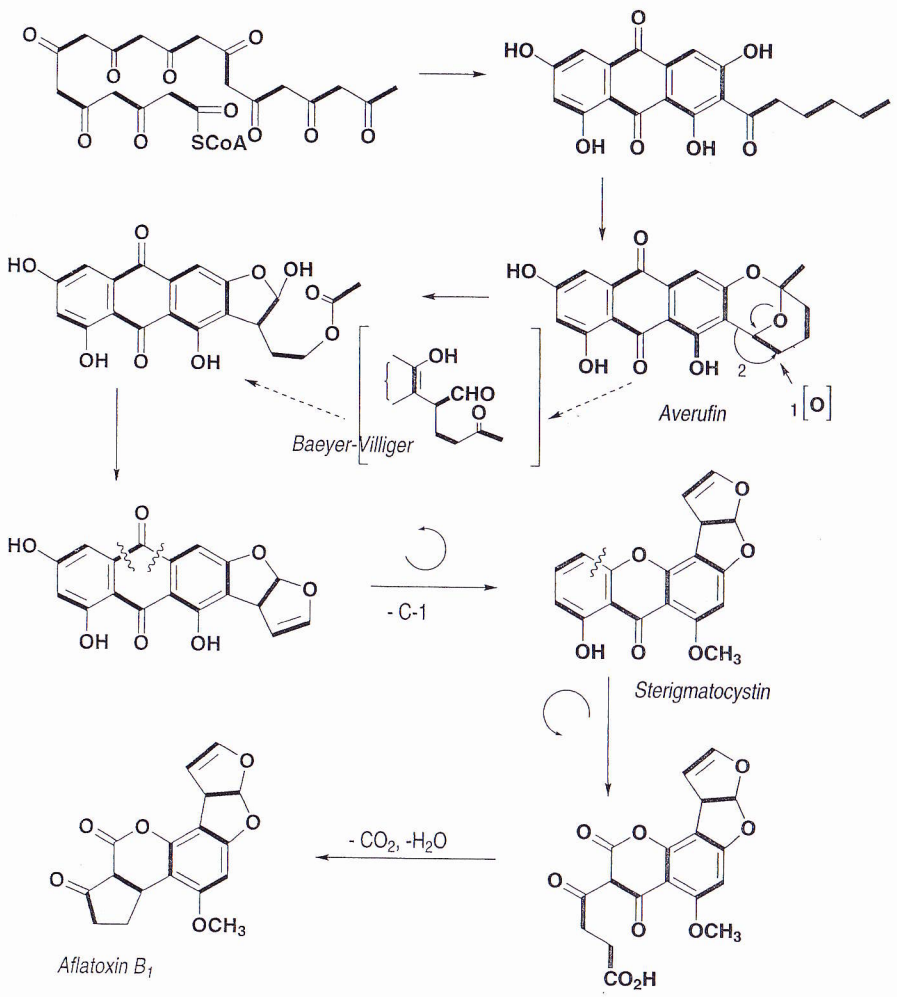


Biosynthetic origin of a complex polyketide: example of aflatoxin B_1
(mycotoxin elaborated by Aspergillus *sp., see p. 275)*

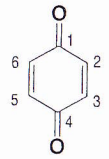
Note: the boldface bonds represent the acetate units incorporated intact into the structure (double-labeled acetate).

Quinones

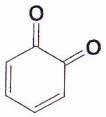
1. INTRODUCTION

Quinones are oxygen-containing compounds which are essentially the oxidized homologs of aromatic derivatives, and are characterized by a 1,4-diketo-cyclohexa-2,5-diene pattern (*para*-quinones), or, possibly, by a 1,2-diketo-cyclohexa-3,5-diene pattern (*ortho*-quinones). In naturally occurring quinones, the dione is conjugated to an aromatic nucleus (benzoquinones), or conjugated to a condensed polycyclic aromatic system: naphthalene (naphthoquinones), anthracene (anthraquinones), 1,2-benzanthracene (anthracyclinones), naphthodianthrene (naphthodianthrones), perylene, phenanthrene, and so on.

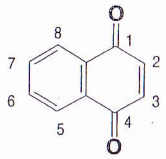
Since quinones arise from the oxidation of phenols, one would expect to encounter the quinone pattern in different classes of secondary metabolites. Thus, several quinone-flavonoids are known (from B ring oxidation), as well as a fair number of quinones with a terpenoid skeleton. In the latter group, the best known are the diterpenoid quinones with an abietane skeleton, which are characteristic of Lamiaceae. In rarer cases, the quinone pattern may be combined with a nitrogen-containing heterocycle (carbazolequinones).



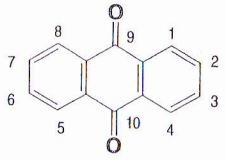
p-Quinone



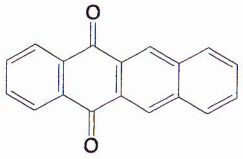
o-Quinone



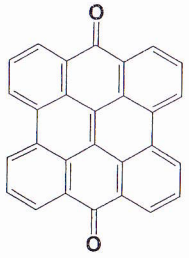
Naphtoquinone



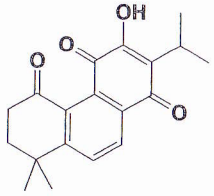
Anthraquinone



Anthracyclinone



Naphtodianthrone



Miltionone
(abietane-quinone)

Benzoquinones and naphthoquinones with a long polyisoprene chain—lipo-quinones or bioquinones—will not be covered here. Indeed, ubiquinones (virtually universal), plastoquinones, and tocopheryl-quinones (in higher plants and algae) are involved as electron carriers in cell respiration and photosynthesis, and therefore may not be considered secondary metabolites. The same comment applies to phyllo- and menaquinones (vitamins K).

2. DISTRIBUTION OF QUINONES

Over 1,200 quinones have been described, mainly in the vegetable kingdom: in Angiosperms, Gymnosperms, Fungi, Lichens, and, very rarely, in Filices. They are not exceptional in the animal kingdom, especially in Echinodermata and Arthropoda.

Simple benzoquinones are characteristic of Arthropoda, and rather rare in higher plants, where they seem specific to a limited number of families: Myrsinaceae, Primulaceae, and Boraginaceae. The very widespread 2,6-dimethoxy-1,4-benzo-quinone is probably a degradation product of lignin.

The distribution of naphthoquinones is limited in Fungi, and is sporadic in Angiosperms. There, again they occur in genera from a rather limited number of families: Bignoniaceae, Ebenaceae, Droseraceae, Juglandaceae, Plumbaginaceae, Boraginaceae, Lythraceae, Proteaceae, Verbenaceae, among others.

Anthraquinones are rather widely distributed: Fungi, Lichens, and, to a lesser extent, Spermatophyta. They are abundant in a small group of Angiosperm families: Rubiaceae, Fabaceae, Polygonaceae, Rhamnaceae, Liliaceae, Scrophulariaceae, and others, in which they frequently occur as glycosides.

3. BIOSYNTHESIS

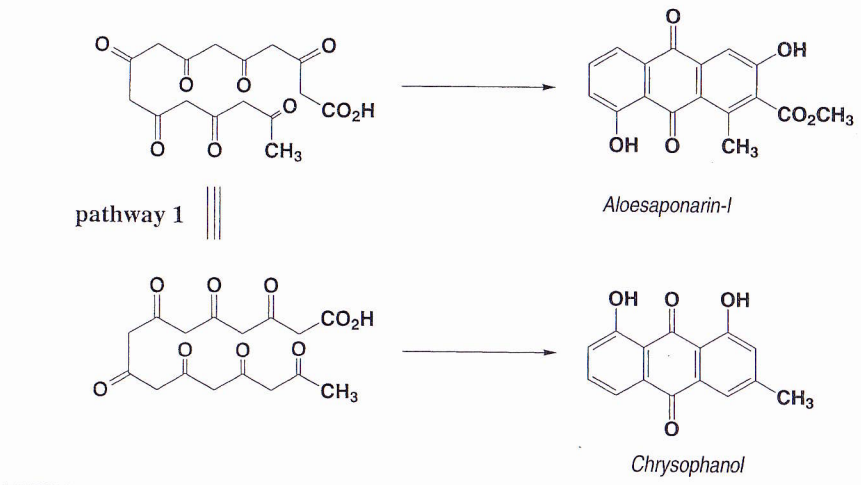
Quinone biosynthesis is characterized by the diversity of the metabolic pathways which allow the various living organisms to elaborate them from a rather limited number of precursors: acetate and malonate, mevalonate, and phenylalanine.

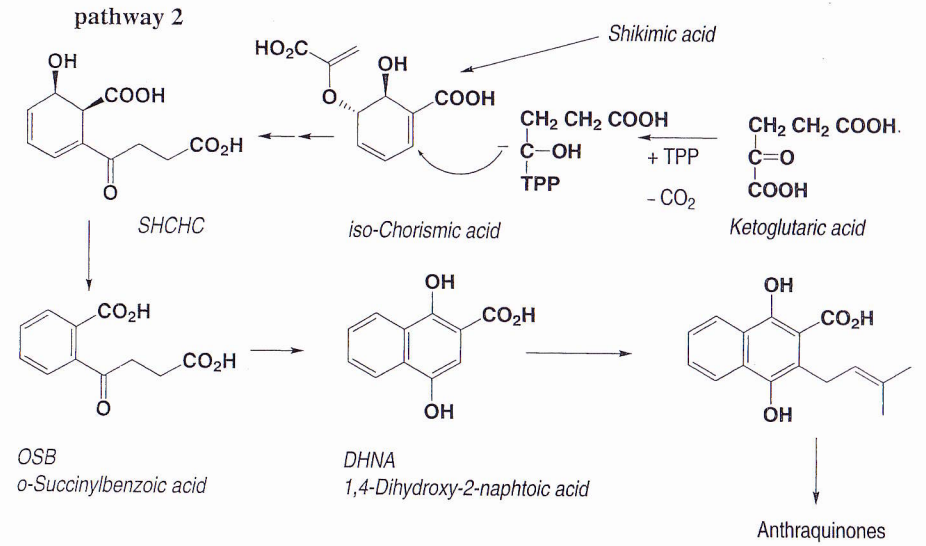
Polyketide Pathway

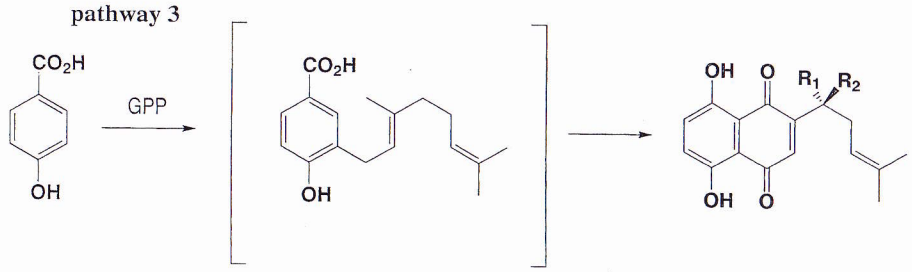
In a large number of cases, the very structure of the quinone reveals how it arose from the cyclization of a poly-β-ketoester: consider chrysophanol, and more generally, 1,8-dihydroxyanthraquinones; consider also aloesaponarin I and related compounds. Some naphthoquinones (for example those of Plumbaginaceae) have such an origin.

Mevalonic and Chorismic Acid Pathway

Another pathway—which is in fact the most common in higher plants—is that of *o*-succinylbenzoic acid (= OSB = 4-(2'-carboxyphenyl)-4-oxobutanoic acid). This acid arises from the reaction of isochorismic acid with α-ketoglutaric acid in the presence of thiamine pyrophosphate. It is then acylated by coenzyme A, and cyclized to 1,4-dihydroxy-2-naphthoic acid (= DHNA), the immediate precursor of naphthoquinones. In several cases, particularly in Rubiaceae, this pathway can be shown to lead to anthraquinones: isoprenylation in the 3-position of DHNA by dimethylallyl pyrophosphate (= DMAPP), cyclization, and aromatization. In other families, DMAPP preferentially alkylates C-2.







Biosynthetic origin of quinones

4-Hydroxybenzoic Acid Pathway

The *p*-hydroxybenzoic acid pathway leads—in Boraginaceae—to naphthoquinones such as shikonin and its isomer, alkannin. 4-Hydroxybenzoic acid, which arises from the metabolism of phenylalanine, acts as an acceptor for the alkylation by a molecule of geranyl pyrophosphate (= GPP).

4. PROPERTIES, EXTRACTION, SEPARATION, AND CHARACTERIZATION

The fundamental properties of quinones are their facile interconversion to hydroquinones (which makes them mild oxidation reagents), and their tendency to add nucleophiles.

Free quinones are practically insoluble in water, can be extracted by the common organic solvents, and their separation requires the common chromatographic techniques. Benzoquinones and naphthoquinones can be steam distilled. Their stability is fair, but the formation of artefacts is always possible, for example the oxidation by silica gel of 7-methyljuglone to methylnaphtharizin and to dimers, or the methoxylation of naphthoquinones by methanol.

Glycoside extraction is achieved with water or with rather dilute hydroalcoholic solutions. Recovering the reduced forms (quinols, anthrones) is delicate: working at low temperature, away from light, and under nitrogen is required to avoid their spontaneous oxidation during the extraction.

Various color reactions can be used to characterize quinones. The main one is Bornträger's reaction, obtained by dissolving the quinone in alkaline aqueous medium: the solution develops a vivid color which ranges, depending on the structure and the substituents of the quinone, from orangy-red to purplish-violet. This reaction is also used to visualize TLC plates. In the specific case of 1,8-dihydroxyanthraquinones, the reaction with magnesium acetate is used very often: it leads to stable colors.

Quinone quantitation is often done by spectrophotometry, and based on one of the color reactions described above. Nowadays, quality control on the drugs of commercial importance is done by HPLC (on reverse phase, in isocratic conditions, and with UV detection).

5. BIOLOGICAL PROPERTIES AND USES OF QUINONE-CONTAINING DRUGS

Natural benzoquinones in the strict sense of the term have no therapeutica application. Note, however, that the reduced form of 1,4-benzoquinone (i.e. hydroquinone) occurs as a glycoside, namely arbutin, and that this molecule possesses strong urinary antiseptic properties (see simple phenol-containing drugs) Synthetic hydroquinone, on the other hand, has dermatological and industria (photography) applications.

Many naphthoquinones are antibacterial and fungicidal (their presence explains the resistance of some tropical woods such as teak to fungi, insects, and generally, to xylophagous organisms). The nucleophilicity of these molecules also explains their cytotoxicity. Antiprotozoal and antiviral activities have been described, and several molecules in the group have non-trivial toxicity. Currently, no natural naphthoquinone is marketed for therapy, and only a very limited number of drugs containing them remain in use to produce galenicals (for example *Drosera* sp.).

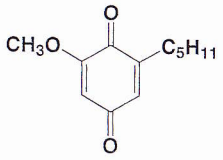
Drugs containing 1,8-dihydroxyanthraquinone derivatives have laxative properties, and have been prized for this activity for centuries (*Cassia, Rhamnus*), or even millennia (*Rheum*). They continue to be widely used, in spite of non-trivial drawbacks.

For a long time, some quinone-containing drugs had been prized as dyes. These included vegetable drugs containing anthraquinones, such as madder root (*Rubia tinctorium* L., Rubiaceae), or containing naphthoquinones, such as alkanna root (= alkanet = Anchusae Radix) (*Alkanna tinctoria* Tausch., Boraginaceae): the former provides chiefly alizarin (the aglycone of ruberythric acid), and the latter provides chiefly alkannin. They also included products of animal origin, such as dyer's kermes from *Kermococcus vermilio*, used to dye textiles. Cochineal, a coloring currently authorized (Eur. id. code E120), is traditionally extracted from the desiccated females of a Central American hemipter, *Dactylopius coccus* = *Coccus cacti* L., which contain approximately 10% of a tetrahydroxylated anthraquinone, carminic acid.

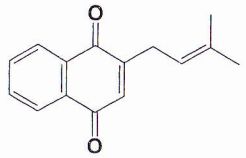
(*R*)-Shikonin (an isomer of (*S*)-alkannin) is found in an oriental Boraginaceae (*Lithospermum erythrorhizon* Sieb. & Zucc.), is currently produced by tissue culture, and was marketed as a coloring in cosmetology.

6. QUINONES AND ALLERGY

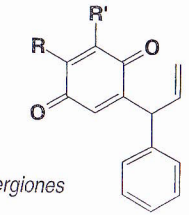
The allergenic potential of many quinones (benzo- and naphthoquinones) is due to the fact that they act as haptens: by combining themselves, through their nucleophilic centers, with amine and thiol functions on macromolecules, they induce dermatitis by sensitization. One of the most notorious examples is that of the horticultural varieties of primroses of Asian origin: the top primrose, *Primula obconica* Hance, and other primroses (e.g., *P. malacoides* Franchet). These species can cause, in gardeners and florists, localized pruriginous reactions and urticaria- or erysipelatous-type rashes on the eyelids, cheeks, chin, neck, fingers, hands, and



Primin



Deoxylapachol



Dalbergiones

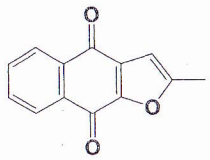
forearms. The affected areas are erythematous and often edematous; the formation of vesicles is common. The molecule responsible for the allergenic activity is an alkylbenzoquinone, primin or 2-methoxy-6-pentylbenzoquinone.

Similar problems occur in the exotic wood industry. Workers exposed to sawdust may develop conjunctivitis and nasal reactions. Erythema and dermatitis with blisters are also frequent on the back of the hands, forearms, eyelids, face, and neck. In very rare cases, an allergy of the same type can be observed following prolonged contact with a musical instrument! Many molecules are incriminated:

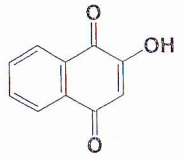
- naphthoquinones such as lapachol, deoxylapachol, and closely related compounds in teak (*Tectona grandis* L., Verbenaceae), *Tabebuia* and *Tecoma* (ipe roxo, páu d'arco, Bignoniaceae), or naphthoquinones of ebonies (from Madagascar, Africa, or the island of Celebes, *Diospyros* sp., Ebenaceae);
- dalbergiones of purple woods from Asia (*Dalbergia latifolia* Roxb.), Africa (*D. melanoxylon* Guill. et Perr.), or South America (*D. nigra* Allem. [Vell. Conc.] Benth., *D. retusa* Hemsley [cocolobo]).

7. NAPHTHOQUINONE-CONTAINING DRUGS

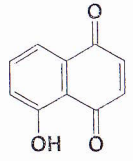
Naphthoquinones are yellow or orangy pigments essentially from plants, and are characteristic of some Angiosperm families, including Ebenaceae, Droseraceae, and Bignoniaceae. They are almost always 1,4-naphthoquinones, and they are, in very rare cases, 1,2-naphthoquinones. The most common substituents are hydroxyl and methyl groups, at C-2, on the aromatic ring, or both. Prenylation is not rare, and in Ebenaceae, dimeric structures are not exceptional. The pharmaceutical interest of this group is very limited.



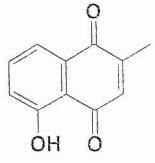
Lapachonone



Lawsone



Juglone



Plumbagin

- SUNDEW,
  Drosera rotundifolia L., *D. anglica* Huds. (= *D. longifolia* L.), *D. intermedia* Hayne (= *D. longifolia* auct. non L.), Droseraceae

The Plant, the Drug. The sundews that used to appear in the French Pharmacopoeia editions, up to but excluding the 10th edition, were species from European peat bogs that have become very rare, and are now protected.

D. rotundifolia is a small plant (5 cm high) with a rosette of leaves with long petioles. The leaf blade is orbicular, and is covered with long red trichomes with

globulous apexes, which secrete a viscous, highly refractive liquid (hence the common name sun*dew*). One or two thin stalks, with no leaves, bear a raceme of flowers with white petals. The other European species differ mainly by the shape of the leaf blade: lanceolate and attenuate into a petiole in *D. anglica*, obovate in *D. intermedia*. The drug consists of the entire plant; it is often falsified by species from Africa or Madagascar.

Chemical Composition. In the fresh plant, a glycoside is found, namely rossoliside, the 4-glucoside of the reduced form of plumbagin (= 2-methyl-5-hydroxy-1,4-naphthoquinone). Plumbagin represents about 0.7 to 1% of the dried drug. The other European species have a similar composition: in *D. intermedia*, plumbagin occurs alongside 2-methyl-5,8-dihydroxy- and 2-methyl-3-chloro-5-hydroxy-1,4-naphthoquinones (1 to 2% of total quinones).

Tests. Quinones can be characterized in sundew by TLC analysis of a tincture. For quantitation, it is possible to take advantage of their amenability to steam distillation: the quinones present in the distillate can be extracted with chloroform, and the absorbance of the organic solution can be measured. An important diagnostic test for drug identification is the detailed microscopic examination of the morphology of the glandular trichomes—especially for the non-European *Drosera* (*ramantacea, peltata*, and others).

Properties and Uses. Experiments in animals show that sundew tincture is an antispasmodic: prevention of acetylcholine-induced bronchospasm, decrease in peristalsis in the isolated guinea pig ileum.

Plumbagin has antibacterial properties: at low concentrations (1/50,000), it is active on Gram positive cocci (staphylococcus, streptococcus, pneumococcus), as well as Gram negative cocci (salmonella). It is also active on certain pathogenic fungi and on some parasitic protozoa (leishmania). At higher doses, plumbagin is cytotoxic.

The common form of utilization of sundew is the tincture (1-3 g/day). Extracts are also used. Tincture and extracts are ingredients of proprietary drugs (especially syrups) promoted as a treatment for spasmodic coughs. Sundew is not listed in the Annex to the 1998 French Explanatory Note. The German Commission E monograph specifies that sundew is used orally for coughing fits and irritating coughs.

- Other *Drosera* species.

Drosera peltata Sm. This species from eastern Asia (Japan, China, Malaysia, Philippines, India) contains plumbagin in its aerial parts, and plumbagin and droserone (2-methyl-3,5-dihydroxy-1,4-naphthoquinone) in the subterranean parts. The species is characterized by its tubercle, its non stipulate leaves (basilar leaves with rounded blade, cauline leaves with asymmetrical peltate blade), and its flowers with white petals.

Drosera ramantacea Burch. ex Harv. & Sond. is from Madagascar and eastern Africa. It is characterized by flowers with purple petals, cauline leaves with lanceolate blade (but no basilar rosette), and by the absence of tubercle. Although it contains quinones, their concentration is very low (about 10 times less than in official *Drosera*), and the chief constituent is 5-hydroxy-7-methyl-1,4-naphthoquinone or ramantaceone, found as a glycoside in the fresh plant. The drug is weakly spasmolytic.

• Walnut Tree, *Juglans regia* L., Juglandaceae

The part of the walnut tree that is used is the dried foliole; it contains not less than 2% total flavonoids (Fr. Ph., 10th Ed.).

The walnut tree, originally from the Near-East, is cultivated in France (in the Perigord and Dauphiné regions) to produce walnuts. The leaves are imparipinnate, have five to nine entire folioles, are ovate-lanceolate, acuminate, and slightly coriaceous. The commercial drug generally consists of folioles, partially cleaned and separated from the rachis (it contains not more than 18% rachis from young stems). The fruit is a drupe with a green exocarp, which blackens by oxidation at maturity (to give walnut stain); the hard, bivalve endocarp surrounds two "cerebriform" and voluminous cotyledons.

The chief known constituent is juglone (5-hydroxy-1,4-naphthoquinone), which occurs in the fresh plant (leaf, stain) as 1,4,5-trihydroxynaphthalene glycoside (2% in the stain, 0.6% in the leaves), but also in the free state, particularly in the epicuticular wax. Alongside juglone are other naphthoquinones (detectable by GC) and reduced derivatives. The leaf and pericarp are rich in hydrolyzable tannins. The leaf also contains a small amount of essential oil, ascorbic acid, and flavonoids. Juglone has antibacterial and fungicidal properties.

The seed cotyledons are used as food and as a source of oil*: indeed they contain 50% and more of an oil rich in linoleic acid (55-65%) and α-linolenic acid (9-15%). The oil has a strong taste, turns rancid rapidly, and its consumption in France is fairly limited. Walnut stain is used to stain woods.

* Monitoring certain populations (Californian Adventists) has shown that the frequent consumption of walnuts appears associated with a decrease in the risk of myocardial infarction and in ischemic heart disease mortality. A controlled crossover single-blind study has helped define the impact of walnut consumption on serum lipids: the subjects on a walnut-rich regimen saw their total cholesterol, LDL-, and HDL-cholesterol drop significantly in comparison to a control group (respectively –12.4%, –16.3%, and –4.9%); the median arterial blood pressure remained unchanged. See Sabaté, J., Fraser, G.E., Burke, K., Knutsen, S.F., Bennett, H. and Lindsted, K.D. (1993). Effects of Walnuts on Serum Lipid Levels and Blood Pressure in Normal Men, *New Engl. J. Med.*, 328, 603-607. On the benefits of dried fruits in general, see the more recent publication: Dreher, M.L., Maher, C.V. and Kearney, P. (1996). The Traditional and Emerging Role of Nuts in Healthful Diets, *Nut. Rev.*, 54, 241-245.

The walnut foliole may be an ingredient of plant-based medications, and may claim the following indications [French Expl. Note, 1998]: 1. orally, traditionally used to treat the subjective symptoms of venous insufficiency, such as fullness in the legs, the symptoms of piles, and the symptoms of mild diarrhea; 2. locally, traditionally used to treat scalp itching, peeling, and dandruff; and as an adjunctive emollient and itch-relieving treatment in skin disorders; as a trophic protective agent for cracks, abrasions, frostbite, chaps, and insect bites; to treat sunburns, and superficial and limited burns; for diaper rashes; as an antalgic in diseases of the oral cavity, pharynx, or both (collutoria, lozenges).

In Germany, the astringent properties recognized by Commission E lead to using the drug only in external application, for superficial skin inflammation, and for excessive foot and hand perspiration. Since the fruit envelope has no demonstrated activity, it must not be used (juglone is mutagenic, maybe even carcinogenic).

Comment. Black walnut tree (*J. nigra* L.) wood shavings are used as horse bedding in North America, and cause the animals to develop laminitis. Apparently juglone is not responsible for the toxicity.

• Henna, *Lawsonia inermis* L., Lythraceae

A shrub that becomes thorny over time, cultivated from North Africa to the Middle East and to India (it followed the spread of Islam), henna is used for its leaves, which are ovate-acuminate, mucronate, and revolute on the edges. Fresh henna leaves contain glycosides, which release lawsone (2-hydroxy-1,4-naphthoquinone) upon hydrolysis. This quinone dissolves in alkaline aqueous solutions to give an intense orangy-red color. It is practically non-toxic, and it is a powerful fungicide. The lawsone level in the dried drug is about 1%. Henna leaf also contains flavonoids, coumarins, and xanthones. The flowers owe their fragrance to an essential oil containing ionones. The ethanolic leaf extract is an analgesic, antipyretic, and anti-inflammatory in rats (0.25-2 g/kg, *per os*).

Henna is used in various ways in Ayurvedic medicine: for the treatment of skin ailments, burns, wounds, and diarrhea, and as a taenicide, an antiepileptic, and an abortifacient agent. As a coloring and cosmetic ingredient, it has been in use for nearly three millennia: as a hair color, nail color, and in the Moslem world, for the (traditional) decoration of the soles of the feet and palms of the hands. The drug is widely used in cosmetology for its dyeing properties, due to the strong binding of lawsone to the hair, probably upon reaction of the thiol groups with keratin (shampoos and hair lotions). Although it is exceptional for hair products to induce a serious allergic reaction, there have been accidents, many of which were fatal, after ingestion of mixtures of tinctures based on henna and *p*-phenylenediamine. It was recently postulated that the lawsone in henna is responsible for the acute hemolytic anemias observed in newborns with congenital G6PD deficiency. The results of an *in vitro* study of the oxidizing power of naphthoquinone were consistent with this hypothesis

8. ANTHRAQUINONE-CONTAINING DRUGS: LAXATIVE HYDROXYANTHRAQUINONE GLYCOSIDES

The different drugs in this group are characterized by the presence of phenolic and glycosidic compounds, derived from anthracene and have a variable degree of oxidation (anthrones, anthranols, anthraquinones): they are the anthraquinone glycosides. No matter what their degree of oxidation, these molecules have in common a double hydroxylation at C-1 and C-8.

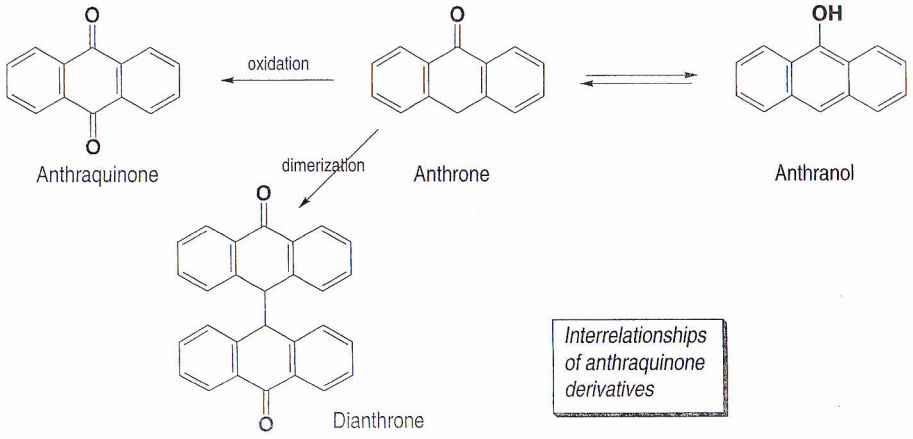
The botanical distribution of the species containing 1,8-dihydroxyanthraquinone glycosides is very limited: Liliaceae (aloe), Polygonaceae (rhubarbs), Rhamnaceae (buckthorn, cascara), and Cæsalpiniaceae (sennas).

A. Structure of Anthraquinone Glycosides

- the aglycones. The degree of oxidation varies. In anthrones (i.e., 10-*H*-anthracen-9-ones), carbon 10 is a methylene carbon. Depending on the pH, these anthrones can occur alongside their tautomeric forms, the anthranols. In practice, anthrones and anthranols are often designated by the term "reduced forms", and anthraquinones by that of "oxidized forms".

Under some conditions (for example during the drying of sennas), anthrones may combine into dianthrones. These are referred to as homo- or heterodianthrones, depending on whether the constituent anthrones in the dimer are identical or different, respectively.

The structural variations observed for these aglycones are limited. Outside of the fact that two phenolic hydroxyl groups are always found in the 1- and 8-positions, only the 3- and 6-carbons may be substituted: the former is always substituted by a carbon of varying degree of oxidation (methyl, hydroxymethyl, or carboxyl), and the latter is sometimes substituted by a phenolic hydroxyl group, which is either free or etherified by methanol. This general substitution scheme clearly indicates that these



Interrelationships of anthraquinone derivatives

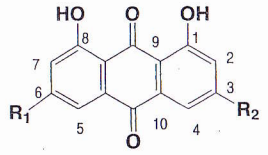
compounds arise biogenetically from the condensation of an octa-acetate (see above).

- the glycosides. Because anthrones are unstable, the free aglycones that occasionally occur in the drugs are always anthraquinones. The reduced forms, on the other hand, exist only in the *combined* state, in other words as glycosides.

The saccharides within these glycosides are commonplace: glucose, rhamnose, and in rare cases apiose. The bond with the aglycone normally involves the phenolic hydroxyl group at C-8 (in the case of glucose), or the one at C-6 (in the case of rhamnose or apiose). The aglycone may be linked to two sugars; thus, glucofrangulin A is emodin 6-*O*-α-L-rhamnosyl-8-*O*-β-D-glucoside.

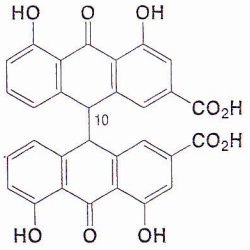
It is not rare for 1,8-dihydroxyanthrones to occur as *C*-glucosides, with the bond forming between the C-1 of glucose and the C-10 of the aglycone, which introduces a chiral center into the molecule (see, among others, aloin A [10*R*] and B [10*S*]). Moreover, these *C*-glycosides may be *O*-glycosides as well. To elaborate on the previous example, aloinosides are aloin 11-*O*-α-L-rhamnosides (the carbon atom of the hydroxymethyl group in the 3-position is numbered 11 in this case).

Comment. There are substantial differences in composition between the fresh plants and the dried drugs. In the fresh vegetable, anthracene-type compounds occur chiefly as glycosides of monomeric anthrones. During desiccation, two transformation processes come into play: oxidation, which leads to anthraquinone glycosides (e.g., oxidation, in buckthorn, of the anthrone forms to frangulin and glucofrangulin), and dimerization, which yields glycosides of dianthrones. It has been shown (in the case of senna) that this dimerization is an enzymatic reaction only observed if drying is accomplished at moderate temperature (40°C). Other

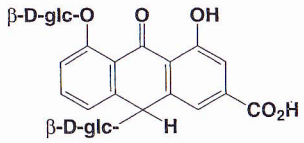


R_1=H, R_2=CH_3: *Chrysophanol*
R_1=H, R_2=CH_2OH: *Aloe-emodin*
R_1=H, R_2=CO_2H: *Rhein*
R_1=OH, R_2=CH_3: *Emodin*

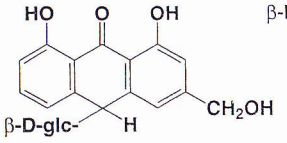
Examples of structures of hydroxyanthraquinones derivatives



Sennidins A, B



Rheinosides A, B



Aloins A, B

Rhein-8-glucoside

authors are of the opinion that these dimers are not only artefacts formed during the drying process, but that they might in part pre-exist and be a part of oxidoreduction systems with potential physiological significance.

B. Physico-chemical Properties and Characterization

Anthraquinones are colored, orangy-red compounds, sparingly soluble in cold water, and soluble in organic solvents and alcohols. The carboxylic aglycones can be extracted with an aqueous sodium bicarbonate solution. The glycosides are soluble in water and hydroalcoholic solutions. Treating the *O*-glycosides in acidic medium causes their hydrolysis, but the cleavage of the carbon-carbon bond of *C*-glycosides can only be obtained in the presence of ferric chloride.. The same reagent, but in neutral conditions, will achieve the transformation of dianthrones into anthraquinones. In practice, the quantitations required by the French Pharmacopoeia are in two steps: reflux in the presence of ferric chloride, acidification with hydrochloric acid, and reflux again.

Characterization. The characterization of hydroxyanthraquinone derivatives applies the Bornträger reaction: upon dissolving the quinones in alkaline aqueous medium (KOH), a red color, more or less purplish, develops. This reaction is only positive with the free anthraquinone forms: to characterize glycosides with this reaction, preliminary hydrolysis is required, and if the aglycones are anthrones, they must first be oxidized to anthraquinones. Another color reaction, specific to 1,8-dihydroxyanthraquinones, uses magnesium acetate in methanol. The resulting red color is more intense and more stable to light than that from the simple reaction with potassium hydroxide. Consequently, it lends itself better to quantitation. Like the Bornträger reaction, this reaction is positive only with the oxidized and free forms.

There is a reaction specific to anthrones: it is based on their ability to react with nitrotetrazolium blue or *p*-nitrosodimethylaniline to form a colored azomethine. The *C*-glucosides of reduced forms can be characterized by the fluorescence of the anthranol forms in the presence of sodium borate (Schouteten reaction).

The identification of the glycosides and aglycones is typically done by TLC: visualization under UV light and by the Bornträger reaction, directly or after oxidation, right on the TLC plate, of anthrones to anthraquinones.

Quantitation. The spectrophotometric quantitation takes advantage of the color obtained with magnesium acetate, or possibly, with potassium hydroxide. Since the free anthraquinone forms have no marked pharmacological activity, they are generally not included in the quantitation (as a general rule, pharmacopoeias only require quantitating the combined forms).

The quantitation of the total combined forms generally includes an extraction, an oxidizing hydrolysis, a color reaction, and a spectrophotometric determination. The drug is powdered and extracted with water or a hydroalcoholic solution; next, the aqueous phase is extracted with [illegible]

anthraquinone forms potentially present. Next, this aqueous solution is oxidized (ferric chloride) and hydrolyzed (hydrochloric acid); the resulting anthraquinones are extracted with an apolar organic solvent. The solvent is evaporated, and the residue redissolved in a methanolic solution of magnesium acetate, whose absorbance is measured at 515 nm (for the variations, see the French Pharmacopoeia and the monographs below).

C. Pharmacological Properties

Depending on the dose administered, 1,8-anthraquinone derivatives exert a more or less violent laxative or purgative activity. At therapeutic doses they are stimulant laxatives: in the past, they were thought to act by irritation of the mucosa, and although this concept is still encountered, it does not seem justified.

The activity is linked to the structure of these compounds: the most interesting derivatives are the *O*-glycosides of dianthrones and anthraquinones, as well as the *C*-glycosides of anthrones, in other words the group of compounds without a $-CH_2-$ in the 10-position. The activity of the glycosides of monomeric anthrones is excessive, which explains why the drugs containing them (for example buckthorn bark) are only used after prolonged storage or after the appropriate heat treatment, during which they are oxidized to anthraquinone glycosides. The free aglycones (anthraquinones) are practically inactive.

The free aglycones found in the drug or formed by initial gastric hydrolysis *, upon reaching the intestine, are absorbed in the small intestine, glucoconjugated in the liver, and almost totally excreted in urine. An enterohepatic cycle is involved. The glycosides of anthraquinone and dianthrones are polar molecules, are water-soluble, and have a high molecular weight, so they are not resorbed nor hydrolyzed in the small intestine. In the colon, they are hydrolyzed by the β-glucosidases of the intestinal flora, and the freed anthraquinones are reduced: thus, the active forms are the anthrones formed *in situ*, which explains the latency observed between compound (or drug) intake and the laxative effect. For some authors, anthraquinone glycosides may be considered *prodrugs*: the sugars would act as transporters by preventing the active moiety from being absorbed prior to being freed in the colon under the influence of bacterial enzymes.

Hydroxyanthraquinone derivatives affect intestinal motility: it has been shown, *in vivo*, that rhein anthrone acts by direct contact with the epithelial cells of the intact intestinal mucosa. These compounds are also known for their cytotoxicity and their ability to induce cellular alterations as well as the formation of insoluble deposits in the cells. Fragments of these deposits are then absorbed by macrophages (clinically, this manifests itself by a melanotic pigmentation of the colon mucosa [= *melanosis coli*] characteristic of the abuse of anthraquinone laxatives). These harsh compounds can cause colon ulceration.

* [illegible]

Anthraquinone glycosides are thought to affect the absorption of water and electrolytes. By inhibiting the Na-K ATPase activity of enterocytes, they cause an inhibition of water, sodium, and chloride resorption, and an increase in the secretion of potassium by the intestinal mucosa. Other mechanisms have also been considered to explain the activity of these derivatives: an action on prostaglandin synthesis (these are involved in the transport of water and electrolytes), or a mechanism involving calcium.

Anthraquinone glycosides are excreted in breast milk, but the risk of diarrhea in nursing infants seems negligible. The administration of sennosides to rats (25 mg/kg/day, *per os*) over 2 years did not reveal any carcinogenicity and experiments in rabbits showed no effects on descendants. Observations in humans—which have yet to be confirmed—have led their authors to suspect a link between the abuse of anthraquinone laxatives and an increased risk of colon cancer. The teratogenicity of anthraquinones has been the subject of much research but the results were contradictory.

D. Uses of Anthraquinone Glycoside-containing Drugs

The different drugs in this group, like all of the laxatives, represent a huge market. They are used crude (as herbal teas), or as galenicals (powders, extracts, and titrated extracts) in which the various components act in synergy.

The use of these drugs and their preparations can certainly have adequate justification (preparation for radiology or coloscopy, softening stool prior to anorectal surgical procedures, treatment of occasional constipation linked to drug treatment or to a change in lifestyle), but it must always include caution, and it must always be limited to short periods of time.

The daily and prolonged use of these stimulant laxatives can cause substantial problems: dependence, and in some cases, "cathartic colon" (spastic colitis with diarrhea and abdominal pains, nausea, vomiting, then a melanotic pigmentation of the colon mucosa [= *melanosis coli*], other alterations of the colon mucosa, water and electrolyte imbalances with hypokalemia leading to a deterioration of the overall health, and risk of drug interactions with cardiac glycosides, diuretics that cause hypokalemia, and more).

The consumption of these drugs often reflects uncontrolled self-prescription, which is useless, sometimes harmful, and frequently induced by a neurotic behavior rooted in a misconception of what frequency of bowel movement is healthy. Depression and anorexia can also be the underlying cause of the abuse.

The considerable drawbacks inherent to this type of compound have led to the statement of specific rules in the context of applications for government authorization to market plant-based medications (see chapter IV and annexes II and IV-A of the 1998 French Explanatory Note). The main points stated in the text are as follows:

1. The packaging of anthraquinone laxative drugs as bulk herbal teas is proscribed.

2. The maximum number of laxative drugs introduced in combinations is limited to five with a maximum of two drugs with anthraquinone principles.

3. Combinations of drugs containing anthraquinone principles with gums, mucilages, pectins, or fibers is allowed. However, the information for physicians, pharmacists, and consumers must be focused on the anthraquinone principles. The mechanisms of action of the different drugs or preparations in the combination must be compatible.

4. The use of drugs with anthraquinone principles must be limited to short periods of time not to exceed eight to ten days; the package size must correspond to that length of time.

5. Knowing that the maximum recommended daily adult dose of anthraquinone glycosides is 25 mg (barbaloin, glucofrangulin A, cascaroside A, sennoside B) or 50 mg (rhein), the daily adult posology is calculated as a function of the maximum anthraquinone glycosides level in the drug as expressed in the French and European Pharmacopoeias. In individual adjustments of the daily posology, each unit dose must not contain more than half the typical daily dose. In the case of combinations, the cumulative effect of the constituents must be taken into account and the quantity of each drug must be decreased accordingly.

6. The administration of laxatives with anthraquinone principles is contra-indicated in children under 10 years of age. It must be discouraged in children between the ages of 10 and 15, or in pregnant or breast-feeding women (senna may be administered to pregnant women with a physician's advice).

7. The information provided to the medical and pharmaceutical profession must mention contraindications (organic inflammatory colopathy [ulcerative rectocolitis, Crohn's disease], fecal impaction, intestinal obstruction, undiagnosed abdominal pain). It must caution against exceeding eight to ten days of treatment, and against prescribing, but exceptionally, for children. The information must also specify that prolonged intake may cause disturbances (cathartic colon, dependence). As for other laxatives, the information must include a reminder that drug treatment for constipation is only an adjunctive measure to a healthy lifestyle (increasing the dietary intake of vegetable fiber and beverages [water], exercising, and training to re-establish normal bowel movements). The simultaneous use of drugs that give wave burst arrhythmias (amiodarone, astemizole, bepridil, bretylium, disopyramide, erythromycin IV, halofantrine, pentamidine, quinidine-type antiarrhythmics, sparfloxacin, sotalol, sultopride, terfenadine, vincamine) must be discouraged. The simultaneous use of cardiac glycosides, diuretics that cause hypokalemia, or corticosteroids requires special precautions (monitoring kalemia). The potential side effects are diarrhea, abdominal pain, hypokalemia, and abnormal urine color.

8. The information for the consumer must echo the above, but in lay language, and must suggest guidelines for a healthy lifestyle and diet that may prevent chronic constipation.

The German Commission E has listed the uses that are common to all anthraquinone laxative drugs: for patients needing easier defecation (anal fissures, hemorrhoids, after anal or rectal surgery) and for constipation. For senna only, the

abdominal surgery. Rhubarb at low doses is used as an astringent and stomachic. All of the drugs in the group are contraindicated in case of intestinal occlusion. Except for aloe, they can be used in pregnant women only with a physician's advice (aloe is formally contraindicated in pregnant women). Preliminary medical advice is also necessary for breast-feeding women. The Commission E monographs describe the side effects. They emphasize the risk of potentiation of the effects of cardiac glycosides (hypokalemia) and the need to limit the treatment to short periods of time. They highlight the potential for a red urine color in the case of aloe, and in the case of buckthorn and cascara, the risk of intense vomiting if fresh bark is used. The package insert must mention that re-establishing normal intestinal function requires a diet rich in fibers, a sufficient water intake, and as much exercise as possible.

Effective January 1, 1997, the state of California started requiring that labels of dietary supplements containing plants with anthraquinones be accurate and informative—apparently California is the only US state to require this.

E. Main Hydroxyanthraquinone Glycoside-containing Drugs

• Senna, *Cassia angustifolia* Vahl. and *Cassia senna* L., Cæsalpiniaceae

Senna leaf, which is listed in the 3rd edition of the European Pharmacopoeia, consists of the dried folioles of *C. senna* L. (= *C. acutifolia* Del.) known as Alexandria senna, or *Cassia angustifolia* Vahl., known as Tinnevelly senna, or a mixture of the two species. The dried fruits of both species are also the subject of a monograph. The leaves and fruits are used for their laxative properties, which are due to anthraquinone glycosides. In 1986, the world production of senna pods exceeded five million tons.

The Plants, the Drugs. Sennas are low shrubs with composite paripinnate leaves. The flowers, tetracyclic, pentamerous, and zygomorph, have a quincuncial calyx, a corolla of yellow petals with brown veins, with imbricate ascendent prefloration, and with a partially staminodial androecium. The fruit is a flattened, parchment-like, dehiscent pod, with six to eight seeds.

Both species have desert origins. Tinnevelly senna, originally from Arabia, is wild in western Africa (Somalia) and in Asia, as far as Punjab. It is currently cultivated in Pakistan and India, in the southwest of the province of Madras. Alexandria senna grows naturally in northeast Africa; it is harvested and cultivated in Sudan.

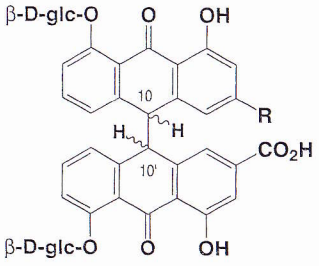
The drugs have very similar morphologies. The Tinnevelly senna folioles are lanceolate, acute, (20-50 mm x 7-20 mm), and slightly asymmetrical at the base; the two sides are smooth, and bear few short hairs. The Alexandria senna folioles are lanceolate (15-40 mm x 5-15 mm), asymmetrical at the base, and mucronate (ending abruptly as a short point). Both sides are finely pubescent.

The Alexandria senna pod is flattened, reniform, rather arched, fairly wide (40-50 mm x 20 mm and more), and its tegument presents a network of prominent wrinkles. The Tinnevelly senna pod is more elongated (35-60 mm x 14-18 mm), and its tegument presents a discontinuous network of transverse and sinuous wrinkles.

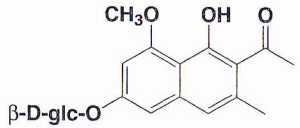
Microscopic characteristics. Of note is the presence, in the leaf section and powder, of long (250 µm) unicellular covering trichomes, with a thick and warty wall, and curved at the base; this base is surrounded by epidermic cells placed radially. The leaf epidermis consists of cells which often contain mucilage that stains in pink with ruthenium red. Determination of the stomatal index may help differentiate the species: *C. senna* = 12.5+/- 2.5 - *C. angustifolia* = 17.5+/- 2.5. Another element is the presence of polygonal epidermic cells with paracytic-type stomata. In the fruit section and powder, note the presence of the highly cutinized polygonal cells of the epicarp and that of crisscrossed fibers, together with cells with calcium oxalate prisms.

Chemical Composition. The composition of the folioles and pods of the two official species is very similar, and the differences are quantitative rather than qualitative. Both species contain flavonoids, a polyol (pinitol), acidic poly-saccharides, 10-12% mineral matter, and naphthalene derivatives. The latter arise, like anthraquinones, from the cyclization of a poly-β-ketoester. Alexandria senna is characterized by 6-hydroxymusizin glucoside, and Tinnevelly senna is characterized by tinnevellin glucoside. The active principles of both drugs are glycosides with 1,8-di-hydroxyanthraquinone-type aglycones.

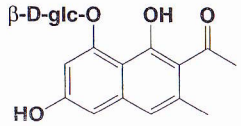
The major components of the **dried drug** are sennosides, which are glycosides of dianthrone-type aglycones, in other words sennidins. Sennosides A and B are major components, and they are the 8,8'-diglucosides of a symmetrical homodianthropic aglycone, dirhein anthrone. Since C-10 and C-10' are chiral, in theory, four isomers are possible for this aglycone. The two *threo* isomers (10*R*, 10'*R* and 10*S*, 10'*S*) are



R	C-10	C-10'	
CO_2H	*R*	*R*	*Sennoside A*
CO_2H	*R*	*S*	*Sennoside B*
CH_2OH	*R*	*R*	*Sennoside C*
CH_2OH	*R*	*S*	*Sennoside D*



Tinevellin (glucoside)



6-Hydroxymusizin (glucoside)

optically active: (+)-sennidin A and (-)-sennidin A1 (= sennidin G); in the *erythro* series, a plane of symmetry reduces the possibilities to only one *meso*, optically inactive derivative (sennidin B). Other dimers found in fair quantities in the dried drug are sennosides C and D, which are the 8,8'-diglucosides of sennidin C and D and the isomers (10*R*, 10'*R* and 10*R*, 10'*S*) of a heterodianthrone, namely rhein aloe-emodin dianthrone.

The **dried drug** also contains traces of free anthraquinones (<0.1%), and a small amount of anthraquinone glycosides (aloe-emodin and rhein mono- and diglucosides) and monomeric anthrone glycosides (rhein-anthrone and aloe-emodin-anthrone glucosides). The average level of the various drugs in hydroxyanthraquinone derivatives ranges from 2 to 5%. In the fruits, they are concentrated in the pericarps. The seeds, considered irritating, are often eliminated from the drug.

The dianthrone derivatives do not exist in **fresh senna**, which mainly contains the 8-glucosides of rhein-anthrone and of aloe-emodin anthrone. It is during the drying process, around 40°C, that the anthrone glucosides are dimerized by an enzymatic process. If drying is conducted at higher temperature, the glycosidic linkage is cleaved, and the anthrones are immediately oxidized to anthraquinones.

Tests

Identification. It is based on the Bornträger reaction: extraction (H_2O) and hydrolysis (HCl) of the glycosides, extraction of the aglycones with ether, and elimination of the solvent. Since good quality senna will contain only few anthraquinones, the color obtained upon addition of aqueous ammonia to the evaporation residue should be yellow or orangy; the characteristic red color is only obtained after heating the mixture.

The assay *per se* includes chromatographic analysis, the verification of the absence of foreign matter, total ashes (<12% [leaf], <9% [fruit]), ashes insoluble in hydrochloric acid, which provides information on the level of silica (<2.5% [leaf], <2% [fruit]), and a quantitation of anthraquinone glycosides. TLC analysis is conducted on a hydroalcoholic extract (50-50). Visualization is achieved by spraying a solution of sodium hydroxide after *in situ* oxidation by HNO_3.

Foreign matter. Senna leaf must contain not more than 3% foreign *parts* and the foreign *matter* must be not more than 1%. In the case of the fruits, the level of foreign *elements* must be not more than 1%.

Quantitation. Anthraquinone glycosides are generally extracted with hot water. The aqueous solution, after acidification to free the sennosides from their combinations as salts, is freed of the free aglycones potentially present by a chloroform extraction. After neutralization *then* centrifugation (to break the emulsion; centrifugation prior to neutralization would cause the loss of part of the sennosides, because they are sparingly soluble in acidic conditions), ferric chloride is added to the anthraquinone glycoside solution, which is refluxed, then acidified to achieve oxidation and hydrolysis. The aglycones, extracted with ether, are redissolved in a solution of magnesium acetate. After measurement of the

leaf must contain a minimum of 2.5% hydroxyanthraquinone glycosides, the fruit of Alexandria senna 3.4%, and the fruit of Tinnevelly senna 2.2%.

Pharmacological Activity. See generalities above.

Uses. Senna and its preparations are used as laxatives. Senna is used as an infusion (5 to 20 g/L), as a powder, and as extracts (particularly as a dried titrated extract [5.5-8% hydroxyanthraquinone glycosides], Eur. Ph., 3rd Ed., 1998 add.). The therapeutic indication is the symptomatic treatment of constipation. The typical daily dose (calculated as sennosides) is 25 mg/day.

● BUCKTHORN, *Rhamnus frangula* L. = *Frangula alnus* Miller, Rhamnaceae

The drug (Eur. Ph., 3rd Ed.) consists of the entire or fragmented dried bark of the twigs and branches. It is used for its laxative properties.

The Plant, the Drug. Buckthorn is a 3 to 5-m shrub with alternate and ovate leaves. These have parallel secondary veins, which curve as they meet the edge of the blade. The flowers, grouped at the base of the leaves, are small and greenish-white. The fruit is a drupe, red at first, then black at maturity, with two or three seeds. Common in the damp woods and bushes of western and central Europe, buckthorn is collected mainly in eastern European countries, from the Balkans to Poland.

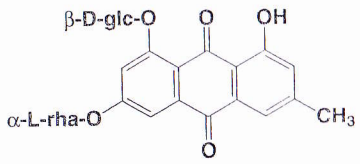
The drug, harvested during the blooming season, consists of thin quills (0.5-2 mm) with a grayish-brown outside surface, wrinkled longitudinally, and covered with grayish lenticels elongated in the transverse direction. The inside surface is reddish-brown, smooth and finely striated, and turns red in the presence of bases. Under the microscope, sclerified elements are absent from the cortical parenchyma which, in young barks, contains mucilage. The thick phloem fibers are surrounded by rows of cells each containing a prism of calcium oxalate, and are separated by wide medullary rays consisting of one to three cells.

Chemical Composition. Buckthorn bark contains traces of cyclopeptidic alkaloids, flavonoids, and 3 to 8% 1,8-dihydroxyanthraquinone derivatives.

Free aglycones are scarce (<0.1%), and mainly represented by emodin. In the dried drug, stored for over a year or heat treated, anthraquinone derivatives occur as mono- or biosidic anthraquinone glycosides. The monosides are frangulin A (= emodin 6-*O*-α-L-rhamnoside) and frangulin B (= emodin 6-*O*-β-D-apioside); the biosides are the corresponding 8-glucosylated derivatives, that is glucofrangulin A and B. Note also the presence of dimers.

Comments: 1. The French suffix *-oside* (as in frangul*oside*) which is customary for glyc*oside* structures, was not adopted by the Pharmacopoeia, which refers to frangul*ine* and glucofrangul*ine* (even though it does refer to sennos*ides* and

cascar*osides*). 2. In the fresh drug, the corresponding anthrone forms (frangularoside and glucofrangularoside) predominate.



Glucofrangulin A

Tests

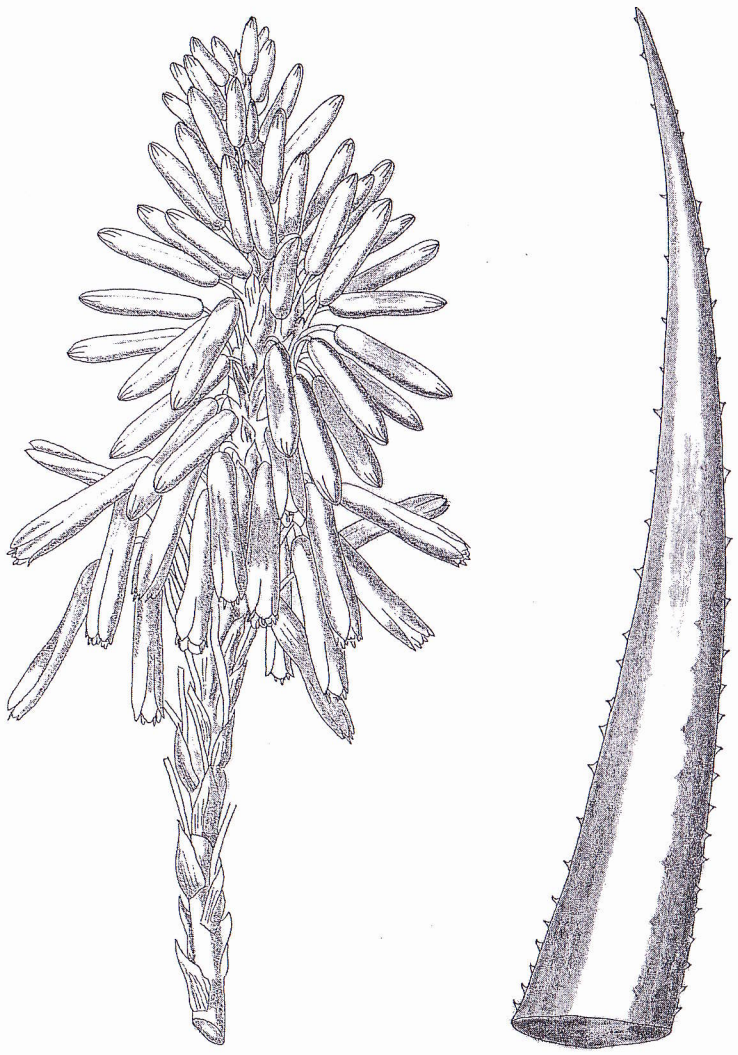
Identification. It is based on the macro- and microscopic characteristics (powder with numerous phloem fibers, with oxalate prisms-containing tubes, but no sclerified cells), on the Bornträger reaction (extraction and hydrolysis [dilute HCl] of the glycosides, extraction of the aglycones [ether], re-extraction, and color reaction [dilute NH_4OH]), and TLC analysis.

The assay *per se* includes foreign elements (<1%), total ash (<6%), and two TLC analyses of a 70% ethanol extract to verify the absence of other *Rhamnus* and of anthrones. Visualization of the first TLC plate with a hydroalcoholic KOH solution shows glucofrangulins and the absence of intense yellow or blue fluorescent bands under UV light. Visualization of the second TLC plate with nitrotetrazolium blue shows the absence of purple or blue-gray anthrone band.

Quantitation. The anthraquinone glycosides are extracted with hot 70% methanol. After filtering, an aliquot of the methanol solution is diluted and acidified, the aglycones potentially present are eliminated by a petroleum ether extraction, the aqueous phase is neutralized, and ferric chloride is added, followed by hydrochloric acid, and refluxing (what is the justification for an oxidizing hydrolysis in the quantitation of anthraquinone biosides?). The resulting free anthraquinones are extracted with ether, and are redissolved in a methanol solution of magnesium acetate. After measuring the absorbance, the glucofrangulin concentration is calculated and expressed as glucofrangulin A: it must be not less than 7% of the dried drug.

Pharmacological Properties. See generalities above.

Uses. Buckthorn bark is widely used as a laxative, in the crude form (herbal tea mixtures), as a powder, or as extracts, which are the ingredients of many proprietary drugs. The dried extract (Eur. Ph., 3rd Ed., 1998 add.) contains 15-30% glucofrangulins. It is sometimes combined with a spasmolytic agent, a bulk laxative, or both. When used crude, the mode of preparation of the infusion undoubtedly influences the final concentration in active principles; the same comment applies to the constituents, for example saponins, potentially present in compound herbal teas. Normally these herbal teas are prepared by a five-minute decoction followed by a two-hour infusion.



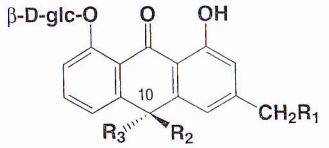
ALOE Sp.

• CASCARA SAGRADA,
Rhamnus purshianus DC
= *Frangula purshiana* (DC) A. Gray ex J.C. Cooper, Rhamnaceae

The drug (Eur. Ph., 3rd Ed.) consists of the dried bark. It is mostly used in the Anglo-Saxon countries.

The Plant, the Drug. Cascara is a tree growing on the west coast of North America. The drug is essentially collected from wild trees in the mountains of the west of the United States and Canada. Collection begins in May, and continues until the end of the summer. The bark, cut into small fragments and dried in the shade, is stored for a long time before use.

The drug consists of rather thick fragments (up to 5 mm) with lenticels on the outside surface, which is typically more or less covered with lichens, mosses, and hepaticas. Under the microscope, this bark can be distinguished from that of buckthorn by the absence of mucilage and the presence of groups of sclereids in the cortical parenchyma and the pericycle. Note the presence, as in buckthorn, of wide medullary rays.



R_1 = OH, R_2 = β-D-glc, R_3 = H, *Cascaroside A*
R_1 = OH, R_2 = H, R_3 = β-D-glc, *Cascaroside B*
R_1 = H, R_2 = β-D-glc, R_3 = H, *Cascaroside C*
R_1 = H, R_2 = H, R_3 = β-D-glc, *Cascaroside D*

Chemical Composition. Cascara bark (dry drug) contains 6 to 9% hydroxyanthraquinone glycosides. The chief constituents (70% and more) are *O*-glycosides of *C*-glycosides, namely cascarosides A, B, C, and D. These compounds are, respectively, C-10 isomers of aloin (= barbaloin *) and chrysaloin 8-*O*-β-D-glucosides. They occur alongside the corresponding *C*-glycosides, which are probably degradation products of cascarosides: aloin (= barbaloin = aloe-emodin

* A certain number of publications—particularly the Pharmacopoeias—use the term barbaloin in place of aloin. Thus, they differentiate *barb*aloin (mixture of isomeric aloe-emodin anthrone 10-*C*-glucosides) and *chrys*aloins (chrysophanol anthrone 10-*C*-glucosides). The corresponding *O*-glycosides are aloinosides (derivatives rhamnosylated on the 3-hydroxymethyl position of [barb]aloins), cascarosides A and B (derivatives glucosylated on the 8-position of [barb]aloin), and cascarosides C and D (derivatives glucosylated on the 8-position of chrysaloins). The constituent isomers of barbaloin having been described under the name of aloins A and B, we found it convenient to keep a single denomination for aloe-emodin derivatives, whether they are pure (**aloins A and B**) or mixed (**aloin**). Note that no one speaks of barbaloins A and B or of barbaloinoside; also to be avoided is the term «isobarbaloin» - this is merely 7-hydroxyaloin.

anthrone 8-*C*-glucoside) and chrysaloin (chrysophanol anthrone 10-*C*-glucoside). Anthraquinone and dianthrone *O*-glycosides are also found.

Tests. The assay takes into account the composition and the particular structure of the glycosides. The macro- and microscopic identification is completed by the stepwise characterization of hydroxyanthraquinone *O*-glycosides (decoction [H_2O], hydrolysis [HCl], aglycone extraction [Et_2O], and characterization), then of the *C*-glycosides (oxidation [$FeCl_3$] of the residual hydrochloric solution, aglycone extraction [Et_2O], characterization). In both cases, the aglycones are characterized by the Bornträger reaction (NH_4OH).

Like for buckthorn, TLC analysis proves the presence of the chief constituents, the absence of anthrone derivatives (visualization by nitrosodimethylaniline), and the absence of contamination by other *Rhamnus* species (analysis of the fluorescence). Foreign elements must be <1%, and total ashes <6%.

The dual quantitation includes the determination of total hydroxyanthraquinone glycosides and that of cascarosides. Thus, a selective extraction is required. To this end, the total glycosides are extracted with boiling water. After cooling and elimination of the free aglycones (ethyl ether-hexane) in acidic medium, the aqueous phase is re-extracted with ethyl acetate; the cascarosides are very polar, and remain in the aqueous phase, whereas the other glycosides go into the organic phase. Next, the classic sequence is applied, on each of the two phases: oxidation and hydrolysis ($FeCl_3$, HCl), aglycone extraction (ethyl ether-hexane), color reaction (magnesium acetate in methanol), and absorbance measurement. The French official drug must contain a minimum of 8% hydroxyanthraquinone glycosides, with a minimum of 60% consisting of cascarosides, and with both groups calculated relative to the dried drug and expressed as cascaroside A.

Pharmacological Properties. See generalities above.

Uses. They are the same as those of buckthorn.

F. Other Hydroxyanthraquinone Glycoside-containing Drugs

- CAPE ALOE, *Aloe ferox* Miller,
- CURAÇAO ALOE, *A. vera* (L.) Burm. *f.*, Asphodelaceae

According to the French Pharmacopoeia, Curaçao aloe consists of the dried concentrated juice from the leaves of *A. barbadensis* Miller (i. e., *A. vera* [L.] Burm. *f.*). Cape aloe comes from various species of aloe, chiefly *A. ferox* Miller and its hybrids (Eur. Ph., 3rd Ed.). Aloes also produce a gel which is said to be healing, and is used in the cosmetics industry. (*Gel* : The French Pharmacopoeia lists among the parts that are used: "concentrated leaf juice, *mucilage*" [IV.7.A1)

The Plants. Aloes (there are over 150 species) are plants with a more or less arborescent habit, with thick and fleshy leaves, most often prickly at the margins, and gathered into a dense rosette at the apex of a hardy "trunk" of variable length. In the case of the official species, the flowers are scarlet red as buds (Cape aloe) or yellow (Curaçao aloe), and are grouped in tight spikes borne by a floral stalk, which is either unique (*A. vera*) or ramified (*A. ferox*).

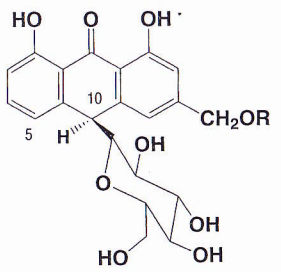
Aloe ferox grows wild in the south of Africa; it is easy to hybridize and cultivate. *Aloe vera* is from north Africa, was introduced as early as in the seventeenth century to the Antilles, and is now cultivated in the United States (Florida).

The Drugs: Aloe Juice and Aloe Vera Gel. The transverse section of the leaf shows, under an epiderm with a very thick cuticle, a parenchyma containing chlorophyll and starch, a central region with mucilage-containing cells, and between the two, isolated vascular bundles with well-marked pericycle and endoderm. Aloe juice ("**aloe**" or "**aloes**") is contained in the pericyclic cells, and flows spontaneously from the cut leaf, whereas **aloe gel** consists strictly of the mucilage from the polyhedral cells of the central region.

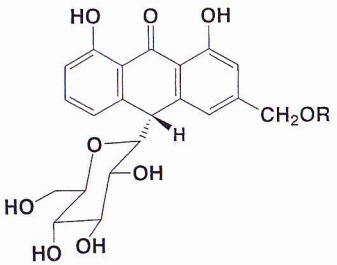
Traditionally, the juice that flows spontaneously from the cut leaves is collected and concentrated by boiling. The thickened juice consists of dark brown masses (Curaçao aloe) with greenish reflections (Cape aloe). The gel is obtained after eliminating the outermost tissues of the leaf.

Chemical Composition.

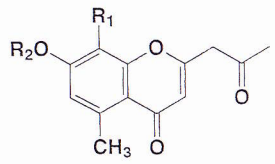
Aloe composition. The drug contains 15 to 40% hydroxy-anthraquinone derivatives, which are aloe-emodin-anthrone 10-*C*-glucosides: aloin (= barbaloin, see footnote *, p. 433), hydroxyaloins, and, in *A. ferox*, aloinoside. Aloin, which is by far the chief constituent, is in fact a mixture of aloin A (10*R*) and aloin B (10*S*), which interconvert through the anthranol form. The same comment applies to aloinoside, a derivative rhamnosylated on the 3-hydroxymethyl group of aloin. The hydroxyaloins allow the differentiation of the two species: 5-hydroxyaloin A characterizes *A. ferox*, whereas 7-hydroxyaloins A and B and their 8-*O*-methylated homologs only occur in *A. barbadensis* (which also contains 10-hydroxylated 10-*C*-glycosides).



R = H, *Aloin A*
R = α-L-Rha, *Aloinoside A*



R = H, *Aloin B*
R = α-L-Rha, *Aloinoside B*



Aloesone, Aloeresins A-C

The juice also contains a resinous fraction from which 2-acetonyl-7-hydroxy-5-methylchromones *C*-glucosylated at C-8 have been isolated: aloesin and aloeresin A. These chromones are the major constituents (*A. ferox*) and sometimes occur alongside small amounts of compounds that are not *C*-glycosides, naphtho[2,3c]-furans, and 1-methyltetralins. *A. barbadensis* contains several 2-(2-hydroxypropyl)-chromones *C*-glucosylated at C-8 (e.g., isoaloeresin D, aloesol derivatives, isorabaichromone). *A. ferox* also contains a tetralin, feroxidin, both in the free state and glycosylated.

Aloe Vera Gel Composition. Very rich in water, it does not appear to contain very specific compounds: amino acids, lipids, sterols, enzymes, and most of all, polysaccharides (pectins, hemicelluloses).

Tests. Aloe is identified by the fact that its infusion fluoresces in the presence of sodium borate. Upon addition of bromine to the aqueous extract, a precipitate forms that is yellow (Cape aloe) or else brownish-yellow with a purple supernatant (Curaçao aloe). The identification is completed by a TLC analysis (methanol extract) to show, alongside "barbaloine", aloesin only (Curaçao aloe) or with aloinosides A and B (Cape aloe). The assay includes ashes (< 2%), loss on drying (<10% [Cape aloe]; <12% [Curaçao aloe]), and a quantitation. The latter is colorimetric (magnesium acetate), and follows aqueous extraction of the drug, oxidizing hydrolysis of the glycosides ($FeCl_3$, HCl), and aglycone extraction (Et_2O). The drug must contain not less than 18% (Cape aloe) or not less than 28% (Curaçao aloe) hydroxyanthraquinone derivatives expressed as "barbaloin" (= aloin) relative to the dried drug. Curaçao aloe must not be found (TLC) in Cape aloe.

Pharmacological Activity and Uses. Both aloes are used to prepare the titrated dried aloe extract (Eur. Ph., 3rd Ed.), titrated to contain 20±1% hydroxy-anthraquinone derivatives. This extract is prepared by aqueous extraction, which eliminates a large part of the resinous material to which most of the side effects of the juice are attributed.

Tradition attributes to Aloe Vera Gel some healing properties that are only partially confirmed by animal experimentation. The basis of this activity remains hypothetical: immunostimulating polysaccharides? Lectins? Antibradykinin glycoproteins? Wound-healing mannose-6-phosphate? Observations in humans are highly contradictory, which may be explained by the variability and instability of the preparations being tested. Several mechanisms have been invoked to explain this activity: stimulation of the complement linked to polysaccharides, or, more simply, high water content imparting hydrating, insulating, and protective properties to the gel. This gel is widely used in cosmetic products as a hydrating ingredient in liquid or creams: sun lotions and shaving creams, lip balms, healing ointments, face packs, and creams. It may be employed in the composition of phytomedicines traditionally used as an adjunct in the emollient and antipruriginous treatment of skin disorders, as a trophic protective agent for cracks, abrasions, frostbite, chaps, and insect bites, for sunburn, superficial and limited burns, and diaper rash [French Expl. Note, 1998].

• RHUBARB, *Rheum* sp., Polygonaceae

Rhubarb consists of the dried subterranean organs of *R. palmatum* L., or *R. officinale* Baillon, or hybrids of the two species, or a mixture of the two species. The subterranean organs are often divided; they are devoid of stem elements and nearly completely devoid of the cortical part that includes the rootlets (Eur. Ph., 3rd Ed.).

The Plant, the Drug. Rhubarbs are tall herbaceous plants, and are perennial by a voluminous rhizome. The leaves have a long fleshy petiole and a wide blade more or less palmatilobate, with prominent reddish veins on the underside. The flowers are small, trimerous, and grouped into a large panicle. The drug is voluminous and brownish-red; it is cut into fragments to facilitate drying. Its appearance varies as a function of the geographical origin of the drug (Sichuan, Guangsi, Qinghai, Korea). Generally, it consists of disc-shaped pieces, 1-5 cm thick, with a diameter reaching 10 cm; the pieces are cylindrical, oval, or planar-convex. The surface is generally covered with a yellowish-brown powder; when the drug is wet, dark crisscrossed lines appear. The odor is characteristic and aromatic. Under the microscope, the powdered drug has starch granules with a star hilum, and in chloral hydrate, large calcium oxalate cluster crystals (100 µm and more) and large reticulate unlignified wood vessels are visible.

Chemical Composition. Many constituents have been isolated from the commercial drugs: galloylglucoses, acylglucoses, phenylbutanones (lindleyin and derivatives), and flavan derivatives (flavan-3-ol mono- and biosides, dimeric and trimeric proanthocyanidins, free or esterified by gallic acid). The specificity of the drug lies in the occurrence of a large number of phenols arising from the cyclization of a poly-β-ketomethylene: naphthalenes, stilbenes (resveratrol glycosides), chromones and chromanones, and especially between 2 and 5% hydroxy-anthraquinone derivatives. In the dry drug, the chief constituents (60-80%) are anthraquinone glycosides, namely emodin, physcion, aloe-emodin, and chryso-phanol glycosides. They occur alongside di-*O,C*-glucosides of the monomeric reduced forms (rheinosides A-B [anthranols] and C-D [anthrones]), and of dimeric reduced forms (particularly sennosides A-D). The level of oxidized forms is maximal in the summer and almost nil in the winter; the interconversion between the two forms is very rapid (three weeks).

Tests. The Pharmacopoeia requires TLC analysis of the aglycones extracted with diethyl ether after reflux in the presence of hydrochloric acid, and visualization under UV light after spraying with NaOH. The anthraquinone glycoside quantitation is done by a conventional method: decoction (H_2O), oxidation ($FeCl_3$), hydrolysis (HCl), aglycone extraction (Et_2O), color reaction (magnesium acetate), and measurement of absorbance. TLC analysis of a methanol extract with visualization by phosphomolybdic acid allows the purification of the chromones of rhapontin, a

stilbene glycoside characteristic of the garden rhubarb, *R. "rhaponticum"* (in fact, *R.x cultorum* Hort. = *R.rhabarbarum* L.). The drug must contain not less than 2.2% anthraquinone glycosides, calculated as rhein; total ashes, <12%; ashes insoluble in HCl, <2%. In addition to the official assay, HPLC analysis, by allowing simultaneous detection of all of the low molecular-weight phenolics, provides useful information on the geographical origin of commercial samples.

Pharmacological Activity. Rhubarb remains in use as a laxative, especially as a powder. Some authors point out that because of the presence of tannins, it is illogical to prescribe it as a laxative. Indeed, at low doses it is an antidiarrheal, and it can even lead to laxative-induced constipation. Outside of its use as a laxative, rhubarb is used (as a purified dry extract combined with salicylic acid) for the local adjunctive treatment of inflammations and for infections of the oral cavity mucosa (irritations due to prostheses, gingivitis, or periodontitis). According to the 1998 French Explanatory Note, it is traditionally used for children's teething pains.

For several years now, many other properties of this major drug in the Chinese Pharmacopoeia have been studied experimentally. Thus, the aqueous extract (administered *per os*) improves the renal function in the uraemic rat: decrease in uraemia and creatininaemia, normalization of various serum and urinary parameters. Rhubarb tannins inhibit the angiotensin converting enzyme; they decrease the plasma concentration of amino acids, and increase the activity of glutamine transaminase in the rat.

• GARDEN RHUBARB, *Rheum sp.*, Polygonaceae

Often referred to as rhapontic rhubarb *, the garden rhubarb (*R. rhabarbarum* L., *R x hybridum* Murray [*R.x cultorum*]) is an ornamental plant also used for its edible petiole **, eaten as stewed fruit or as jam. It is a rhubarb substitute, but it is seldom used (despite being officially classified in the category of stimulant laxatives [French Expl. Note, 1998]). Chemically, it contains anthraquinone derivatives and the stilbene glycoside rhaponticin. This highly fluorescent compound is absent in authentic rhubarb, and this property allows verification of its absence in official rhubarb. The excessive consumption of rhubarb can, because of the high concentration of corrosive oxalic acid, cause a more or less serious intoxication: there have been case reports of fatalities in young children (gastrointestinal distress, hematemesis, kidney tissue alterations).

* *Rhaponticum* Hill. are Asteraceae. *Rheum rhaponticum* L. is a rare Bulgarian species.

** The petiole and leaves contain a substantial amount of soluble oxalate, hence the potential for intoxications (with gastrointestinal distress, vomiting and bloody diarrhea, tetany, or kidney damage). A few rare fatalities have been described: Sanz, P. and Reig, R. (1992). Clinical and Pathological Findings in Fatal Plant Oxalosis–A Review, *Amer. J. Forensic*

• GURMALA, *Cassia fistula* L., Cæsalpiniaceae

Gurmala is a tropical tree whose fruit (cassia) is used. This is a cylindrical indehiscent pod with a blackish pulp rich in pectins and mucilages, and containing approximately 2% anthraquinone glycosides. The pulp is a stimulant laxative [French Expl. Note, 1998], sometimes used in pediatrics, in contradiction with the recommendations listed above (however, the anthraquinone glycoside level is low).

• BUCKTHORN, *Rhamnus catharticus* L., Rhamnaceae

The fruit of this subshrub with thorny branches native to southern Europe was formerly described in the French Pharmacopoeia (1965). The size of a pea, black, shiny, and fleshy, it gets wrinkled upon drying. It contains flavonoids, tannins, and traces of incompletely identified 1,8-dihydroxyanthraquinone glycosides. The German Commission E monograph describes its laxative properties. It is used for constipation and when soft stool is desired (hemorrhoids, after anal or rectal surgery). The contraindications and side effects are the same as for other anthraquinone-containing drugs. In France, buckthorn fruit pulp is on the list of laxative herbal remedies eligible for an abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM*, under "bulk laxatives". (The anthraquinone glycosides are thought to be concentrated in the seeds).

9. OTHER DRUGS: NAPHTHODIANTHRONE-CONTAINING DRUGS

• SAINT JOHN'S WORT, *Hypericum perforatum* L., Clusiaceae *

The flowering tops of Saint John's wort (Fr. Ph., 10th Ed.) are becoming increasingly popular in Germany where it is widely prescribed, as a standardized extract, for the treatment of mild depression. In France, Saint John's wort is still used in phytotherapy for their antiseptic and healing properties. It contain polycyclic quinones that are photodynamic sensitizers and antiviral agents.

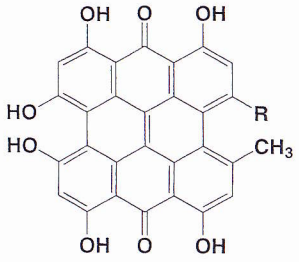
The Plant, the Drug. This perennial herb grows in the neglected fields and along the country roads of Europe and North America. It is highly branched and has erect stems. The leaves are small, opposite, and sessile, with no stipules (1-2.5 x 0.5-1.5 cm). The blade is dark green, scattered with transparent points (schizogenous secretory cavities), and has tiny black dots on the edges (pigment-filled cell clusters).

* Clusiaceae = Hypericaceae

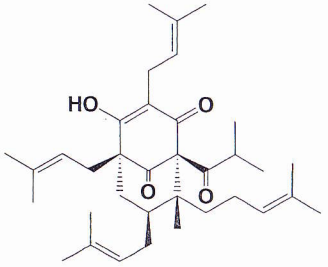
The flowers, grouped in corymbiform racemes, are easy to identify by their five, slightly asymmetrical, yellow petals, by their numerous stamens fused into three bundles, and by three divergent dark red styles that surmount three carpels. The fruit is a capsule that opens by three valves.

Chemical Composition. Saint John's wort contains approximately 0.6-3 mL/kg essential oil (terpenoid hydrocarbons, 2-methyloctane, *n*-alkanols), triterpenes, and sterols. It is rich in phenolics: caffeic acids, chlorogenic acid, proanthocyanidins (dimers [B-2] and oligomers of catechin and epicatechin), prenylated derivatives of phloroglucinol (found in the flower and fruit where they are concentrated at maturity: hyperforin (2-4.5%), adhyperforin (0.2-1.8%), and flavonoids. The flavonoids are abundant (2-4%): hyperin, rutin, quercitrin, isoquercitrin, and, concentrated in the flowers, biflavonoids (bis-apigenins C-3'– C-8" [i.e., amentoflavone, 0.01-0.05%] and C-3–C-8" [0.1-0.5%]). Also found is a trace of xanthones in the flowering stems (1,3,6,7-tetrahydroxyxanthone).

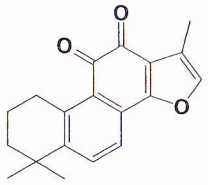
The constituents responsible for the color of the juice contained in the black dots on the leaves and flowers are naphthodianthrones (0.06-0.15%): hypericin, biogenetically derived from emodin anthrone, occurs alongside pseudohypericin, and, in the fresh plant, alongside protohypericin and protopseudohypericin.



R = CH_3, *Hypericin*
R= CH_2OH, *Pseudohypericin*



Hyperforin



Tanshinone-IIA

Tests. Identifying Saint John's wort may be difficult, due to the occurrence of hybrids and of multiple intermediate morphological types between *H. perforatum* and close species. Differentiating the main species (*barbatum* Jacq., *maculatum* Crantz, *tetrapterum* Fries, *hirsutum* L., *montanum* L.) is possible by TLC analysis and careful morphological examination: round stems, glabrous (*montanum*, *barbatum*) or covered with yellowish hairs (*hirsutum*), marked by two lines (*perforatum*), four lines (*maculatum*), or four wings (*tetrapterum*); the leaf and sepal shapes are also of diagnostic value. HPLC analysis allows the specific quantitative estimate of hypericin. The French Pharmacopoeia only requires the TLC analysis of a methanol extract; this allows the characterization of hyperin but since the active substance(s) have not been identified, this standardization has limited meaning, if any.

The French Pharmacopoeia requires only a TLC analysis on a methanolic extract to characterize hyperin (visualization by aminoethanol diphenylborate and PEG 400), and, due to their fluorescence, hypericin, pseudohypericin, and the chlorogenic acids.

Properties and Uses. Saint John's wort has a reputation for having healing properties. The antibacterial properties of extracts have been demonstrated *in vitro*. The same can be said of the properties of hyperforin, which is structurally quite close to the bactericidal keto-enols found in the hop cone.

The conventional animal experiments used to detect an antidepressant activity show that Saint John's wort has a stimulant effect on the CNS. The MAO inhibiting activity of hypericin initially shown *in vitro* could not be confirmed. This MAO-A inhibiting-type activity appeared to be concentrated in the fractions rich in flavonoids; it could also be due to xanthones, but their concentration is too low (0.0004%). In addition, the MAO inhibiting activity was not observed *in vivo* (rat) and other mechanisms—and/or other compounds—may be responsible for the properties of the extracts. The crude extract in particular has a strong affinity for GABA receptors. Amentoflavone was recently shown to have a strong affinity (IC_{50} = 15 nM) for the binding site of benzodiazepines* *in vitro*, although no inhibition of flunitrazepam binding was observed *in vivo* (mouse). Saint John's wort extract may also interfere with serotoninergic mechanisms: inhibition of serotonin uptake at synapses, long-term effect on the density of 5-HT_2 receptors (but again, what happens *in vivo*?). Hypericin has antiretroviral properties, *in vitro* and *in vivo*. It acts directly on the virus envelope and the viral proteins. Its activity develops just as well in the light (singlet oxygen is generated) as in the dark. The discovery of an anti HIV-1 activity has led to experiments of limited scope in humans.

In 1996, a meta-analysis of 23 randomized clinical trials (20 double-blind) including 1,757 patients suffering from mild or moderate depression was published. The conclusions of the analysis were that the placebo-controlled trials show that Saint John's wort extract alone (14 trials) or in combination with other plant extracts (1 trial) has an activity superior to that of a placebo. Conditions: 300- 900 mg/day of a standardized extract, i.e., depending on the medicine being prescribed, 0.45-2.7 mg/day hypericin; treatment for 4-8 weeks in 22 of 23 trials The authors of the meta-analysis and others have pointed out the heterogeneity of the published studies, the lack of long-term trials, and the fact that the inclusion criteria often correspond to a very mild form of depression. Nevertheless all experts agree that this promising drug must undergo further research and may yet be recognized as a true antidepressant **. The trials conducted in comparison with a known treatment (imipramine, maprotiline, amitriptyline) failed to establish whether Saint John's wort

* The interaction between plant components and benzodiazepine receptors was mentioned above for apigenin from feverfew and chrysin from passion flower.

** Beyond clinical trials of greater depth, a rational standardization of the extracts would require the formal identification of the active principle(s)...

extracts are as efficacious as the other treatments. Recent clinical trials with homogeneous groups of patients confirmed that Saint John's wort extract is significantly more active than a placebo. The extracts do not induce sedation. They are apparently well tolerated and seem devoid of toxicity (in the short term, and as far as it is known to date *), therefore many experts consider them to be an interesting alternative to synthetic antidepressants (except for severe depression).

Uses. In Germany, 2.7 million prescriptions were written in 1993 and 66 million packages were sold in 1994: German practitioners use medicines based on Saint John's wort extract extensively to treat mild and moderate depression. Saint John's wort itself—available in tea bags—is used for the same indications and to prepare a wound-healing oil. Commission E warns consumers about the risk of photosensitization, especially in fair-skinned individuals. In fact, phototoxic manifestations have been observed only after ingestion *of the plant* and only in sheep and bovines.

In France, where folk medicine has long used the flower *digesté* in oil (i.e., the oil in which the flowers were soaked) to treat burns and where the *Faculté* approved its use as an ingredient of *the* vulnerary spirit or *alcoolat vulnéraire*, phytomedicines based on Saint John's wort flowering tops may only claim indications for topical use [French Expl. Note, 1998]: as an adjunct in the emollient and antipruriginous treatment of skin disorders; as a trophic protective agent for cracks, abrasions, frostbite, chaps, and insect bites; for sunburns, superficial and limited burns, and diaper rash; as an antalgic in diseases of the mouth, pharynx, or both (collutoria, lozenges). These preparations are not to be used before exposure to sunlight.

The use of Saint John's wort in foods and beverages is authorized in Europe, but the hypericin concentration must be not more than 0.1 mg/kg (1 mg/kg for confectionery products, 10 mg/kg for alcoholic beverages [European directive CEE88/388]).

10. BIBLIOGRAPHY

Generalities

Hausen, B.M. (1986). Contact Allergy to Woods, *Clin. Dermatol.*, **4**, 65-76.

Van den Berg, A.J.J. and Labadie R.P. (1990). Quinones, *in* "Methods in Plant Biochemistry, vol. **1**, Plant Phenolics", (Harborne J.B., Ed.), p. 451-491, Academic Press, London.

Naphthoquinone-containing Drugs

Ali, B.H., Bashir, A.K. and Tanira, M.O.M. (1995). Anti-inflammatory, Antipyretic, and Analgesic Effects of *Lawsonia inermis* L. (Henna) in Rats, *Pharmacology*, **51**, 356-363.

* One case report of photosensitization was published in 1997: Golsch, S., Vocks, E., Rakoski, J., Brockow, K. and Ring, J. (1997). Reversible Erhöhung der Photosensitivität im UV-B-Bereich durch Johanniskrautextrakt-Präparate, *Hautarzt*, **48**, 249-262.

Kandil, H.H., Al-Ghanem, M.M., Sarwat, M.A. and Al-Thallab, F.S. (1996). Henna (*Lawsonia inermis* L.) Inducing Haemolysis among G6PD-deficient Newborns. A New Clinical Observation, *Ann. Trop. Paediatr.*, **16**, 287-291.

Michelitsh, A., Wurglics, M., Schubert-Zsilavecz, M. and Likussar, W. (1999). Determination of 5-Hydroxynaphthoquinones in Phytotherapeutic *Drosera* Preparations by Differential Pulse Polarography, *Phytochem. Anal.*, **10**, 64-68.

Schilcher, H. and Elzer, M. (1993). Drosera–der Sonnentau: ein bewährtes Antitussivum, *Z. Phytother.*, **14**, 50-54.

Zinkham, W.H. and Oski, F.A. (1996). Henna: a Potential Cause of Oxidative Hemolysis and Neonatal Hyperbilirubinemia, *Pediatrics*, **97**, 707-709.

Hydroxyanthraquinone Glycoside-containing Drugs

Brusick, D. and Mengs, U. (1997). Assessment of the Genotoxic Risk from Laxative Senna Products, *Environ. Mol. Mutagen.*, **29**, 1-9.

Lemli, J. (1996). Mécanisme d'action des sennosides, *Ann. Gastroentérol. Hépatol.*, **32**, 109-112.

Siegers, C.-P., Hertzberg-Lottin, E. von, Otte, M. and Schneider, B. (1993). Anthranoid Laxative Abuse – A Risk for Colorectal Cancer? *Gut*, **34**, 1099-1101.

van Gorkom, B.A.P., de Vries, E.G.E., Karrenbeld, A. and Kleibeuker, J.H. (1999). Review Article: Anthranoid Laxatives and their Potential Carcinogenic Effects, *Aliment. Pharmacol. Ther.*, **13**, 443-452.

Senna

Kabelitz, L. and Reif, K. (1994). Anthranoide in Sennesdrogen – Ein analytischer Beitrag zur Risikobewertung, *Dtsch. Apoth.-Ztg.*, **134**, 5085-5088.

Kinjo, J., Ikeda, T., Watanabe, K. and Nohara, T. (1994). An Anthraquinone Glycoside from *Cassia angustifolia* Leaves, *Phytochemistry*, **37**, 1685-1687.

Mereto, E., Ghia, M. and Brambilla, G. (1996). Evaluation of the Potential Carcinogenic Activity of Senna and Cascara Glycosides for the Rat Colon, *Cancer Lett.*, **101**, 79-83.

Verma, R.K., Uniyal, G.C., Singh, S.P., Sharma, J.R. and Gupta, M.M. (1996). Reverse-phase High Performance Liquid Chromatography of Sennosides in *Cassia angustifolia*, *Phytochem. Analysis*, **7**, 73-75.

Aloe

Okamura, N., Hine, N., Tateyama, K., Nakazawa, M., Fujioka, T., Mihashi, K. and Yagi, A. (1998). Five Chromones of *Aloe vera* Leaves, *Phytochemistry*, **49**, 219-223.

Park, M.K., Park, J.H., Kim, N.Y., Shin, Y.G., Choi, Y.S., Lee, J.G., Kim, K.H. and Lee, S.K. (1998). Analysis of 13 Phenolic Compounds in *Aloe* species by High Performance Liquid Chromatography, *Phytochem. Anal.*, **9**, 186-191.

Rauwald, H.W. and Sigler, A. (1994). Simultaneous Determination of 18 Polyketides Typical of *Aloe* by High Performance Liquid Chromatography and Photodiode Array Detection, *Phytochem. Anal.*, **5**, 266-270.

Speranza, G., Fontana, G., Zanzola, S. and Di Meo, A. (1997). Studies on Aloe. 15. Two New 5-Methylchromones from Cape Aloe, *J. Nat. Prod.*, **60**, 692-694.

van Wyk, B.-E., van Rheede van Oudtshoorn, M.C.B. and Smith, G.F. (1995). Geographical