Anthocyanins

1. Introduction. 355
2. Structure and Biosynthetic Origin of Anthocyanins. 356
3. Physico-chemical Properties 358
4. Extraction and Characterization 359
5. Pharmacological Activity and Uses....360
6. Chief Anthocyanin-containing Drugs....361
Bilberry.. 361
Cranberry 363
Black Currant....364
Vine....365
European Elder....366
7. Bibliography....367

1. INTRODUCTION

The term anthocyanin, initially coined to designate the substance responsible for the color of the cornflower (from the greek *anthos*, flower and *kuanos*, blue), applies to a group of water-soluble pigments responsible for the red, pink, mauve, purple, blue, or violet color of most flowers and fruits. These pigments occur as glycosides (the anthocyanins), and their aglycones (the anthocyanidins) are derived from the 2-phenylbenzopyrylium cation, more commonly referred to as the flavylium cation, a name that emphasizes the fact that these molecules belong to the vast group of flavonoids in the broad sense of the term.

Rare in Gymnosperms, anthocyanins are found in all of the Angiosperms, except for most Caryophyllales: of the dozen families in this order (defined according to

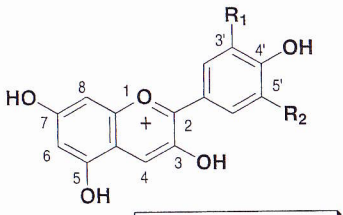
other families (e.g., Chenopodiaceae, Cactaceae, Phytolaccaceae, Nyctaginaceae), the pigmentation of the various organs is due to betalains (examples are the beet root and the bougainvillea or amaranth flowers).

Although they are generally characteristic of flower petals (poppies, high mallow, hibiscus) and fruits (cherry, European elder, eggplant) , anthocyanins can also be found in bracts (Bromeliaceae), leaves (*Coleus* sp.), petioles (rhubarb), and even roots (radish) or bulbs (red onion). Most often, they accumulate in the vacuoles of the epidermal tissue cells, in solution, and less commonly as anthocyanoplasts.

Anthocyanins, whose vivid colors attract insects and birds, play a major role in pollination and seed dispersal. A high coloring power and the absence of toxicity lend to these natural coloring glycosides the potential to replace synthetic colors in food technology: their harmlessness—a fruit or vegetable extract does not require extensive safety testing—and their acceptability to the consumer compensate for their instability (pH, temperature, light) and their production cost, sometimes high. In current practice, the therapeutical applications of anthocyanins are limited to the treatment of vascular disorders: the drugs containing them are used for the extraction of anthocyanins for the preparation of galenicals designed to treat the symptoms linked to capillary and venous fragility.

2. STRUCTURE AND BIOSYNTHETIC ORIGIN OF ANTHOCYANINS

Anthocyanidins occur in acidic medium as cations. With exceptions (apigeninidin), they are always hydroxylated at C-3 and, most often, penta- (3,5,7,3',4') or hexasubstituted (3,5,7,3',4',5') by hydroxyl groups, or methoxyl groups, or both. The most common aglycones (they are virtually ubiquitous) are pelargonidin (scarlet), cyanidin (crimson), and delphinidin (purple). No anthocyanin is known in which all of the hydroxyl groups are methylated or glycosylated: at least one hydroxyl group at C-5, C-7, or C-4' must remain free to allow the formation of the colored quinonoid structures.



Structures of the chief anthocyanidins

$R_1 = R_2 = H$: *Pelargonidin*

$R_1 = OH$, $R_2 = H$: *Cyanidin*

$R_1 = OCH_3$, $R_2 = H$: *Peonidin*

$R_1 = R_2 = OH$: *Delphinidin*

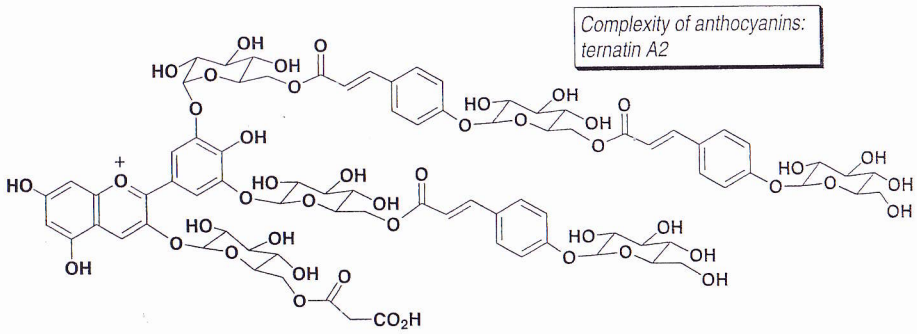
$R_1 = OCH_3$, $R_2 = OH$: *Petunidin*

$R_1 = R_2 = CH_3$: *Malvidin*

The rare 3-deoxyanthocyanidins that are known (Gesneriaceae, Poaceae) are relatively stable. In contrast, anthocyanidins are unstable because their 3-hydroxyl

group makes the flavylium ion very reactive. In fact, the 3-hydroxyl group is never found in the free state—it is always linked to a sugar (very often glucose) to form a stable and water soluble anthocyanin. The most common anthocyanins are 3-monosides and 3,5-diglycosides. Also known are 3,7-diglycosides and triglycosides (for example 3,5,3'-triglycosides). The sugar residue of anthocyanins can be a monosaccharide (e.g., glucoside, galactoside, rhamnoside), a disaccharide (e.g., rutinoside, xylosylglucoside), or, less often, a trisaccharide.

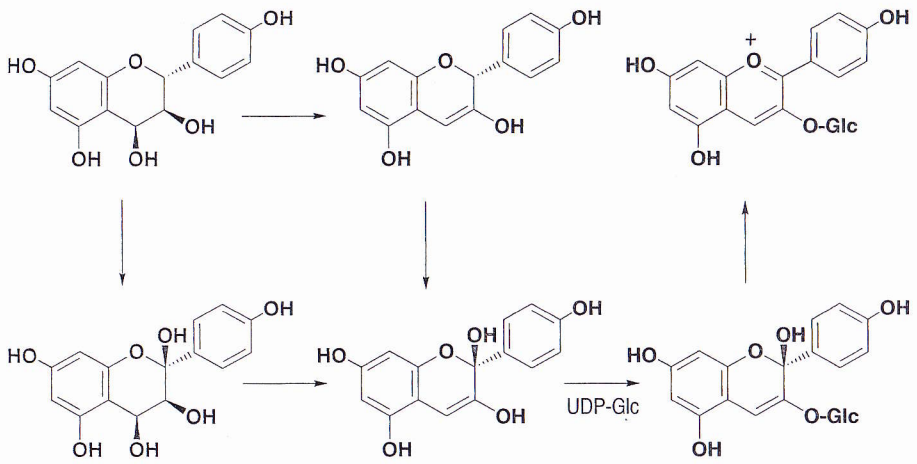
Many anthocyanins are acylated by phenylpropanoic acids (e.g., *p*-coumaric, caffeic, ferulic,sinapic acids) or benzoic acids (gallic acid), which esterify one or several hydroxyl groups on the saccharide(s), generally at C-6". Also known now—and their number is increasing rapidly—are anthocyanins acylated by dicarboxylic aliphatic acids (e.g., malonic, malic, oxalic, succinic acids); molecules acylated simultaneously by both types of acids have also been described. The standard extraction procedures do not allow proper isolation of these zwitterionic molecules (they are very labile in the dilute hydrochloric acid traditionally used to extract them), and a good number of long known anthocyanin structures probably occur as zwitterions, even if they have not yet been characterized as such. This is confirmed by electrophoresis, which is an easy way to show their presence, and by the use of milder methods of extraction and purification (neutral alcohols, acetic acid, formic acid). Finally, high molecular weight metallo-anthocyanins are known, which comprise six anthocyanin molecules and six flavonoids chelating two metal atoms (Mg, or Fe, or both) (e.g., in the cornflower, *Centaurea cyanus* L.).



Complexity of anthocyanins: ternatin A2

Biosynthetic Origin

Anthocyanins arise from the general metabolism of flavonoids. Experimental data obtained from acyanic mutants and from white-flower genotypes show that the 2,3-*trans*-dihydro-3,4-*cis*-dihydroxyflavonols are the precursors of anthocyanidins. The final steps in the formation of anthocyanins have not yet been elucidated: in all likelihood, the diols undergo a hydroxylation (at C-2) and a double dehydration. Glucosylation (which requires the involvement of UDP-glucose) probably occurs late.

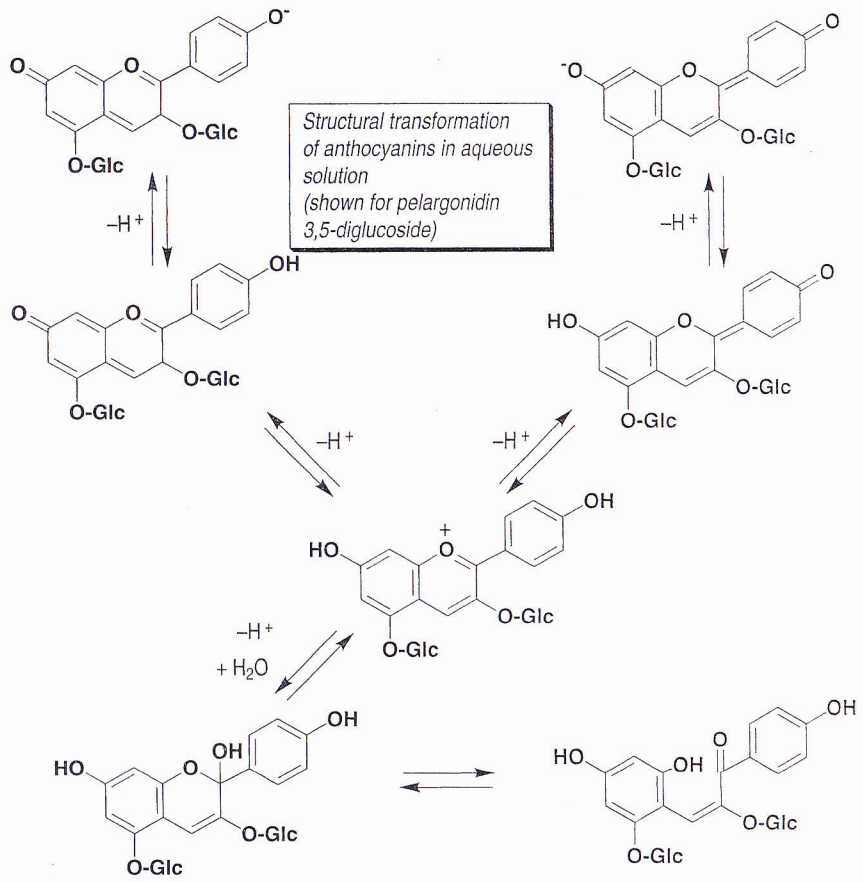


Possible biosynthetic origin of anthocyanins

3. PHYSICO-CHEMICAL PROPERTIES

The behavior of anthocyanins in aqueous solution results from the properties of the 2-phenylbenzopyrylium cation, which is at the same time a weak diacid and a good electrophilic reagent. In strong acidic medium (pH<3), the cationic form is red and stable. In weak acidic medium (at pHs between 4 and 6), the cation loses one or two protons, and this leads to an anhydrobase, which is neutral or ionized, respectively, and stabilized by resonance; these quinonoid forms are blue. Hydrating the molecule (at C-2, by simple dilution) leads to a carbinol pseudobase, which is colorless. The latter is in equilibrium with the corresponding chalcone (also colorless), and if the pH increases, it becomes ionized (as a phenate), and the anthocyanin structure is destroyed. As a consequence of these properties, neutral or slightly acidic anthocyanin solutions lose their color fairly rapidly (although the anhydrobase is colored, the hydration of the cation is faster than the loss of a hydroxyl hydrogen). To explain the color of anthocyanins at the pHs commonly found in living media—since the pH of vacuoles is only very rarely lower than 3—, it is necessary to take into account the stabilization of their structure and its protection against the nucleophilic addition of water. Several mechanisms may be at play: 1. intramolecular copigmentation, in which the acids that acylate the saccharides form a sandwich around the anthocyanidin (most polyacyl-polyglycosyl-anthocyanins are remarkably stable in solution); 2. self-association, also referred to as autocopigmentation, in which stacked anthocyanin molecules share mutual protection; 3. intermolecular copigmentation with flavonoids. Adsorption onto proteins, pectins, and other macromolecules also adds to the stability.

Among the other properties of anthocyanins, note their instability to oxygen, heat, and light, and their susceptibility to nucleophilic attack at C-2 (hydration) and



especially at C-4 to form flav-2-enes substituted at C-4: the reaction with sulfur dioxide, which leads to the (reversible) formation of stable sulfonic derivatives at C-4, results in a rapid loss of color of the corresponding solutions.

4. EXTRACTION AND CHARACTERIZATION

Anthocyanins are soluble in water and alcohols, insoluble in apolar organic solvents, and unstable in neutral or alkaline medium. They are generally extracted with an alcohol (methanol, preferrably ethanol if the product is intended for use in food) in the presence of a small amount (0.1-1%) of hydrochloric acid. To avoid esterification of the free carboxyl group of acylated anthocyanins by a diacid, and especially to prevent their deacylation, it is better to use other acids, either weak acids (acetic, tartaric, citric) or volatile acids (trifluoroacetic), or to work in a neutral medium (alcohol mixtures), and to work at low temperature (< 30 °C). Anthocyanin solutions are very unstable, and they can only be kept under nitrogen, at low temperature, and in the dark.

Different procedures are available for the industrial preparation of anthocyanin extracts. The oldest one is an extraction in aqueous medium in the presence of sulfur dioxide, followed by acidification to regenerate the anthocyanins. Among the more recent procedures are ultrafiltration on cellulose membranes and chromatography on ion-exchange resins.

The separation of anthocyanins is achieved by chromatographic techniques (column chromatography on polyamide supports, on polyvinylpyrrolidone supports, or on ion-exchange resins, preparative TLC on cellulose-coated plates, or semipreparative HPLC).

HPLC is the method of choice to analyze anthocyanin-containing drugs. The separations are most often carried out on reverse phases with acidic water and alcohol gradients, in which the cationic forms can be detected at about 500-550 nm. As for flavonoids, diode array detectors represent a considerable method enhancement. The more complex methods (LC-MS, MS-MS) are only available in specialized research laboratories.

As a general rule, anthocyanin quantitation is performed by spectrophotometry. At the wavelengths of maximum absorption of these compounds, interferences are exceptional: quantitation can be done directly on an acidic solution in alcohol (cationic form) or on an acidified juice. To prevent anthocyanin self-association, which would result in a positive deviation from the Beer-Lambert Law, dilute solutions must be used. Quantitative estimates of the constituents of an anthocyanin mixture are now obtained directly by HPLC.

5. PHARMACOLOGICAL ACTIVITY AND USES

As in the case of flavonoids in the strict sense of the term, biological tests on animals based on the diffusion of dyes indicate that anthocyanins decrease capillary permeability and fragility. The activity of these glycosides is thought to be linked to the participation of the collagen of the vascular wall in the control of the permeability of that wall. It may be due, in part, to an inhibition of the proteolytic collagen degradation enzymes (elastase, collagenase); in fact this inhibitory activity has been shown *in vitro* for black currant extracts (*median inhibitory concentration* = IC50 0.16 and 0.56 mg/mL). Other properties have been shown: antiedema activity and increase in regeneration of 'visual purple' or rhodopsin (see bilberry). Like many other phenolic coumpounds, anthocyanin pigments act like radical scavengers *in vitro* (antioxidant activity).

These actions on capillaries and veins lead to the use of anthocyanin-containing drugs and the preparations based on them, for the symptomatic treatment of venous and lymphatic insufficiency and capillary fragility (in phlebology, proctology, or gynecology). Anthocyanins are also promoted in ophthalmology to treat circulatory disorders of the retina or choroid, and to improve vision at dusk.

Other uses. The chief industrial application of anthocyanins is coloring. Indeed,

chronic. In general, crude extracts of must of grape (unfermented grape juice), a plentiful and inexpensive starting material, are used. These extracts are either liquids titrated to contain 0.5-1% anthocyanins, or nebulisates titrated to contain 1-5% anthocyanins. Other fruit juices are also used (elderberry) as well as red cabbage leaves, which are more expensive, but provide a more stable coloring agent. The instability of these pigments in aqueous media is a handicap; it results in color changes as a function of the pH, and in sensitivity to heat, light, sulfites (often used as preservatives), and metals (food cans). The common occurrence of proanthocyanidins and gallotannins in the extracts can also be a problem (for example it makes gelatin precipitate in jams). The insolubility of anthocyanins in lipids also restricts the scope of their applications.

Anthocyanins are extracted from edible fruits and vegetables and may be used as food additives (Eur. id. code E163), for example in beverages (30 mg/L), jams, and confectionery products, to name only a few.

6. CHIEF ANTHOCYANIN-CONTAINING DRUGS

• BILBERRY, *Vaccinium myrtillus* L., Ericaceae

The bilberry (or blueberry), well-known for its tasty fruits, is used by the pharmaceutical industry for the extraction of anthocyanins. The leaf and the dried fruit are used in phytotherapy. The fresh and dried fruits were added to the French Pharmacopoeia in January of 1992. The leaf or the official fruit may be used in the composition of phytomedicines [French Expl. Note, 1998].

The Plant, the Drug. The bilberry is a subshrub with coriaceous leaves. The bell-shaped flowers grow solitary or in pairs at the base of the leaves. The fruit is a multiseeded tetra- or pentalocular globose berry with a fleshy mesocarp; on the flattened top, the remains of the style and the calyx form a small disc with a dull edge. Blueberries are particularly abundant in the woods that grow on siliceous soils in the mountains of the northern hemisphere. The French market is largely dominated by imports (from Poland). Other species (e.g., *V. corymbosum*, cultivated in Germany) are also used in the food industry.

The microscopic examination of the drug reveals calcium oxalate rosettes, punctate sclerenchymatous cells, and epidermal cells with evenly thickened walls. This examination is useful to detect other species (e.g., *Vaccinium uliginosum* L.). The official drug must contain not more than 2% leaves and twigs; the dried extract must contain not less than 9% anthocyanins, which are analyzed by TLC.

Chemical Composition.

Blueberries are rich in water (up to 90%), sugars (3 to 7%), and organic acids. Phenolic acids, flavonoids (hyperin [= hyperoside], quercitrin), proanthocyanidins

have been identified. The anthocyanin level in the fresh fruits is about 0.5%. These glycosides, about fifteen of them, are C-3 *O*-glucosides, *O*-galactosides, and *O*-arabinosides of cyanidin, peonidin, delphinidin, malvidin, and petunidin.

The **bilberry leaf** contains phenolic acids, flavonoids (rhamnoglucosyl-, arabinosyl-, and glucuronylquercetin) and traces of quinolizidine alkaloids (myrtine, epimyrtine). It is rich in proanthocyanidins and catechin (up to 10%) and in the 1950s, hydroquinone and arbutin were isolated. Later on, it was not possible to characterize them again. This is explained either by the occurrence of chemotypes, or—this is very likely—by contamination of the batches initially analyzed by closely related species that grow in the same habitat (*V. vitis-idaea* L. or *V.* x *intermedium* Ruthe [= *V.myrtillus* x *V. vitis-idaea*]), species rich in arbutin (3.3-6.6 and 2%, respectively), hydroquinone, and pyroside (= 6-*O*-methylarbutin).

Pharmacological Activity. Despite their low bioavailability shown in rats (it is linked to poor digestive absorption), bilberry anthocyanins have, in animals, a vascular protective and antiedemic activity by the oral, as well as the IP or IV route. *In vitro* or *in vivo* (in rabbits), they inhibit collagen- or *adenosine diphosphate*- (= ADP-) induced platelet aggregation, and stimulate (in rats, *per os*) the prostacyclin (= PGI2) activity of the vascular walls. They are also inhibitors of cAMP phosphodiesterase and are free radical scavengers. When administered orally to rats, cyanidin chloride counteracts the ulcer-causing action of various triggering mechanisms (stress, *non-steroidal anti-inflammatory drug* = NSAID, ethanol, histamine, and more). It is generally accepted (and is confirmed by electroretinography) that anthocyanins, by facilitating the regeneration of rhodopsin, improve vision in poor light. In humans, experimental data and a large body of clinical observations indicate favorable results in the treatment of vascular disorders (venous disorders, capillary disorders secondary to diabetes, ecchymoses, purpura, and gingival hemorrhage). Anthocyanins have also been tested for the treatment of retinopathies of hypertensive or diabetic origin. The bilberry leaf is reputed to lower glycemia, but to date, this has not been clearly demonstrated, nor has experience provided any substantiation for the other virtues that are traditionally celebrated. In the diabetic rat, the hydroalcoholic extract of bilberry is hypolipidemic.

Uses. The pharmaceutical industry produces and markets a water-soluble bilberry powder titrated to contain 70% anthocyanins. The first step in the manufacturing process is the biological elimination of the sugars by *Saccharomyces* sp.; the medium recovered from fermentors is clarified by on-line centrifugation and concentrated under vacuum at low temperature. The purification of this crude extract entails repeated treatment with boiling ethanol. Upon cooling of the ethanol solution, the insoluble anthocyanins precipitate and are recovered by centrifugation (the free anthocyanidins, organic acids, and other impurities remain in solution).

Extracts obtained from the bilberry fruits and enriched in anthocyanins are ingredients of drugs used to treat the functional symptoms of venous and lymphatic vessel insufficiency, cutaneous capillary fragility, and mesopic and scotopic vision (nyctalopia, myopia).

In France, the bilberry fruit, fresh or dried, and the bilberry leaf may claim the following indications (orally or topically): traditionally used to treat the subjective symptoms of venous insufficiency, such as fullness in the legs, and to relieve the symptoms of piles. The fruit, fresh or dried, is traditionally used for the adjunctive therapy of the painful component of functional dyspepsia. Only the fresh fruit may claim indications for the symptomatic treatment of the functional symptoms of capillary fragility, but the bilberry leaf or dried fruit may be used for the symptomatic treatment of mild diarrhea [French Expl. Note, 1998].

In Germany, Commission E considers the bilberry fruit to be an astringent, used as such for the symptomatic treatment of diarrhea and as a topical anti-inflammatory in case of irritation of the mucous membranes of the mouth and throat. As far as the bilberry leaf is concerned, the Commission considers that none of the activities traditionally attributed to it have been demonstrated. Furthermore, the risk of poisoning by high doses or chronic use is emphasized, therefore the use of the leaf is not recommended.

With regards to the risk of poisoning, there is a lack of data: although symptoms in cats are reminiscent of hydroquinone poisoning, it is unlikely that this benzoquinone (or its derivatives) occur in the bilberry leaf. In the absence of additional toxicological investigations, and considering the lack of established properties for the bilberry leaf, the Commission E position seems wise. Yet the French Explanatory Note requires safety testing (basic tests) only for the leaf powder, not for the extracts, regardless of their titer.

● CRANBERRY*, *Vaccinium macrocarpon* Aiton, Ericaceae

Cranberry grows wild in eastern North America, from the Carolinas to Canada. Cultivated in the United States since the beginning of the nineteenth century, it produces small dark red fruits, widely consumed as such (fresh or frozen) and as cranberry juice (pure or as a cocktail sweetened with corn syrup), cranberry sauce, and so on. The fresh fruit is very rich in acids (citric, quinic, benzoic); it also contains anthocyanins (3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin), catechin, and flavonoids.

* Cranberry is the American name. The (common) cranberry is typically American, but it is also found in Europe where it is cultivated and, according to the Flora Europea, naturalized locally. The (small) cranberry is known (*V. oxycoccos* L.) in Europe. In fact, the adjectives common or small are rarely used and most publications do not specify the species. In a recent North American review, the author included, under cranberries, *V. macrocarpon* and *V. oxycoccos* (Siliciano, A.A (1996). Cranberry, HerbalGram, n° 38, 51-53. In general, the English terminology for the *Vaccinium* species seems very confusing: *V. myrtillus* L. is the bilberry, (black) whortleberry, or blueberry; *V. uliginosum* is the bog bilberry; *V. vitis idaea* L. is the cowberry, (red) whortleberry, foxberry, mountain cranberry, or red bilberry *V. corymbosum* L. is the blueberry or highbush blueberry; *V. angustifolium* Aiton is the (lowbush) blueberry, and so on.

The beneficial—bacteriostatic—effect of cranberry juice in the treatment of urinary infections is confirmed by secular use; it was first attributed to urine acidification by the fruit acids and their metabolites. Actually, the pH remains unchanged, and it is now postulated that the activity is due to the inhibition of bacterial adhesion onto mucous membranes. This has been demonstrated in the case of *E. coli* adhesion onto urinary tract epithelial cells, using cranberry juice as well as the urine of mice or humans collected after administration of cranberries. A non-dialyzable polymer that contains no nitrogen and inhibits the adhesins specific to the pathogenic strains of *E. coli* has since been isolated from cranberry juice—and also from bilberry (blueberry) juice (preliminary analyses do not exclude the possibility that the polymer is of a procyanidin type). Fructose is found in large quantities in cranberry juice and may contribute to the activity. More recently, a placebo-controlled, double-blind clinical trial showed that the daily consumption of 300 mL of a commercially-available cranberry juice induced, in elderly women (average age 78.5 years), a very significant decrease in the frequency of urinary bacterial contamination, after 4-8 weeks of treatment, a delay which may correspond to an initial action on the intestinal bacterial flora (but the effect of cranberry juice on the adhesion of intestinal bacteria has yet to be studied). In the United States, the recommended use of cranberries is as a dilute juice; dried juice capsules are also available.

• Black Currant, *Ribes nigrum* L., Grossulariaceae

This bushy shrub is cultivated for its edible fruits (in the Burgundy region of France and in central Europe). The black currant leaf as well as the fruit are used in pharmacy (Fr. Ph., 10th Ed.).

Black currant is characterized by tri- to pentalobate leaves, the underside of which are pale, pubescent, and scattered with yellow secretory glands. The flowers are reddish, grouped in dangling racemes, and have a pubescent calyx which is longer than the corolla. The fruit is a fragrant black berry on top of which the remains of the calyx can be seen.

The fruit, rich in sugars (10-15%) and organic acids, contains, among other constituents, flavonol glycosides and anthocyanins (cyanidin and delphinidin glycosides). The leaves contain a small amount of essential oil, many flavonoids (hyperin, astragalin, rhamnoglucosides and glucoxylosides of quercetin and kaempferol; the French Pharmacopoeia specifies that it contains not less than 1.5% flavonoids), and dimeric and trimeric prodelphinidins, which would be in part the basis of their anti-inflammatory activity.

The fruit is used to prepare extracts enriched in anthocyanins; these extracts, and the proprietary drugs that contain them, have therapeutic indications identical to those of the bilberry.

Based on tradition, phytopharmaceuticals containing black currant leaves may claim the following indications(orally, French Expl. Note, 1998): to facilitate urinary and digestive elimination functions, to enhance the renal excretion of water, and as an adjunct in weight loss programs. Leaf-based preparations are also traditionally used, orally and topically, for the symptomatic treatment of minor painful symptoms of the joints. In Germany, the black currant leaf is used to increase the volume of urine and the "antirheumatic" use is found exclusively in folk medicine. Contemporary phytotherapy prescribes the preparations based on the buds in the same fashion. These are rich in diterpenoid acids (hardwickiic acid), and are prized for their essential oil, which is used in food technology. The composition of this essential oil varies with cultivars, but the chief constituents are almost always hydrocarbons (Δ^3-carene, sabinene, phellandrenes, and limonene).

• Vine, *Vitis vinifera* L. (*tinctoria* varieties), Vitaceae

The term "vine" designates cultivars with black grapes, red pulp, and leaves that turn red in the fall, partially or completely (for example *Alicante, Gamay teinturiers*). The dried vine leaf "*dite à cépage teinturier*" is the subject of a monograph in the 10th edition of the French Pharmacopoeia. The pharmaceutical industry also uses grape seeds (see note * p. 152).

The color of the vine leaves is of course linked to a high anthocyanin concentration, which varies as a function of time: it is maximal when the fruits are mature, and can reach, in some cultivars, 0.3% of the dry weight. The chief constituents are 3-*O*-glycosides of cyanidin and peonidin. They occur alongside other phenolics: monocaffeoyltartaric acid, phenylpropanoic acids, flavonol glucosides, hydrolyzable tannins (esters of glucose and of gallic and dehydrohexahydroxydiphenic acids), and proanthocyanidins.

The French Pharmacopoeia describes the microscopic characteristics of the transverse leaf section and of the powder (covering trichomes with lumen divided into small compartments, calcium oxalate raphides). The qualitative assay is focused on the TLC analysis of the phenolics: the plate is examined in daylight (anthocyanins) and after spraying with aminoethanol diphenylborate (flavonoids, monocaffeyltartaric acid). Considering the crop treatments commonly applied in viticulture, a verification of the absence of abnormal quantities of copper in the ashes is in order. In addition, the assay includes two quantitations: that of total polyphenols (on an aliquot of a decoction, by colorimetry after reaction with phosphotungstic acid; standard: pyrogallol), and that of anthocyanins (by measurement of absorbance of an acidic methanol extract). The required levels are: polyphenols >4% and anthocyanins >0.2% (expressed as glucoside-cyanidin).

Vine leaf-based phytopharmaceuticals are traditionally used (orally and topically, French Expl. Note, 1998) to treat the functional symptoms of capillary fragility such as ecchymosis and petechiae, the subjective symptoms of venous

insufficiency such as fullness in the legs, and the symptoms of hemorrhoids. Topically, they are traditionally used for eye irritation or discomfort of various etiologies (e.g., eye strain, seawater or swimming pool water, or smoky atmospheres).

• EUROPEAN ELDER, *Sambucus nigra* L., Caprifoliaceae *

Two organs of this species are of interest, the flower—it is the subject of a monograph in the European Pharmacopoeia (3rd Ed., 1998 add.) and the fruit, a source of extracts used as food coloring.

Flowers. The European elder is a shrub widespread in western Europe. Its bark has small cracks and its leaves are imparipinnate. It is easy to identify by its large (20 cm) inflorescences of strong-smelling flowers, and later in the year, by its black berries with their purplish-red juice and three seeds. The flower is fairly easy to identify. However, to verify the absence, in the drug, of flowers of dwarf elder (*S. ebulus* L.)—with red instead of yellow anthers—the French Pharmacopoeia requires a TLC analysis of the flavonoid content of a methanol extract. In addition, the flavonoids must represent not less than 0.8% of the dried drug. The flowers are rich in flavonoids (rutin, isoquercitrin) and derivatives of caffeic acid, free and esterified. They also contain triterpenes and give an essential oil by steam distillation, which smells like muscat grapes, has a pasty consistency, and contains fatty acids, 3,7-dimethyl-1,3,7-octatrien-3-ol, linalol, *cis*-hexenol, and rose oxides, among others.

Despite the fact that pharmacological data are rare (anti-inflammatory?) and most often uninterpretable (mixtures), and in the absence of clinical data., elder flower is traditionally used orally to enhance the urinary and digestive elimination functions, as an adjunct in weight loss programs, and to enhance the renal elimination of water [French Expl. Note, 1998].

The indications reflect one traditional facet: historically, this drug was mostly used as a sudorific *. It is this very "property" which is mentioned in the United Kingdom (BHP 1990) and recognized, in Germany, by Commission E (alongside another effect, an increase in bronchial secretions). Therefore, elder flower has different indications in Germany, including colds and coughs. What is the compound responsible for the activity? A flower constituent? Or very hot water...?

* But some authors now tend to classify this genus, as well as the genus *Viburnum*, in another family within Dipsacales, namely Adoxaceae.

** The authors of the French Explanatory Note were masterfully (?) subtle: although the information for the medical profession mentions urinary and digestive elimination, the corresponding consumer information uses the phrase "the body's elimination functions". Isn't the concept of a sudorific written between the lines?

The Belgian authorities recognize the use as a traditional diuretic "despite the fact that the activity has not been proved".

Fruits. In addition to cyanidin glycosides (3-*O*-glucosyl-, 3-*O*-sambubiosyl- *, 3,5-diglucosyl-, and 3-sambubiosyl-5-glucosyl-cyanidins), the berries contain flavonoids, acids (citric, malic), saccharides, and 0.1 mL/kg essential oil; the seeds contain cyanogenic glycosides. The ripe fruit, edible fresh or as jam, is the source of an extract used as food coloring (e.g., to color cherry or pomegranate syrup). The unripe fruit can cause minor gastrointestinal distress. Despite a glaring lack of pharmacological data, the fruit of the European elder is traditionally used—in France—for medicinal purposes, for the same indications as the leaf. The same is true for the bark of the stem, which is known to contain a potentially toxic lectin.

Other Anthocyanin-containing Drugs

The cornflower (*Centaurea cyanus* L., Asteraceae, Fr. Ph., 10th Ed.) owes its color to anthocyanins; it also contains polyalkynes. Traditionally used topically (without specific justification) for eye irritation or discomfort of various etiologies (for example eye strain, seawater or swimming pool water, or smoky atmospheres). as an adjunct in the emollient and antipruriginous treatment of skin disorders, and as a trophic protective agent [French Expl. Note, 1998], cornflower capitulums are mostly of interest for the touch of color that they impart to herbal teas. The same comment often applies to other vividly colored drugs cited elsewhere for other constituents, such as the rose (p. 394), high mallow (p. 111), or hibiscus (p. 24).

7. BIBLIOGRAPHY

Generalities

Dangles, O., Saito, N. and Brouillard, R. (1993). Anthocyanin Intramolecular Copigmen Effect, *Phytochemistry*, **34**, 119-124.

Hoshino, T. and Tamura, H. (1999). Anthocyanidin Glycosides: Color Variation and Colo Stability, *in* "Naturally Occurring Glycosides", (Ikan, R., Ed.), p. 43-82, John Wiley & Sons, Chichester.

Jackman, R.L. and Smith, J.L (1996). Anthocyanins and Betalains, *in* "Natural Foo Colorants", (Hendry, G.A.F. and Houghton, J.D., Eds.), p. 244-309, Blackie Academic Professional, London.

, Strack, D. and Wray, V. (1994). The Anthocyanins, *in* "The Flavonoids – Advances i Research since 1986", (Harborne, J.B., Ed.), p. 1-22, Chapman & Hall, London.

* Sambubiose is 2-O-β-D-xylopyranosyl-β-D-glucopyranoside.