rosidae, Dileniidae, Hamamelidae) except for Asteridae in which they do not commonly occur.

Condensed tannins. Condensed tannins or *proanthocyanidins* are polymeric flavans. They consist of flavan-3-ol units linked together by carbon-carbon bonds, most often 4–>8 or 4–>6, which result from coupling between the electrophilic C-4 of a flavanyl unit from a flavan-4-ol or flavan-3,4-diol and a nucleophilic position (C-8, less commonly C-6) of another unit, generally a flavan-3-ol.

Proanthocyanidins have been isolated or identified in all plant groups, including in Gymnosperms and Pteridophyta.

3. STRUCTURES OF HYDROLYZABLE TANNINS

In general, gallotannins are esters of gallic acid and glucose. However, mono- and digalloylglucoses are devoid of the classic properties of tannins, because their molecular weight is too low. These properties, particularly the ability to precipitate proteins, are only true for triesters and their higher homologs.

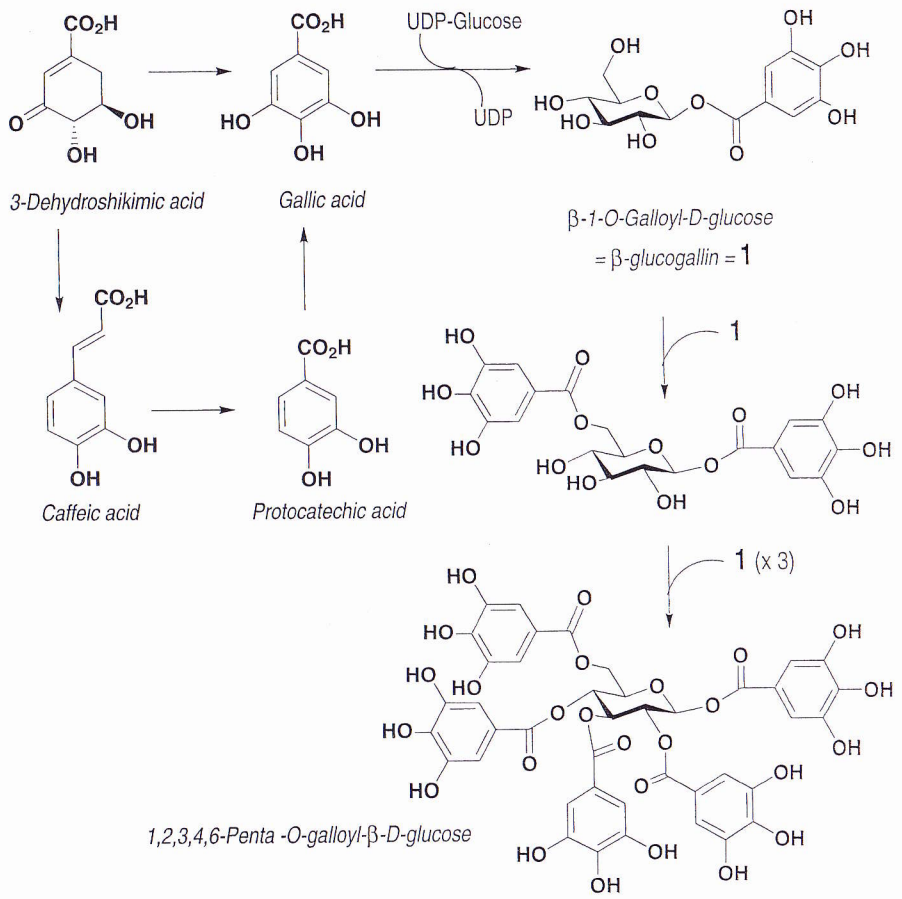
Biogenetically, gallic acid (= 3,4,5-trihydroxybenzoic acid) arises from the metabolism of shikimic acid. It is accepted that it is generally formed by direct dehydrogenation of 3-dehydroshikimic acid, or, in some cases, by oxidation of protocatechic acid (itself derived from a C_6-C_3 acid, caffeic acid). The glucosylation involves *uridine diphosphate glucose* (UDP-glucose). It is generally accepted, on the basis of *in vitro* experiments, that the resulting monogalloylglucose—β-glucogallin—units can function as glucose donors or acceptors, which leads to a diester, 1,6-di-*O*-galloyl-β-D-glucose, and that these steps repeat themselves *via* 1,2,6-tri-*O*- and 1,2,3,6-tetra-*O*-galloyl derivatives, until pentagalloylglucose is formed.

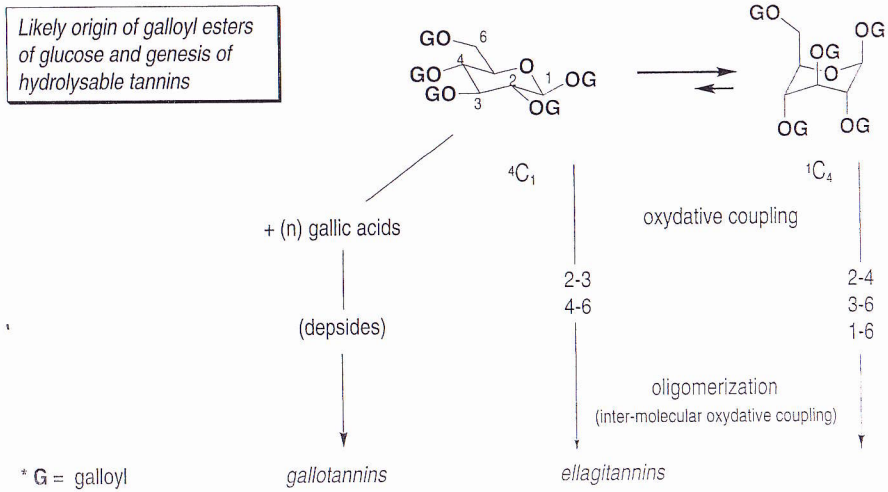
A. Monomeric Hydrolyzable Tannins

The above pentaester (1,2,3,4,6-penta-*O*-galloyl-β-D-glucose) is the most common tannin: it plays a central role in the metabolism of tannins, because most plants can metabolize it, following one of two types of pathways:

1. Evolution toward a heavier molecule (hexa- to undecagalloylglucose) by the formation, at C-3, C-4, C-6, or a combination of those, of side chains consisting of several gallic acids bonded by *meta*- or *para*-depsidic linkages (the two forms are in equilibrium in solution through acyl migration). These gallotannins are characteristic of a small group of families, including Anacardiaceae (*Rhus*), Fagaceae (*Quercus*), Ericaceae, Geraniaceae, and Aceraceae.

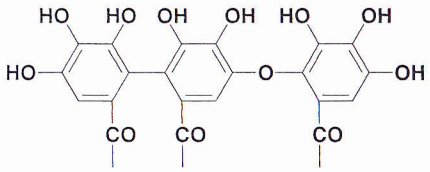
2a. Formation of ellagitannins. The C-2–C-2' oxidative coupling of the galloyl units of the molecule yields a dicarboxylhexahydroxybiphenyl moiety. The



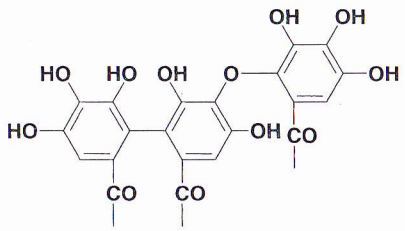


pentasubstitution of the glucose makes possible several couplings between the galloyl residues in the relative 1,2- or 1,3-positions, to form hexahydroxydiphenoyl esters; the molecule may be a mono- or bis-HHDP ester, with the other hydroxyl groups on the glucose unit remaining in the free state, or else becoming esterified by gallic acid, or by a dehydrodigallic acid (e.g., A and B agrimonic acids).

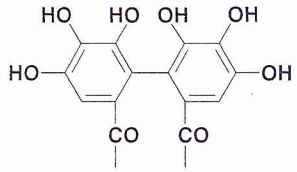
Various ellagitannins comprise in their structure a trigallic sequence—valoneyl, macaranoyl, or tergalloyl—arising from the etherification of the HHDP group by a gallic acid (e.g., rugosins A and B). This type of sequence can also lead to a depsidone (e.g., praecoxin D) or a dilactone (e.g., tirucallin A). The most frequent oxidative couplings involve the galloyl residues at C-2–C-3 and at C-4–C-6 of a glucose molecule in the $^4C_1$ conformation. Due to atropisomerism, HHDP can occur as *R* or *S*, and the chirality can be determined by circular dichroism.



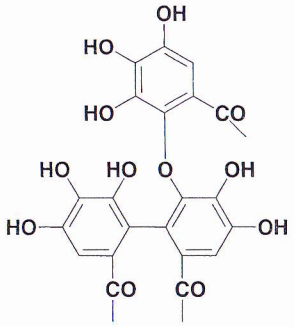
Valoneoyl group (—> m-DOG)



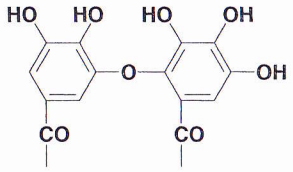
Tergalloyl group (—> p-DOG)



Hexahydroxydiphenoyl group



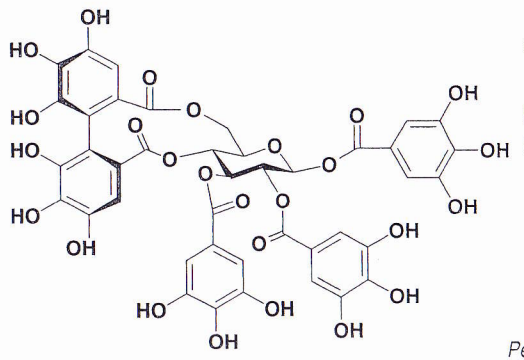
Macaranoyl group



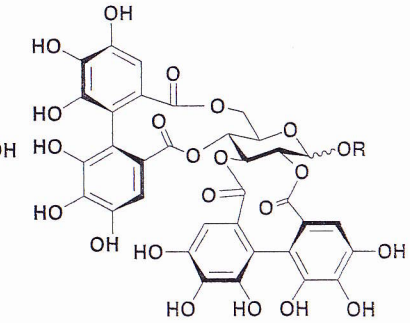
Dehydrodigalloyl group (—> m-GOG)

2b. Subsequently, the metabolism of these HHDP esters may go further, with the oxidation of HHDP to DHHDP (dehydrohexahydroxydiphenoyl homologs, characteristic of dehydroellagitannins, e.g., geraniin), or the opening and rearrangement of the DHHDP rings (to chebuloyl homologs, characteristic of chebulinic and chebulagic acid in myrobalans). The oxidative coupling most often involves the galloyl residue at C-2–C-4 or C-3–C-6 of the glucose unit, which imposes upon the latter the less favorable $^1C_4$ configuration (e.g., in geraniin or chebulagic acid).

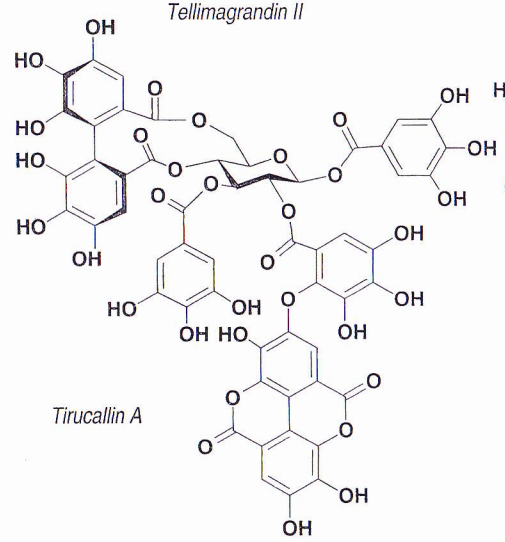
In some cases, the glucose pyran ring opens, allowing the free aldehyde function to react with the aromatic ring of a galloyl residue (and form, for example, castalagin, vescalagin, casuarinin, and other compounds commonly found in Fagaceae, Myrtaceae, Rosaceae, and Lythraceae). Finally, it is the condensation of this type of molecule (*via* its C-1) with the C-8 or the C-6 of a flavan (or flavone) which leads to



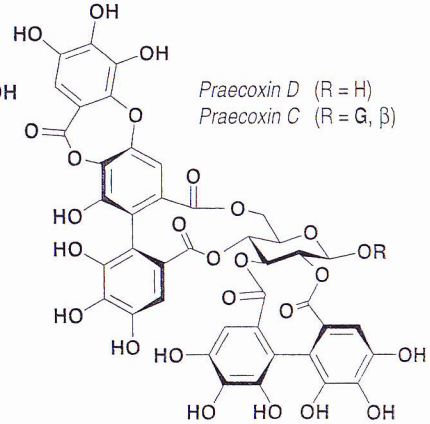
Tellimagrandin II



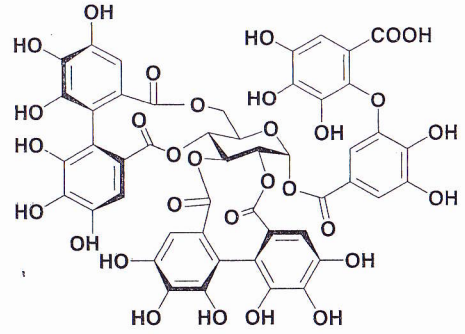
Pedunculagin (R = H)
Casuarictin (R = G, β)
Potentillin (R = G, α)



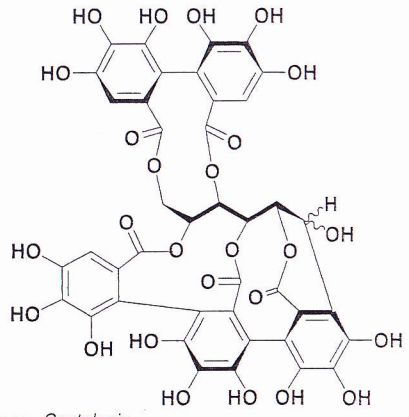
Tirucallin A



Praecoxin D (R = H)
Praecoxin C (R = G, β)

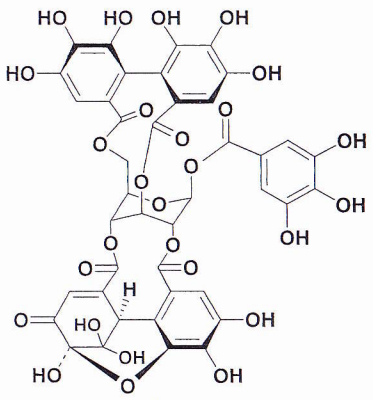


Agrimonic acid A

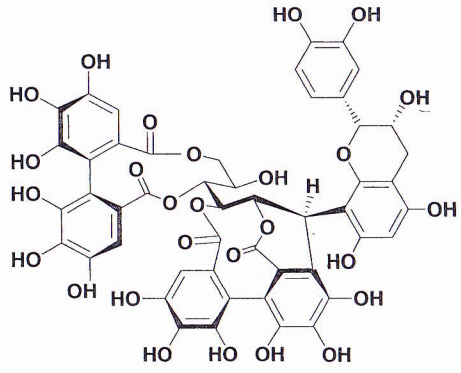


OH *Castalagin*
OH *Vescalagin*

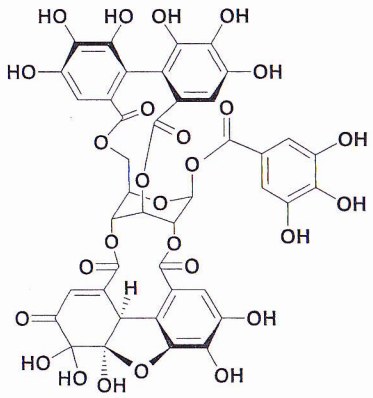
Examples of structures of hydrolyzable tannins

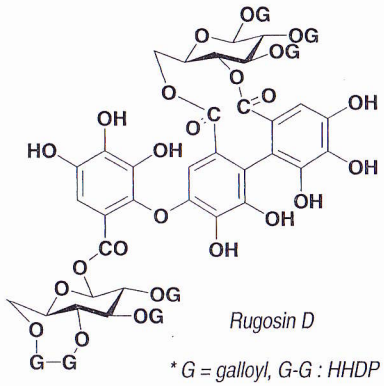


Geraniin



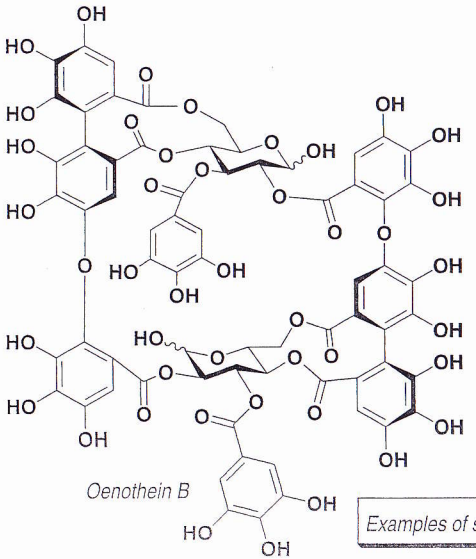
Cameliatannin



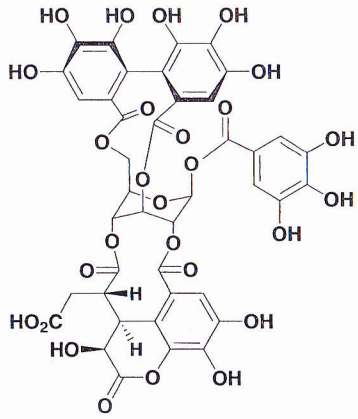


Rugosin D

* G = galloyl, G-G : HHDP



Oenothein B



Chebulagic acid

Examples of structures of hydrolyzable tannins (continued)

the complex tannins mentioned above, which are common (e.g., Fagaceae, Combretaceae, Myrtaceae, Melastomataceae [e.g., camelliatannin A]).

B. Oligomeric Hydrolyzable Tannins

Intermolecular oxidative coupling (C-C or C-O-C) explains the existence of a large number of oligomeric ellagitannins of molecular weight between 2,000 and 5,000. Thus, rugosin D, isolated from *Filipendula ulmaria* and from other Rosaceae, has a molecular weight of 1,874; it is the "dimer" of tellimagrandin II or 1,2,3-trigalloyl-4,6-hexahydroxydiphenoyl-β-D-glucose.

The distribution of the oligomeric forms of hydrolyzable tannins seems limited to Dicotyledons, excluding gamopetalous plants. The knowledge of their structure has advanced quite rapidly: ten years after the description of the first dimer (agrimoniin, 1982), 150 structures were described (about 85% dimers and 10% trimers). In view of this great structural diversity, some authors have proposed to classify these compounds as a function of the nature of the residues engaged in the monomeric unit linkages (e.g., gallic acid or HHDP), and as a function of the linkage type. These criteria distinguish five groups:

1. GOG (or GOGOG). The linkage unit is composed of two (or three) galloyl residues (G), linked by an ether bond involving the hydroxyl group *meta* (*m*-GOG = dehydrodigalloyl), or *para* (*p*-GOG = isodehydrodigalloyl) to the carboxyl group of one unit, and the hydroxyl group *ortho* to the carboxyl group of the other unit. An example is agrimoniin (*m*-GOG) in Rosaceae, including *Agrimonia*, *Potentilla*, and *Rosa*; another example is nupharine in Nymphaeaceae;

2. DOG. The linkage unit is of the tergalloyl (i.e., *p*-DOG) or valoneyl (i.e., *m*-DOG) type. The ether bond involves a hydroxyl group that is *meta* or *para* to a hexa-hydroxydiphenoyl residue (HHDP = D). Examples of this type include rugosins, and œnothin (which has an additional macrocycle) from various Onagraceae (*Oenothera, Epilobium*) and Lythraceae;

3. GOD. Oxidative coupling between the carbon atom of a HHDP residue and the oxygen atom of the hydroxyl group of a galloyl residue links the two monomers: the resulting moiety is referred to as "sanguisorbyl" (e.g., sanguiin of *Sanguisorba* and *Rubus*);

4. $D(OG)_2$ (*m,m'*-, *m,p*-). The unit that links the two monomers involves two ether bonds between the hydroxyl groups of two galloyl units and one HHDP unit, for example in euphorbins. The structure may also be a macrocycle, for example in woodfordins;

5. Oligomeric *C*-glycosidic ellagitannins, for example roburins of oak wood.

Comments

1. Compounds built upon a polyol other than glucose are rare, but examples exist and include the following: "hamamelitannin" or 2',5-di-*O*-galloyl-α-D-hamamelose, which may not be considered a tannin in the strict sense of the term, due to

its low molecular weight; aceritannin or 2,6-di-*O*-galloyl-1,5-anhydro-D-glucitol and its tri- and tetragalloyl derivatives; the polygalloyl derivatives of quinic acids, constituents of tara tannin, extracted from the pods of *Caesalpinia spinosa* (Molina) Kuntze; and polygalloylshikimates (*Castanopsis* sp.).

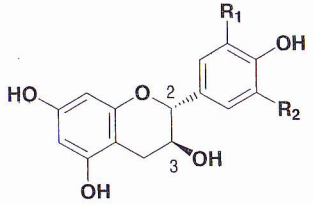
2. Sometimes the term tannin is applied to polymers of phloroglucinol, halogenated or not, isolated from several genera of Phaeophyceae Algae. These polymers are also known as phlorotannins.

3. Intentionally left out of the present section are flavanol gallates (see tea "tannin", p. 1077), glycosyl-flavonol gallates (even though some of them have biological properties similar to those of hydrolyzable tannins), and the gallates of phenolic glycosides, such as the galloyl derivatives of arbutin isolated from *Arctostaphylos* sp. (Ericaceae) or *Bergenia* sp. (Saxifragaceae).

4. STRUCTURES OF CONDENSED TANNINS (PROANTHOCYANIDINS)

The proposed proanthocyanidin nomenclature was initially based on the name of the anthocyanidin that is formed when the polymer is treated with an acid at high temperature: procyanidin, prodelphinidin, or propelargonidin. Now it is more often based on the name of the monomer from which the molecule is built. Although it is convenient to use common names, one recommendation is to name the structures according to a rule inspired by the nomenclature of oligo- and polysaccharides: name the units, and indicate the position and the direction of the interflavanoid bond(s) in parentheses. For example, aesculitannin A (isolated from the seminal tegument of the horse chestnut) is epicatechin-(4β–>8)-epicatechin-(4β–>8, 2β–>7)-epicatechin-(4β–>8)-epicatechin.

2*R*, 3*S* series:
$R_1 = R_2 =$ H: *Afzelechin*
R_1 = OH, R_2 = H: *Catechin*
$R_1 = R_2 =$ OH: *Gallocatechin*



2*R*, 3-*R* series: (OH 3-α): *Epiafzelechin, Epicatechin, Epigallocatechin*

The basic structural element of these polymers is a flavan-3-ol: catechin and epicatechin (3,5,7,3',4'-pentahydroxylated, constituents of procyanidins), gallocatechin and epigallocatechin (3,5,7,3',4',5'-hexahydroxylated, constituents of prodelphinidins), fisetidinin (3,7,3',4'-tetrahydroxylated, constituents of profisetidinins), and the less common afzelechin and epiafzelechin (3,5,7,4'-tetrahydroxylated, constituents of propelargonidins), to name only the main examples.

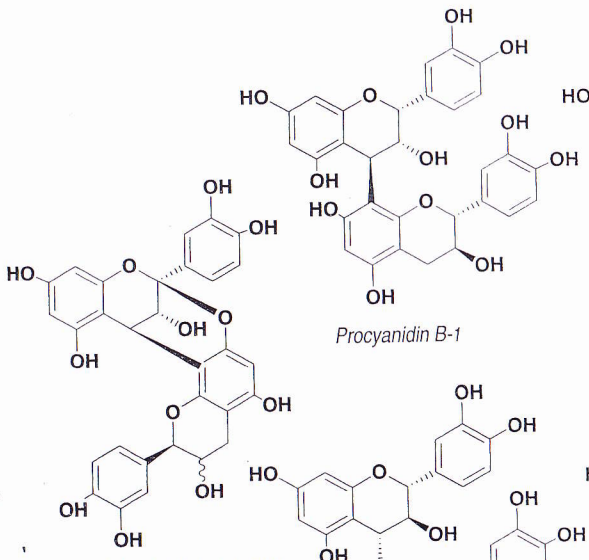
Biogenetically, these flavan-3-ols arise from the metabolism of flavanoids (see previous chapter). They are formed by the 3-hydroxylation of a flavanone. The

resulting 2,3-dihydroflavon-3-ols are subsequently reduced to flavan-3,4-diols, then to flavan-3-ols by a mechanism that remains, in part, hypothetical. The common configuration of the flavan-3-ols is (2*R*,3*S*) or (2*R*,3*R*) (in the *epi* series). Enantiomers such as *ent*-catechin (2*S*,3*S*) are far less common.

Chemically, the formation of oligomers and polymers involves flavan-3,4-diols: these molecules, highly reactive because of the benzylic character of their 4-hydroxyl group, readily form a carbocation which immediately reacts, either with the nucleophilic C-8 or C-6 of a flavan-3-ol. The repetition of this mechanism leads to oligomers and polymers.

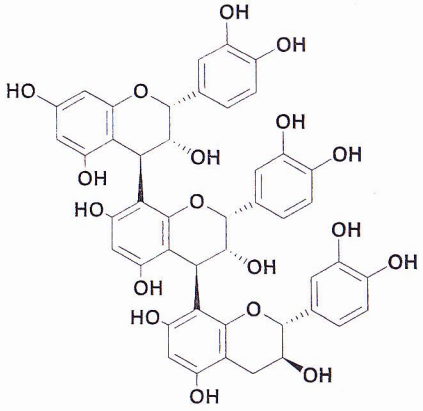
Type B proanthocyanidins. The simplest dimers are procyanidins B-1, B-2, B-3, and B-4, in other words proanthocyanidins consisting of two units of (2*R*,3*S*)-(+)-catechin, (2*R*,3*R*)-(–)-epi-catechin, or one of each, with a 4–>8 linkage in an α (B-3 and B-4) or β (B-1 and B-2) configuration. These procyanidins occur in the free state, and are widely distributed.

Type A proanthocyanidins. Another important group of procyanidins consists of dimers with a double interflavanoid linkage: C-4–>C-8 and C-2–>*O*–>C-7. The best known are æsculitannins, which are procyanidins from the seminal tegument of the horse chestnut (*Aesculus*, see p. 694); they are also found in the bark of cassia cinnamon (see p. 549).

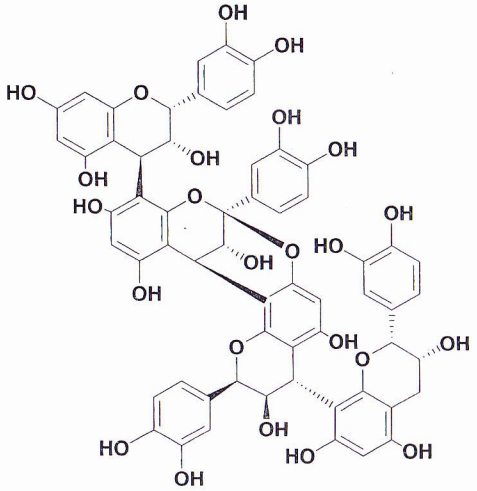


Examples of "dimeric" proanthocyanidins.

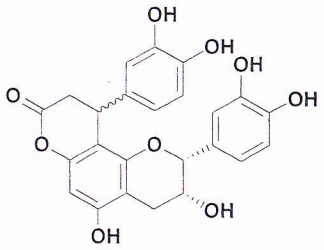
In group A, as in group B, the C-4–>C-8 linkage may be replaced by a C-4–>C-6 bond (e.g., procyanidin B-5). The other dimers (propelargonidins, prodelphinidins) are less common. Also known are procyanidin *O*- and *C*-glucosides (in official rhubarb and tea), C-6'–>C-6' dimers (in *Camellia sinensis* (L.) O. Kuntze), dimers involving a chalcane (assamicains), and enantiomeric dimers such as *ent*-procyanidin,



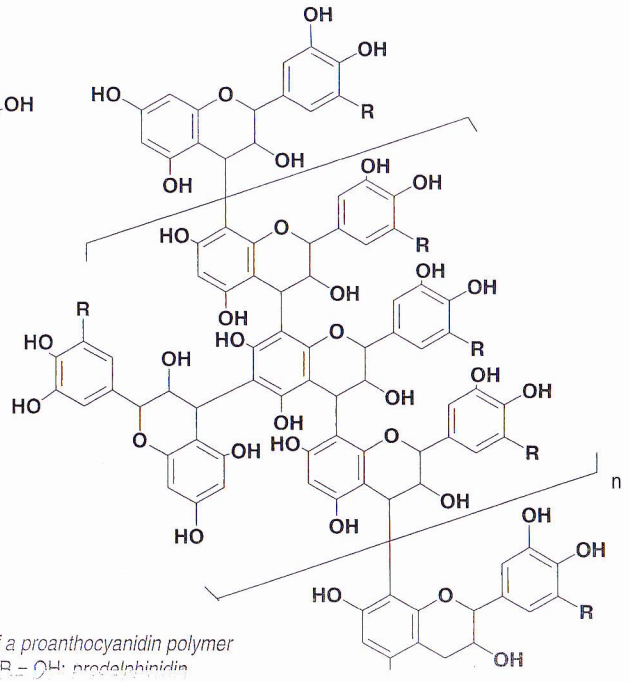
Epicatechin-(4β–>8)-epicatechin-(4β–>8)-catechin (trimer)



Epicatechin tetramer



Cinchonains



General structure of a proanthocyanidin polymer
R = H: procyanidin; R = OH: prodelphinidin

Examples of proantho-cyanidins structures

characterized in the bark of *Byrsonima crassifolia* (L.) Kunth, a tropical America Malpighiaceae that produces edible fruit.

Oligomers. Oligomers are formed by the stepwise addition of flavanoid units. Many structures are now known in group B: C-1 trimers (three epicatechins with 4β–>8 linkages), C-2 trimers (three catechins with 4β–>8 linkages), and the corresponding oligomers. Group A includes trimers and oligomers formed by the addition of a flavanoid unit onto a dimer whose monomeric units are linked by two bonds (e.g., æsculitannins and cinnamtannins).

Polymers. The polymers may include up to fifty monomer units. The most widespread are polyepicatechins and procyanidin-prodelphinidin copolymers. The interflavanoid linkage is chiefly of the C-4–>C-8 type, and is always *trans* relative to the 3-hydroxyl group (i.e., if the monomer unit is epicatechin (3*R*), then the C-4 is *R*). The examination of molecular models reveals a partial hindrance to rotation about the interflavanoid linkage, which results in the polymer being a left or right helix, as a function of the precursor (B-1, B-2 or B-3, B-4). The other polymers (propelargonidins and *ent*-flavan-3-ol polymers) are rather rare, and appear limited to Monocotyledons.

Finally, note that 1. flavanoid monomers can form bonds with caffeic acid to yield lactones (e.g., cinchonains and homologs) and that 2. condensed tannins can occur as galloyl esters. An example is the tannin from the unripe fruit of *Diospyros kaki* L. *f.* (Ebenaceae): half of the flavanoid units have a 3-*O*-galloyl substituent. This is also true of the condensed tannins of official rhubarb (p. 437).

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

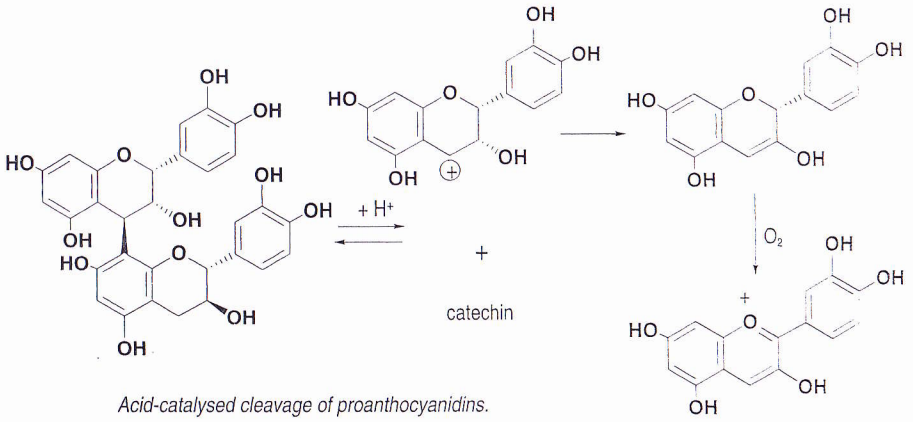
A. Properties

Tannins dissolve in water to form colloidal solutions, but their solubility varies with their degree of polymerization. They are soluble in alcohols and acetone. The stability of the aqueous solutions varies with the structure, and is generally moderate. For example, during the extraction with boiling water (i.e., in the conditions of a decoction), a tannin such as geraniin decomposes in 30 minutes to gallic acid, ellagic acid, and corilagin (= 1-galloyl-3,5-HHDP-glucose). The dimeric and oligomeric forms of galloyl and HHDP esters of glucose are also rather unstable. Like all phenols, tannins react with ferric chloride. Heavy metal salts and gelatin make them precipitate out of aqueous solutions. Hydrolyzable and condensed tannins may be distinguished based on their behavior in acidic medium at high temperature:

• **Hydrolyzable tannins** are polyesters of glucose, and upon hydrolysis, they release the sugar, and either gallic acid, hexahydroxydiphenic acid, or both. The

terminology of ellagitannins). Oligomer hydrolysis also yields compounds with three or four aromatic rings, whose structures vary depending on the nature of the bond between monomers. For example, since rugosin D (see above) results from the formation of a biphenylether linkage between a galloyl residue and a hexahydroxydiphenoyl residue, its hydrolysis gives a product with three rings, namely valoneic acid (actually its dilactone). Comment: in the case of polygalloylglucoses with a depsidic lateral chain, the depsidic bonds can be cleaved in the presence of mild acid at ambient temperature, and these conditions leave intact the ester linkages that involve the glucose hydroxyl groups.

• **Condensed tannins**, under the same experimental conditions, will see their interflavanoid bond cleaved, and in the presence of air, the carbocation that is formed leads to an anthocyanidin. Under controlled conditions, this reaction may be used for structural elucidation; the reactive intermediate may be trapped by an appropriate nucleophile. This nucleophile may be an alcohol or a thiol (toluene-2-thiol), but also a flavanoid derivative, which explains why the reaction also yields insoluble, deeply colored polymers: the phlobaphenes.



Acid-catalysed cleavage of proanthocyanidins.

B. Extraction

Tannins are generally extracted with a water and acetone mixture (methanol is to be avoided because it causes the methanolysis of galloyl depsides). The optimal yield is obtained from the fresh tissues, or from the frozen or lyophilized tissues, because in the dried drugs, part of the tannins is irreversibly combined to other polymers. After eliminating the acetone by distillation, the pigments and lipids are removed from the aqueous solution by a solvent extraction (e.g., with dichloromethane). Next, an ethyl acetate extraction of the aqueous solution separates the dimeric proanthocyanidins and most gallotannins. The polymeric proanthocyanidins and high molecular weight gallotannins remain in the aqueous phase. To obtain pure compounds, the appropriate chromatographic techniques are used, most often one of the gel filtration techniques, followed by reverse phase chromatography, again in water and alcohol, or water, alcohol and acetone mixtures.

C. Characterization

With ferric salts, gallotannins and ellagitannins give bluish-black colors and precipitates, and condensed tannins give greenish-brown precipitates.

Gallotannins give a pink color with potassium iodate (whereas free gallic acid gives an orange color with this reagent). Ellagitannins are colored by nitrous acid in the presence of acetic acid (pink at first, the color turns purple, then blue), and condensed tannins turn red with vanillin and hydrochloric acid.

To analyze extracts, conventional techniques are used: TLC (on cellulose or silica gel) with visualization by fluorescence under UV light and with the reagents mentioned above, or HPLC (reverse phase, with slightly acidic alcoholic solvents).

D. Quantitation

Tannin quantitation is delicate: complete extraction is difficult to achieve, and not all of the methods based on the phenolic character of these compounds are specific. Nevertheless, some of the methods are somewhat specific, particularly for condensed tannins. The best methods for tannin detection and quantitation measure their—specific—ability to precipitate proteins.

Quantitation methods involving protein precipitation

- One of the most frequently used methods is the hemolyzed blood method. It is based on the fact that tannins form a combination with hemoglobin, and that the residual, non-precipitated hemoglobin can be estimated colorimetrically against a blank, in a part of the spectrum where interferences are unlikely. A variation on this procedure makes use of a standard (for example tannic acid or geraniin) to determine the relative astringency (i.e., relative to that of the standard).
- There is a closely related method in which the hemoglobin is replaced with bovine serum albumin. The protein is solubilized at its isoelectric pH, then precipitated by the tannins contained in the extract to be quantitated; the protein concentration of the precipitate is determined colorimetrically after alkaline hydrolysis and reaction with ninhydrin. The protocol is simplified if the albumin is coupled with a dye beforehand.
- The traditional method using hide powder is also based on the fact that tannins react with proteins. This method is still used, especially to assay certain French official drugs (see purple loosestrife, bramble, and others, Fr. Ph., 10th edition). First, an infusion of the drug is prepared; one aliquot is evaporated to determine the total soluble matter (S), and in another aliquot the tannins are precipitated by the addition of hide powder; the precipitate is eliminated, and the supernatant is evaporated to dryness (residue N). The difference in weight between the two residues (S-N) corresponds to the weight of tannins contained in the aliquot.

Quantitation of condensed tannins. Proanthocyanidins can be quantitated by measuring the color (absorbance) obtained upon conversion to anthocyanidins by boiling in *n*-butanol in the presence of hydrochloric acid. The depolymerization is

accelerated and the reproducibility is improved by addition of ferric salts to th reaction medium (but the absorption at a given wavelength varies depending on th structure of the anthocyanidin that is obtained).

Proanthocyanidins can also be quantitated as vanillin addition products i methanol in the presence of hydrochloric acid. Vanillin, an aldehyde, forms a adduct at the 6-position of the flavanoid units, which are dihydroxylated at C-5 an C-7. *p*-Dimethylaminobenzaldehyde can be used instead.

Thiolysis is primarily used for structure elucidation and mean DP determination thiolysis followed by HPLC is a quantitation method (including for nonextractibl proanthocyanidins).

Quantitation of hydrolyzable tannins. Gallotannins can be hydrolyzed in th presence of sulfuric acid; following the reaction of the resulting gallic acid wit rhodamine, the absorbance of the product can be measured. The reaction wit potassium iodate can be be used instead.

The conventional quantitation procedure for ellagitannins involves a reactio with nitrous acid after sulfuric hydrolysis. The unsubstituted carbon atoms of th resulting ellagic acid are susceptible to electrophilic attack, therefore a quinon oxime is formed.

Total phenol quantitation. The general methods for total phenol quantitation ar sometimes used, in conjunction with the hide powder precipitation technique, t measure the total tannins in a drug. (See, among others, "total polyphenols" an "polyphenols not adsorbed by hide powder" in the European Pharmacopoeia, 3r Ed., under witch hazel or rhatany). In brief, this is a procedure derived from th Folin-Denis method: the phenolate ion—formed by adding sodium carbonate—i oxidized by a mixture of phosphotungstic and phosphomolybdic acid, which i simultaneously reduced to give a blue solution whose absorbance is determined.

6. BIOLOGICAL PROPERTIES OF TANNINS

Most of the biological properties of tannins are linked to their ability to for complexes with macromolecules, particularly with proteins (digestive and oth enzymes, fungal or viral proteins). This also explains the problems that they cause i food technology (cloudiness of beers), or in agriculture (humic acid formatio nutritional value of fodder).

- Reversible complexation. Under non-oxidizing conditions and physiological pH, complexation (by hydrogen bonds and hydrophobic interaction is reversible. The mechanism of this complexation appears to be a non-specif surface phenomenon. The tannins form, on the surface of the protein, a layer whic is less hydrophilic than the protein itself, and this causes precipitation; in additio they establish (in concentrated protein solutions) bonds between the protei

residues in the proteins, and with the flexibility of the protein conformation (salivary proteins, collagen). This affinity is highly dependent on the tannin molecular weight, and is maximal for pentagalloylglucose and its oligomers. The formation of biphenyl linkages (HHDP) decreases the conformational freedom of the molecule, and reduces its affinity for proteins. Molecules that are virtually rigid, such as vescalagin, have a very low affinity for proteins. For the same reason, proanthocyanidins, which have hindered rotation about the interflavanoid bond, have a lower affinity for proteins than do polygalloyl esters.

• Irreversible complexation. Given their marked tendency to spontaneously oxidize, the polyphenols that are tannins yield *o*-quinones, which react with the nucleophilic groups on proteins to form covalent bonds: the combination becomes irreversible (in the case of proanthocyanidins in acidic conditions, flavanoid carbocations might be involved in the formation of these bonds).

Advances in the field of purification and structural analysis of tannins have made pure substances available for research on their biological properties, many of which have been shown, at least *in vitro*: little is known on the intestinal absorption and metabolism of tannins, thus it is a stretch to even discuss their therapeutic (or health *) effects. At this time, no chemically defined molecule from the tannin group is used in therapeutics and there is virtually no relevant clinical study demonstrating the benefit of tannin-containing drugs. Yet these drugs remain in use, especially in phytotherapy.

Therapeutic activities due to the astringency. The applications of tannin-containing drugs are limited, and result from their affinity for proteins. Externally, they waterproof the external layers of the skin and the mucosas, thus protecting the underlying layers; they also have a vasoconstrictor effect on small superficial vessels. By limiting fluid losses, and by preventing external aggressions, tannins enhance tissue regeneration in case of superficial wound or burn. Internally, they are undoubtedly antidiarrheals. Regardless of the route of administration, these molecules have clearly demonstrated antiseptic effects (antibacterial and antifungal), which are of interest in the treatment of infectious diarrheas and dermatitis.

Antioxidant activity. Many tannins, especially hydrolyzable tannins, inhibit the lipid peroxidation induced by ADP and ascorbic acid in rat hepatic mitochondria. *In vitro*, they are (especially HHDP esters of glucose) free radical scavengers and inhibitors of superoxide ion formation, and some of them inhibit lipoxygenase, but not cyclo-oxygenase, in rat peritoneal granulocytes. Several compounds inhibit the autoxidation of methyl linoleate. *In vitro*, geraniin (or a metabolite?) lowers the serum levels of peroxidized lipids in rats.

* Except for some epidemiological data—on dietary tannin intake—and to the extent that a relationship can be found between the incidence of the disorder and the intake of one particular microconstituent.

Antioxidant flavanols and proanthocyanidins from grape juice and wine are widely considered to be the main principles responsible for the preventive effect on cardiovascular disease of a moderate and regular intake of red wine—the French paradox *.

Enzymatic inhibition. Generally speaking, tannins are enzyme inhibitors: they block 5-lipoxygenase (geraniin, corilagin); they inhibit angiotensin converting enzyme, hyaluronidase activation, and the glucosyltransferases of microorganisms involved in the formation of cavities; sanguiin H-6 or chebulagic acid inhibit topoisomerases; ellagitannins and complex tannins inhibit protein-kinase C, and so forth.

Other activities. A few ellagitannins counteract the mutagenicity of certain carcinogens, and are unfavorable to the transplantation of experimental tumors (by stimulating immune mechanisms). Inhibitory effects on virus replication have also been described *in vitro*: inhibition of virus adsorption onto cells, and inhibition of reverse transcriptase by dimers and galloyl derivatives.

Are some of the activities attributed to tannins the consequence of a simple, nonspecific tannin-protein interaction? Research published in 1997 showed, on the basis of radiolabeled-ligand receptor-binding experiments, that 1. for 10 of the 16 receptor types in the study, the binding of the ligand was not inhibited by tannins or phenols; 2. some polyphenols inhibited one or two types of receptors selectively (e.g., selectivity of pedunculagin, but not tellimagrandin, for the β-adrenergic receptor; of rugosin D for the α-2 receptor)

Procyanidin dimers, considered responsible for the positive inotropic and coronary vasodilator activities of the flowering tops of hawthorn (p. 396) also have

* The "French paradox" is (at first approximation) the lower cardiovascular mortality among residents of certain French cities, whose fat intake and tobacco use, combined with a distinct propensity for physical inactivity, would be expected to result in a mortality at least similar to that of residents of other westernized cities. The only difference of note between the populations of interest is the much higher alcohol consumption in France—mostly as wine.

Grape is rich in antioxidant phenolics: concentrated in the skin, the rachis, and most of all in the seeds, these compounds are monomers, dimers, and mostly polymers of catechin, epicatechin, and related derivatives, sometimes galloyl derivatives. Depending on the variety, they occur alongside anthocyanin and flavonoids. In red wines, the composition varies depending on the winemaking method (the major part remains in the marc, i.e., the expression residue) and the aging of the wine, which induces a variety of chemical transformations (not all of which are known). The cultivar, production year and site, and degree of ripeness of the grape all contribute to differences in initial composition.

Polyphenols are not the only active constituents of wines. Alcohol is known to have antiatherogenic properties, at least experimentally, and grapes—and wine—are known to contain trihydroxystilbenes and their glycosides (*Z*- and *E*-resveratrol, piceins) capable of inhibiting *in vitro* oxidation of LDL, platelet aggregation, and eicosanoid synthesis.

The controversy continues to generate an abundant literature. See, among others,: Watkins, T.R., Ed. (1997). Wine – Nutritional and Therapeutic Benefits, ACS Symposium series 661, American Chemical Society, Washington DC.

properties similar to those of flavonoids: decrease in capillary fragility and permeability, increase in vascular tone, and stabilization of collagen. Their inhibitory activity on histidine decarboxylase, elastase, and the angiotensin converting enzyme has been shown experimentally. Procyanidins B-2, B-3, and B-4 inhibit (10^{-5} M, > 80%) the binding of serotonin onto 5HT1 receptors.

Toxicity. The potential toxicity of tannins to humans is not very well known. In contrast, acorns and young oak leaves (*Quercus* spp.) are infamous for the risk that they represent for cattle. In most cases, the prognosis of the intoxication—initially marked by persistent constipation, then severe liver damage—is bleak. Again, it is not known whether the tannins or their metabolites are the actual toxins.

7. CHIEF TANNIN-CONTAINING DRUGS

• Oaks, *Quercus* spp., Fagaceae

(French) official tannin. Official tannin—it was described in the previous, 9th edition of the French Pharmacopoeia—can be prepared from the nutgalls of an oak found on the eastern side of the Mediterranean rim (*Quercus infectoria* Olivier). The formation of the galls is a consequence of the deposition of the eggs of hymenopterous insects (*Cynips*) in the oak tissues: the developing larva induces cellular proliferation in the host tissues. This proliferation manifests itself by the growth of a globose, hard, and dense mass—the gall—, the color of which varies with the stage of development. Within it, galloyl esters of glucose accumulate.

Galls contain hydrolyzable tannins (*m*-depsides of pentagalloylglucose, pedunculagin, tellimagradin-II, or casuarictin) in high proportion (50-70% in the case of *Q. infectoria*), free gallic and ellagic acids, sterols and triterpenes, and starch. Official tannin, also known as "tannic acid" (or in France, as ether-extracted tannin or *tanin "à l'éther"*), is prepared by extracting the galls with an ether and alcohol mixture saturated with water, separating the phases, and evaporating the aqueous layer.

Tannin is official in Switzerland (Helv. VII) and may be used externally as an astringent (for burns and dermatitis); it is also a hemostatic agent. It is incompatible with many other products (e.g., ferric salts, oxidants, proteins, alkaloids, glycosides).

Oak bark. Official in Switzerland for human as well as veterinary use, formerly listed in the United States National Formulary, oak bark is obtained from *Q. robur* L. (= *Q. pedunculata* Ehrh.), *Q. petræa* (Mattuschka) Liebl (= *Q. sessilis* Ehrh.), and *Q. pubescens* Willd. or, formerly in the United States, from *Q. alba* L. The drug contains tannins in a wide range of concentrations (8-20%, not less than 12% for the Swiss Pharmacopoeia). The composition of the bark of *Q. petraea* is best known, and includes pedunculagin, vescalagin, castalagin, mongolicanin—procyanido-ellagitannin—, flavano-ellagitannins (acutissimins, guajavin), oligomeric

proanthocyanidins, flavanoid monomers, and their galloyl esters. In Germany, Commission E recognizes astringent and virostatic properties for oak bark and specifies under uses: externally, for skin disorders (except in case of extensive damage); internally, for acute diarrhea; on the mucous membranes (throat, vagina) in case of moderate inflammation. There are no contraindications for internal use, but the absorption of basic medicines may be decreased and medical advice must be sought for diarrhea if it persists beyond 3-4 days. Bathing in oak bark preparations is contraindicated in case of hyperthermia, cardiac insufficiency, and weeping eczema. Package inserts may list the following indications: throat inflammations, foot perspiration, and the adjunctive treatment of anal fissures and chilblains.

• Witch Hazel, *Hamamelis virginiana* L., Hamamelidaceae

Dried Hamamelis leaf or Witch Hazel Leaves (Eur. Ph., 3rd Ed.) are alternative names for a drug used for its astringent and vasoconstrictor properties. The stem bark is also used for the same properties, mostly in Germany.

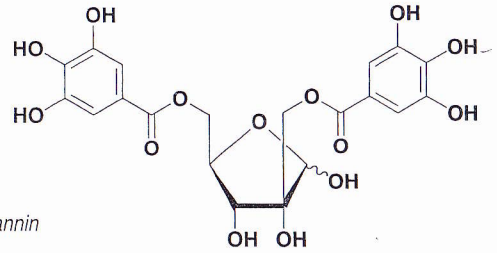
The Plant, the Drug. *Hamamelis* is a shrub or small tree, somewhat similar to the hazelnut tree. Known as witch hazel, it is common in the woods of the eastern American continent (Quebec and northeast United States, down to Virginia).

The branches are ramified, and bear leaves (5-12 x 3-8 cm) on short petioles, which are asymmetrical at the base, have crenate or sinuate blade margins, and have prominent pinnate veins on the underside. The tetramerous flowers blossom after the leaves fall. This imparts an ornamental character to this species. The drug, dull green or brownish-green, tastes slightly astringent. It can be identified by its microscopic characters, particularly by unicellular covering trichomes in groups of 4 to 12, located on the lower surface, near the veins; they can reach 250 µm in length and their lumen, which is clearly visible, often contains brown material. There are sclerites in the mesophyll, which are barely ramified, and enlarged at the ends.

Chemical Composition. Hamamelis leaves contain 0.05% essential oil (characterized by 2-hexen-1-al, acetaldehyde, and ionones), flavonol glucosides (astragalin, myricitrin), and up to 10% tannins (in the broad sense of the term): gallic acid, polygalloylglucose, hamamelitannin and analogues, monomeric flavanes, free and esterified (epicatechin gallate), and proanthocyanidins. Hamamelitannin is 2',5-di-*O*-galloyl-α-D-hamamelofuranose (as a 2:1 mixture with its β-anomer). It is found in the leaves and only in small amounts. The chief polyphenolic constituents of the leaves are procyanidins and procyanidin-prodelphinidin copolymers.

The bark of the stems or Hamamelis Bark is also rich in tannins, and hamamelitannin is the major one. It occurs alongside its very unstable α and β anomers and its 1-*O*-(4-hydroxybenzoyl) derivative—which may be the true

1-*O*-(4-hydroxy-benzoyl) ester, and an analog of hamamelitannin, 2',4-di-*O*-galloyl-D-hamamelopyranose. The bark also contains proanthocyanidins: dimeric procyanidins and prodelphinidins, as well as an oligomer. Some of these proanthocyanidins are esterified at C-3 by a gallic acid or a 4-hydroxybenzoic acid.



Hamamelitannin

Tests. Mostly broken, crushed, and compressed, Hamamelis leaves are pulverized for identification by microscopic examination (covering trichomes, see above; different from those of hazel leaves which are unicellular [l = 600 µm] and very tapered, with a thick wall). TLC of the extract (in 60% alcohol) shows gallic acid and other phenolics (visualization by ferric chloride). The French Pharmacopoeia requires a quantitative estimate of phenolics in an aqueous extract. The tannins are quantitated by calculating the difference between total polyphenols (determined by colorimetry after reaction with phosphotungstic acid) and polyphenols not absorbed by hide powder (same method). The minimum is 7%.

Pharmacological Properties. *In vitro*, the *Hamamelis* extract is bacteriostatic (on Gram – bacteria) and toxic to molluscs. The hydroalcoholic extract fraction enriched in oligomeric proanthocyanidins by ultrafiltration has a modest antiviral effect (herpes virus, ED_{50} = 11 µg/mL; acyclovir, ED_{50} = 0.42 µg/mL) and a marked anti-inflammatory effect (oil of *Croton*-induced edema of the mouse ear). It also has an inhibitory effect on human polynuclear leucocyte elastase, but it is one-tenth of that of the hamamelitannin-containing fraction. The latter is a 5-lipoxygenase inhibitor and an antioxidant: it is an efficacious hydroxyl, superoxide anion, and singlet oxygen free radical scavenger. *In vitro*, it protects fibroblasts against damage induced by all these agents. The conventional galenical preparations, such as the fluid extract, are vasoconstrictors (rabbit artery).

Uses. In France, in the absence of rigorous clinical trials, Hamamelis leaf-based phytomedicines are traditionally used, orally as well as locally, to treat the subjective symptoms of venous insufficiency such as fullness in the legs, and the symptoms of piles. Locally, the medicines are also used to relieve eye irritation or discomfort of various etiologies (for example eye strain, seawater or swimming pool water, or smoky atmospheres), and as a mouthwash for oral hygiene [French Expl. Note, 1998]. Unlike the total drug powder, herbal tea preparations and aqueous or hydro-alcoholic extracts (regardless of their titer) are not required to undergo any

The German Commission E monographs for Hamamelis leaf and bark list astringent, anti-inflammatory, and local hemostatic properties, and the uses are the same for both (phlebitis, hemorrhoids, skin disorders). The insert of packaged Hamamelis (leaf or bark) products is required to mention the following: for the adjunctive treatment of acute diarrhea; for inflammation of the gums and mucous membranes of the mouth. In some patients, this drug can upset the stomach. Medical advice must be sought in case of persistent diarrhea. In the United Kingdom, Hamamelis leaf and bark are approved for external use only.

The drug is also used in cosmetic formulation as Hamamelis Water or Distilled Witch Hazel Extract, and promoted as an astringent, even though in theory it contains no tannins.

• RHATANY, *Krameria lappacea* (Dombey) Burdet & Simpson (= *K. triandra* Ruiz & Pav.), Krameriaceae

Classified in a small monotypic family related to the Polygalaceae, rhatany is a subshrub with red flowers that grows at high altitude, from Chile to Peru. The dried subterranean parts are used, generally as fragments, and are listed in the 3rd edition of the European Pharmacopoeia. The roots are almost straight, and come out of a thick and knotty crown; the wood is dense, and the dark reddish-brown bark is smooth on the younger parts and rough on the older parts.

The drug contains 10 to 15% condensed tannins. These are propelargonidins (65%) and procyanidins (35%), and comprise 2 to 14 flavanoid units linked (4–>8). In the most condensed molecules, a few (4–>6) links correspond to ramifications. Tetramers are the chief constituents. Also found are benzofuranoid derivatives (0.3%, ratanhiaphenols, conocarpane). The hydroalcoholic extract is an antibacterial (*Streptococcus aureus*). The assay includes tannin quantitation on a decoction of the drug. The French Pharmacopoeial method consists of quantitating the total phenols (by colorimetry after reaction with phosphotungstic acid, relative to pyrogallol as a standard) before and after precipitation with hide powder. The difference (the tannins) must be not less than 10% of the drug.

A marketing authorization from the French government (abridged application dossier or *dossier abrégé d'AMM*) can be obtained for rhatany root-based medications for one of the following indications [French Expl. Note, 1998]: "traditionally used" (orally and locally) 1. for the symptomatic treatment of cutaneous capillary fragility (ecchymoses, petechiae); 2. for the subjective symptoms of venous insufficiency, such as fullness in the legs, and for the symptoms of piles. Locally, the drug may be used as a mouthwash for oral hygiene. In Germany, Commission E authorizes only one use for the decoction, namely the treatment of inflammations of the mucous membranes of the mouth and throat (gingivitis, stomatitis). It recommends against using the drug longer than 2 weeks without medical advice and mentions that local allergic reactions have been

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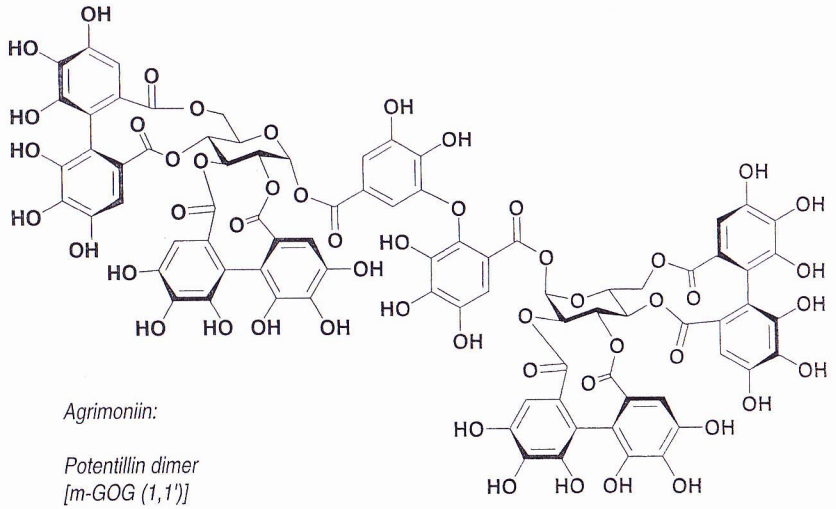
8. OTHER TANNIN-CONTAINING DRUGS USED IN PHYTOTHERAPY

The 1998 French Explanatory Note includes, in Annex 1, several drugs to which tradition attributes properties that might be due to tannins, at least in part and to extent that they have been substantiated. The majority of these drugs is provided by the Rosaceae family: agrimony, lady's mantle, herb bennet, wild strawberry, blackberry, (French) rose, tormentil, but other species are also used (bistort, herb Robert, hazel, purple loosestrife).

For all of these species, relevant clinical data are nonexistent and pharmacological data are more than rare. The chemical composition of some of them is almost unknown. Although the composition of some individual species cannot be described, a chemotaxonomic study has shown, in the case of Rosaceae, that 1. H-6 (dimer) and H-11 (tetramer) sanguiins are characteristic of the genus *Rubus*; 2. gemin A, of the genus *Geum*; 3. agrimoniin, of the genera *Agrimonia*, *Fragaria* and *Potentilla*. Most Rosaceae also contain monomers (e.g., casuarictin, pedunculagin, tellimagrandins). Their use is often limited.



Agrimoniin:

Potentillin dimer [m-GOG (1,1')]

Rosaceae

• **AGRIMONY**, *Agrimonia eupatoria* L., dried flowering tops (Fr. Ph., 10th Ed.). The official drug contains not less than 5% tannins. It also contains flavonoids, quercetin glycosides, kaempferol, luteolin, and apigenin. It is thought to be hypotensive in the cat. The subterranean parts of *A. pilosa* Ledeb. (*xianhecao*) are used in Chinese medecine as an antidysenteric and hemostatic.

• **LADY'S MANTLE**, *Alchemilla glabra* Neygenf. (= *A. vulgaris* L.) and closely related species (the genus is very polymorphic), dried aerial parts (Fr. Ph., 10th Ed.)

The drug contains 6-8 % tannins (pedunculagin) and flavonoids. *A. xanthochlora* Rothm. (= *A. vulgaris* auct. non L.) contains only hydrolyzables tannins (agrimoniin, pedunculagin, laevigatin F) and no condensed tannins; it contains triterpenes. In past centuries, the drug enjoyed a flattering reputation for the treatment of "women's ailments". Lady's mantle extracts are antibacterials.

• **Herb Bennet**, *Geum urbanum* L. The rhizome of herb bennet, a widespread species, may contain over 25% tannins (gallotannins); it also contains 3 mL/kg essential oil (mainly eugenol) and an eugenol arabinosidoglucoside.

• **Wild Strawberry**, *Fragaria vesca* L., dried rhizome (Fr. Ph., 10th Ed.). The official drug contains not less than 8% tannins. The wild strawberry rhizome contains condensed tannins. The wild strawberry leaf also contains hydrolyzable tannins (pedunculagin, agrimoniin) and flavonoids; the drug enjoys a reputation for being a panacea, which is completely unjustified.

• **French Rose**, *Rosa gallica* L. (red rose), *Rosa centifolia* L. (cabbage rose), dried petals and flower buds (Fr. Ph., 10th Ed.). French rose petals were used for ages to manufacture the French "*Miel Rosat*" (= *mellite de rose rouge*). Cabbage rose petals are used to obtain distilled rose water. It is official, and its concentration in phenylethanol ranges from 0.03 to 0.1% (determined by GC). Antimicrobial agents are sometimes added to it and it is used for its mild astringent properties (dermatology, collutoria, gargles, eye drops).

• **Blackberry**, *Rubus* sp., compound leaf or foliole (Fr. Ph., 10th Ed.). The official drug—pinnatisect compound leaf with prickles on the midrib—contains not less than 5% tannins. Among them are dimeric ellagitannins. The drug also contains organic acids, flavonoids, and triterpenes. In the early 1900s, the bark of the subterranean parts was used as a decoction for diarrhea. In Germany, fermented blackberry leaf is used in the composition of a home substitute for tea (*Frühstücketee*, *Deutscher Haustee*). Blackberries, like raspberries (*R. idaeus* L., leaves white on the underside), are edible and can be used as a flavor.

Folk medicine attributes many virtues to the raspberry leaf: it prepares women for birthing, calms painful periods, is an antidiabetic, and is a blood "cleanser". In the 1950s, its activity was studied on isolated organs (including uterine fragments) but the results were not conclusive. The German Commission E states that since none of these actions have been substantiated, the therapeutic use of blackberry leaf cannot be recommended. Like blackberry leaf, raspberry leaf is still used to treat diarrhea.

• **Tormentil**, *Potentilla erecta* (L.) Räusch. (= *P. tormentilla* Stokes). The dried rhizome, very hard and not very ramified, is rich in tannins: condensed tannins (70%, oligomers of two to six units) and hydrolyzable tannins (pedunculagin, agrimoniin, B and F laevigatins).

Other species

• **Bistort**, *Polygonum bistorta* L. (Polygonaceae). Bistort is a perennial plant of mountain pastures. It is thought to contain 15-20% tannins in the subterranean parts. The hydroalcoholic extract is anti-inflammatory *per os* at high doses (rat, carrageenate-induced edema). It is used in Chinese medicine as an antidiarrheal (*quanshen*).

• **Herb Robert**, *Geranium robertianum* L. (Geraniaceae). Like other species of western Europe (*G. sanguineum* L.) or North America (*G. maculatum* Bieb., alun-root), herb Robert is reputed to be rich in tannins (entire plant).

• **Hazel**, *Corylus avellana* L. (Betulaceae-Coryloideae [Corylaceae]), leaf, (Fr. Ph., 10th Ed.). This shrub native to western Europe is most interesting for the oily character and dietary value of its seed, the hazelnut or filbert, which produces an oil of composition similar to that of almond oil. The composition of the leaf is not well known: however, it is known to contain proanthocyanidins and a flavonoid which is a myricetin rhamnoside, namely myricitrin. The official drug contains not less than 2% tannins.

• **Purple Loosestrife**, *Lythrum salicaria* L., (Lythraceae), flowering tops (Fr. Ph., 10th Ed.). The official drug contains not less than 10% tannins. Found in damp locales, this herbaceous plant is easy to identify by its purplish-pink flowers, grouped in elongated spikes that are terminal or lateral. The drug contains anthocyanins (in the flowers), flavone *C*-glucosides (orientin, vitexin), and gallotannins.

Uses of the above drugs

For the sake of simplicity, the indications that the above drugs may claim are summarized below:

• traditionally used for the subjective symptoms of venous insufficiency such as fullness in the legs; for the symptoms of hemorrhoids (orally and topically): agrimony, lady's mantle, herb bennet, bistort, hazel, blackberry, purple loosestrife, tormentil;

• traditionally used for the symptomatic treatment of mild diarrhea (orally): agrimony, lady's mantle, herb bennet, bistort, wild strawberry, herb Robert, hazel, blackberry, (French) rose, purple loosestrife, tormentil; in France, for this indication and for all of these drugs, the package insert is required to include a warning about the risk of dehydration that accompanies profuse diarrhea, especially in infants and children under 30 months of age, and the advice to consult a physician in the event of such diarrhea;

• traditionally used locally (collutoria, lozenges) as an antalgic in disorders of the mouth, pharynx, or both: bistort, hazel, blackberry, purple loosestrife;

• traditionally used locally as a mouthwash for buccal hygiene: agrimony, lady's mantle, herb bennet, bistort, wild strawberry, herb Robert, (French) rose;

• rose petals and flower buds may also be used locally as an adjunct in the emollient and antipruriginous treatment of skin disorders and as a trophic protective agent for cracks, abrasions, frostbites, chaps, and insect bites.

In Germany, Commission E approves the use of the following drugs to treat mild diarrhea: agrimony, lady's mantle, blackberry, and tormentil. The Commission specifies that if the diarrhea persists longer than 3-4 days, it is necessary to consult a physician. For inflammation of the mucous membranes of the mouth and throat (or skin), agrimony, tormentil, or blackberry can be used. In addition, the Commission indicates that the therapeutic use of strawberry leaf is not justified, but that there is no reason to not use it in herbal tea mixtures (as an additive).

9. DIMERIC PROANTHOCYANIDIN-CONTAINING DRUGS

• HAWTHORN, *Crataegus monogyna* Jacq., *C. laevigata* (Poiret) DC., Rosaceae

Hawthorn berry is the subject of a monograph in the 3rd edition of the European Pharmacopoeia (1998 add.), and as far as the French Pharmacopoeia is concerned, the decree or *arrêté* of 7 November 1996 specified that effective 1 January 1997, hawthorn flower and flowering tops were to remain listed. Both drugs are obtained from *Crataegus monogyna* Jacq. emend. Lindman, or *C. laevigata* (Poiret) DC (= *C. oxyacantha* auct. non L. = *C. oxyacanthoïdes* Thuill.). The flowering tops contain not less than 20% flowers and not more than 10% lignified parts. The flower (dried, harvested before blooming) contains not more than 9% foreign matter, including not more than 7% lignified parts. *C. pentagyna* Waldst. & Kit. ex Willd., *C. nigra* Waldst. & Kit., and *C. azarolus* L. may soon be added to the European Pharmacopoeia (they are listed in the Swiss Pharmacopoeia).

The Plant, the Drugs. Hawthorn is a thorny shrub common in almost all of the temperate areas of the northern hemisphere. The leaves are bright green, and have three to five shallow obtuse lobes (*C. laevigata*), or five to seven acute lobes, deeper and further apart (*C. monogyna*). The flowers, grouped into branchy corymbs, have five triangular sepals, five white petals, and an androecium of 15-20 stamens inserted on the edge of a monocarpellate, brownish-green receptacle (*C. monogyna*), or else bi- or tricarpellate (*C. laevigata*). In *C. laevigata*, the floral peduncles and the sepals are glabrous, the stamens have red anthers, and they typically have two or three styles. In *C. monogyna*, the floral peduncles and sepals are pubescent, the stamen anthers are black, and the style is solitary.

The pseudo-fruit of *C. monogyna* is obovate (6-10 x 4-8 mm). It is brownish-red to dark red and surmounted by the remains of the reflexed sepals. It contains only one seed, smooth and glossy. The fruit of *C. laevigata* is more elongated—it can reach 13 mm—and contains 2-3 drupes.

Chemical Composition. Along with pentacyclic triterpenoid acids, the drugs * contain aromatic amines, a trace of essential oil, phenolic acids, 1-2% flavonoids, and 2-3% proanthocyanidins. The chief flavonoid constituent of the leaves is hyperin, the 3-galactoside of quercetin; other constituents include, among others, spirein and rutin. Flavone *C*-glycosides are also found: vitexin, orientin, and most of all, their 2"-*O*-rhamnosylated derivatives (2"-*O*-rhamnosylvitexin is the principal flavonoid in the flowers [accompanied in *C. monogyna* by its derivative acylated at C-4''']). Di-*C*-glycosides of apigenin (vicenin, shaftoside) have also been identified. The composition of the proanthocyanidoid fraction is characteristic: the dimeric procyanidin B-2 (epicatechin (4β –>8) epicatechin) and the trimeric procyanidin C-1 (epicatechin (4β–>8) epicatechin (4β–>8) epicatechin) are the chief constituents, and procyanidin B-5 (epicatechin (4β–>6) epicatechin), a tetramer, and oligomers are also found. (–)-Epicatechin itself is found in substantial quantities.

Tests. Hawthorn berries are identified by their macroscopic and microscopic characteristics (in the powder, long covering trichomes, unicellular, flexuose, ending in a point, with smooth thick wall) and by TLC analysis of a methanolic extract (identification of chlorogenic acid, caffeic acid, hyperin, and rutin). The drug contains not more than 2% foreign matter and not more than 5% spoiled pseudo-fruits. It must not contain pseudo-fruits with more than three drupes (i.e., *C. nigra*, *C. pentagyna*, *C. azarolus*). The procyanidins are quantitated by depolymerization (HCl), extraction of the resulting anthocyanidins (butanol), and absorbance measurement, and must represent not less than 1% of the dried drug.

The flowering tops are identified by their macroscopic characters and the microscopic characters of the powder: unicellular covering trichomes with wide lumen, numerous calcium oxalate macles included in the parenchyma fragments, highly papillose cells from the petals, and pollen cells with three pores. The assay also includes TLC analysis to show the presence, in a methanolic extract, of flavonoids and chlorogenic acid. The flavonoid concentration is determined by spectrophotometry (methanolic extract with aluminum chloride added), expressed as hyperin, and is not less than 1.5%.

The identification of the flower, its assay, and its quantitative test are similar. The flavonoid concentration is not less than 1.5% (Fr. Ph., add. n° 38, *arrêté* or decree of 25 August 1997).

Pharmacological Properties. Hawthorn has the reputation for being active on the myocardium, and this activity may result from a synergy involving several components of the drug, mainly the procyanidins. Published experimental results show negligible toxicity, and despite substantial experimental gaps, they show the positive effect of hawthorn extract on myocardial contractility and output (on isolated organs and whole animals), as well as its hypotensive activity and its

* The hawthorn berry and flower have very similar compositions. The differences are in the relative concentrations of certain constituents (e.g., hyperin, vitexin derivatives) and depend on the species (*monogyna*, *laevigata*), the organ (leaf, flower, fruit), and the harvest season.

propensity to decrease peripheral vascular resistance. A potential antiarrhythmic activity has also been shown (rabbit). Experiments on isolated organs also show that hydroalcoholic extracts and proanthocyanidins increase coronary output; the same is observed *in vivo* (*per os* in several animal species). The activity is thought to involve inhibition of the cAMP phosphodiesterase, and an effect on β-adrenergic receptors has been shown .

Observations in humans and a few clinical trials conducted according to strict protocols (placebo-controlled, double-blind, randomized) tend to confirm the experimental results obtained in animals: improvement of the subjective symptoms (e.g., feeling of breathlessness, palpitations) in patients with mild cardiac insufficiency upon long term treatment (aqueous extract, *per os*), decrease in rhythm, improvement of systolic contraction, coronary output, and muscular work capacity.

Uses. In France, hawthorn flowering tops and flowers may be used in the formulation of plant-based phytomedicines [French Expl. Note, 1998] with the following indications (orally): traditionally used to treat abnormalities of the cardiac rhythm in the adult (normal heart) and in the symptomatic treatment of neurotonic disorders in the adult and in the child, for example, minor sleeplessness. The consumer information must specify that the drug is used "to soothe nervous adults, particularly those who have an exaggerated perception of their heartbeats (palpitations), after all possible cardiac diseases have been excluded".

Although self-medication for cardiac symptoms—even if they are minor—is not recommended, for the physician, hawthorn alone or in combination seems to be a useful alternative drug to prescribe. The drug (powder, extracts, tincture) is commonly available in combination with other plants: passion flower, valerian, even lime tree or balm. In several proprietary drugs, hawthorn is combined with phenobarbital (treatment of minor signs of anxiety). The drug has no acute toxicity, and no major side effects, drug interactions, or contraindications are known. Its chronic toxicity has apparently never been studied. In France, only the powder-based preparations are required to undergo safety testing (basic tests).

In Germany, Commission E recognizes that hawthorn (flowering tops) has positive inotropic and dromotropic activities, a negative bathmotropic activity, and the ability to increase coronary and myocardial circulation. Therefore, since 1994, the Commission has approved the use of only the flowering tops for cardiac insufficiency corresponding to NYHA (*New York Heart Association*) stage II. Prior to that date, other approved indications used to be the feeling of pressure in the cardiac region, mild bradycardia, and the aging heart that does not yet require cardiac glycosides.

Other Species, Sources of Dimeric Proanthocyanidins

• Vine, *Vitis vinifera* L., Vitaceae

Vine is used in phytotherapy (vine leaf) and in the pharmaceutical industry, which manufactures from grape seeds a purified extract standardized for oligomeric procyanidins. The seeds are also the source of grape seed oil.

Experimentally, grape seed procyanidins have an angioprotective activity on several models for alteration of capillary permeability. They are *in vitro* inhibitors of collagenase, elastase, hyaluronidase, xanthine-oxidase, and angiotensin converting enzyme, as well as free radical scavengers, and they apparently protect collagen and elastin from degradation. Despite their methodological bias, several studies in humans tend to indicate that these proanthocyanidins (150-300 mg/day) improve the functional symptoms of venous insufficiency, increase visual sensitivity (after being blinded by bright light), and improve the subjective symptoms of visual fatigue.

In therapeutics, the procyanidins of grape seed have two indications: treatment of the functional symptoms of venous and lymphatic vessel insufficiency; treatment of the lymphedema of the arm subsequent to breast cancer radiation therapy and chemotherapy (to complement physical methods, or alone if the latter are not possible). In addition, low-dose formulations are promoted as a treatment for problems involving retinal or choroidal circulation.

Like many other free radical scavengers, proanthocyanidins are of interest for the formulation of "dermoprotective" cosmetic products.

• Pinaster or Maritime Pine, *Pinus pinaster* Soland, Pinaceae

Although the interest in pine is due, among other reasons, to turpentine (see oleoresin-containing drugs), its bark is also a good starting material for the preparation of proanthocyanidinoid oligomers (like many other Coniferae whose barks can contain up to 5% of dimeric procyanidins B-1-B-4). Pine proantho-cyanidins are promoted as a treatment for symptoms related to venous and lymphatic vessel insufficiency.

• Cypress, *Cupressus sempervirens* L., Cupressaceae

The cypress is a tree with opposite, decussate, and triangular leaves, which are tightly imbricate, and are pressed against the stem like scales. The different varieties of this Mediterranean species are widely used for their ornamental qualities. The female cone (also referred to as galbulus) is the part used in phytotherapy. It is

formed of peltate and fleshy scales that gradually become lignified. Chemically, the cones contain an essential oil (5 mL/kg, including α-pinene and Δ^3-carene), proanthocyanidinoid dimers and oligomers, and diterpenoid acids, whereas the twigs are characterized by biflavones and 0.3-0.8% essential oil containing mono-, sesqui-, and diterpenoid hydrocarbons. Pharmacological experiments in the rat show that the oligomers have a vascular protective activity; in addition, these molecules inhibit angiotensin converting enzyme (in the rabbit, by the IV route), elastase, and trypsin activity (*in vitro*). Medications based on cypress cones are traditionally used to treat the subjective symptoms of venous insufficiency such as fullness in the legs and the symptoms of hemorrhoids (orally and locally [French Expl. Note, 1998]).

• Black Catechu, Pale Catechu

Black catechu or cutch is an aqueous extract of the heartwood of *Acacia catechu* (L. *f.*) Willd. (Mimosaceae), concentrated by boiling. Upon cooling, crystals separate: these constitute *katha* or *kath*, which contains more than 55% catechin; concentrating the supernatant yields *cutch*. Several thousand metric tons of these products are used annually in southeast Asia to tan hides, protect ropes, dye textiles, and more. The distribution area of this species ranges from the south of the Himalayas (Pakistan, India) to Myanmar and Thailand. Catechu contains a gum, flavonoids, and flavanoid derivatives, both monomeric (catechin and epicatechin, 10-12%) and polymeric with a variable degree of condensation (25-30%). Catechu finds limited uses (confectionery).

The term catechu also applies to the aqueous extract of the leaves and twigs of a creeping Rubiaceae cultivated in Malaysia and Indonesia, namely gambir (*Uncaria gambir* [Hunter] Roxb.). This extract is known as pale catechu or cube gambir. The distinction between the two catechus (black or pale) can be made on an alcoholic extract alkalinized with sodium hydroxide: pale catechu gives, after re-extraction of the filtrate by petroleum ether, a green fluorescence (this intense fluorescence is due to 0.05% indole alkaloids, gambirtannin and derivatives). In the United Kingdom, pale catechu is used as an antidiarrheal. In the United States and the European Union, pale catechu is an authorized flavor.

• Industrial Sources of Tannins

There are multiple industrial sources of tannins, and although usage in the leather industry has become negligible (at least in the so-called developed countries), other uses have surfaced: protective coatings, adhesives, plastics, taste improvement of wines and other alcoholic beverages, dyes, and more. Oaks (*Quercus* sp.) and chestnut trees (*Castanea* sp.) are still used for their bark. The other drugs range in importance, depending on the country under consideration. Some are still widely used, for example *Acacia mearnsii* De Willd. (in South Africa, Brazil, Kenya), with plantations still accounting for 350,000 hectares (= over 0.8

billion acres) in the world in the early 1980s; another example, it seems, is that of the various *Schinopsis* of South America (Anacardiaceae): *S. balansae* Engl., *S. haenkeana* Engl., and *S. quebracho-colorado* (Schldl.) F. Barkley and T. Meyer = *S. lorentzii* (Griseb.) Engl. In China and in 1987, 60,000 metric tons of wood of *Myrica esculenta* Buch.-Ham (Myricaceae) were used for tanning. In India, 100,000 metric tons of fruits of *Terminalia chebula* Retz (black myrobalan, Combretaceae) were produced in 1981.

Other drugs continue to be used, but do not seem to be traded internationally, for example:

- Rhizophoraceae from southeast Asia, Indonesia, Malaysia, and the Philippines: *Bruguiera gymnorhiza* (L.) Savigny, *Rhizophora mucronata* Poiret, *Ceriops decandra* (Griffith) Ding Hou, and other species among these genera characteristic of mangrove swamps;
- Anacardiaceae of North America such as sumacs: *Rhus hirta* (L.) Sudw., f. *hirta* and f. *typhina* (L.) Reveal, (= *R. typhina*, velvet sumac);
- Caesalpiniaceae: divi-divi of tropical America (= *Caesalpinia coriara* [Jacq.] Willd.) whose pods contain 40-45% tannins, *C. digyna* Rottler, *C. brevifolia* Baillon, *C. paraguariensis* (Parodi) Burkart, and more.

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