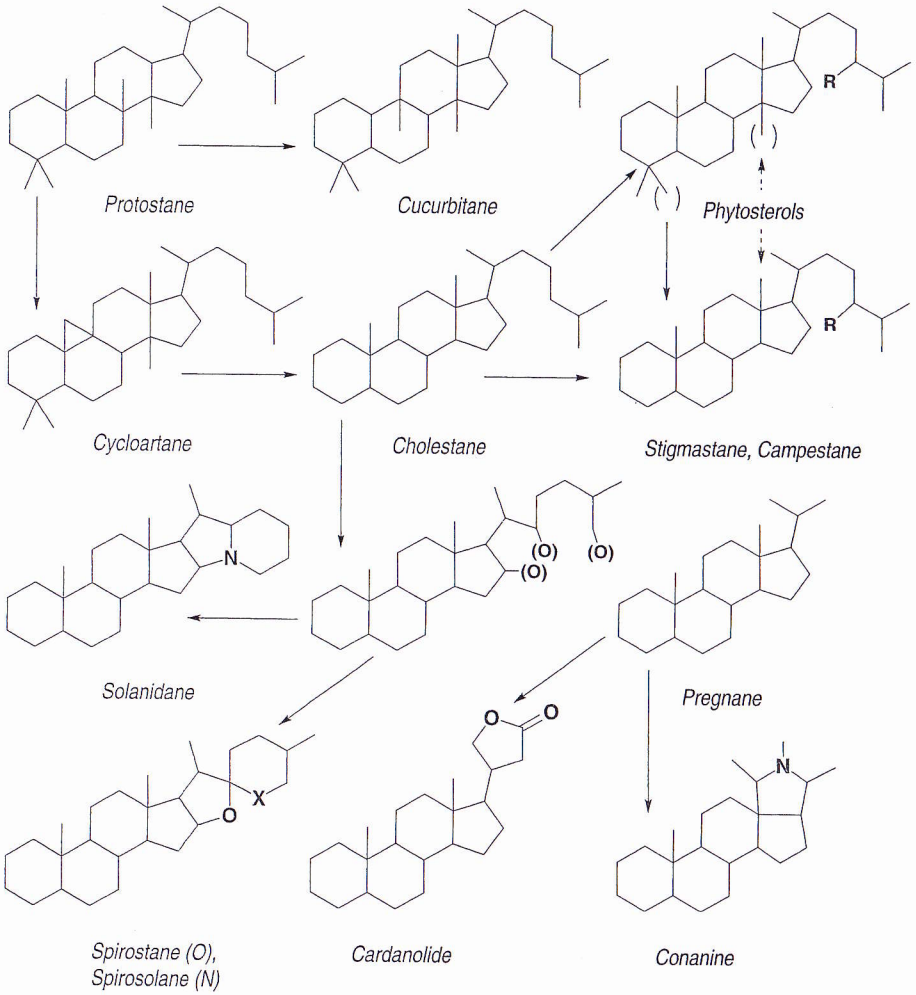


Chief basic steroidal skeleton found in higher plants

Triterpenes and Steroids

Saponins

1. INTRODUCTION

Saponins constitute a vast group of glycosides which are ubiquitous in plants. They are characterized by their surface-active properties: they dissolve in water to form foamy solutions. In fact, it is because of this surface activity that some drugs containing them have been used throughout history: the soapwort (*Saponaria officinalis* L. from the Latin *sapo, saponis* = soap) was widely used in Europe and for ages as a household detergent; in the tropics, the fruits of various "Indian soaps" were used instead (*sapo* + *India* —>*Sapindus, S. saponaria* L., *S. marginatus* Willd.,). Most saponins have hemolytic properties and are toxic for coldblooded animals, especially for fish. These properties are not common to all of the saponins, therefore they cannot be used to define these compounds: it is better to develop a structural definition, since a simple and unambiguous chemical definition is not possible either*.

Saponins deserve attention because of their industrial applications—some are starting materials for the semisynthesis of steroidal drugs—and because of their pharmacological properties. Several saponin-containing drugs are used in the pharmaceutical industry to prepare galenicals, and others have applications in phytotherapy. The cosmetics industry takes advantage of their detergent properties.

2. STRUCTURE OF SAPONINS

Structurally, saponins may be classified into two groups based on the nature of their aglycone.

- Steroidal saponins. They are found almost exclusively in the Monocotyledon Angiosperms: Liliaceae (*Allium*, *Smilax*, *Asparagus*), Agavaceae (*Agave*, *Yucca*), and

* In addition to the problem of deciding where to classify steroidal "alkaloids", to state—without further detail and as some authors do summarily—that saponins are glycosides with a triterpenoid or steroidal aglycone would amount to considering most plant steroids to be

Dioscoreaceae (*Dioscorea*). Some are known, however, in the Fabaceae (fenugreek), Solanaceae (tobacco), or Scrophulariaceae (foxgloves).

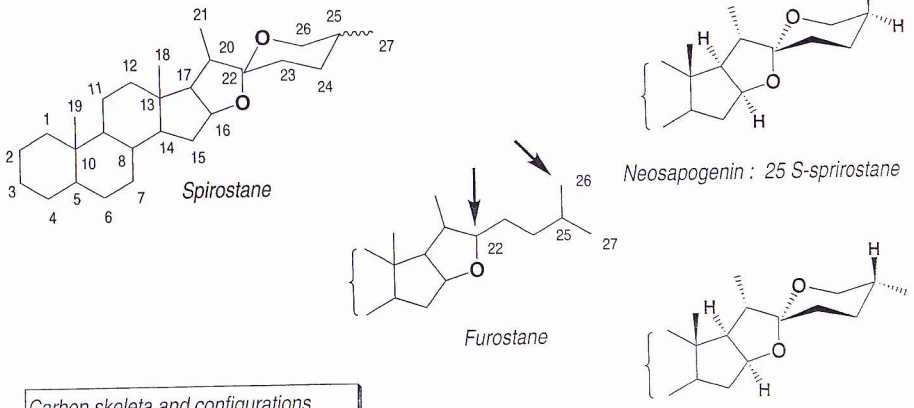
- Triterpenoid saponins. By far the most common, they occur in some marine animals and some Pteridophyta. They are practically never found in Gymnosperms and otherwise they are found mainly in the Dicotyledon Angiosperms, e.g., Araliaceae, Caryophyllaceae, Cucurbitaceae, Fabales, Primulaceae, Ranunculaceae, Rosaceae, and Sapindaceae.

Hostettmann and Marston, who are widely recognized saponin experts, distinguish a third category of saponins, namely that of steroidal amines which are treated by others as steroidal alkaloids. Biogenetically, they are undoubtedly merely pseudoalkaloids (they are not derived from an amino acid), and in terms of their properties, their behavior is reminiscent of that of saponins. Nevertheless, the origin of the nitrogen atom brings them closer to other nitrogen-containing terpenoid metabolites which are unanimously considered to be alkaloids (e.g., aconitine): therefore, they will be treated as alkaloids in this book (p. 1052).

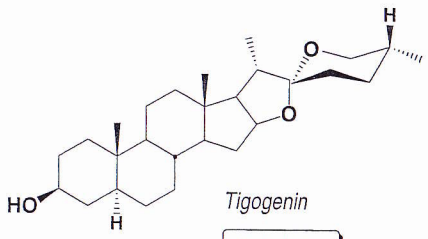
A. Structure of the Aglycones

- ***Steroidal Aglycones***

Steroidal aglycones all possess a skeleton with 27 carbon atoms which generally comprises six rings: rings E (furan) and F (pyran) are, theoretically, the result of an intramolecular ketalization which occurs after the oxidation of a cholestane-type precursor at C-16, C-22, and C-26. Because of the spiro character of C-22, this type of hexacyclic skeleton is commonly referred to as a spirostane. In fresh plant material, it is not uncommon to find the hydroxyl group at C-26 engaged in a glycosidic linkage, in which case the structure remains pentacyclic, and is referred to as a furostane. This type of structure can only occur as a glycoside: its hydrolysis leads spontaneously to a spirostane-type derivative. Spirostane-type glycosides are mostly distributed in the bulbs, roots, and seeds.

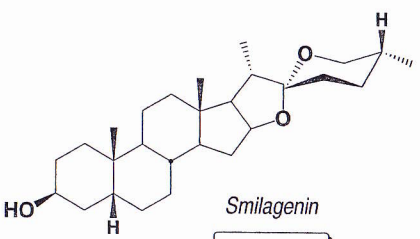


Carbon skeleta and configurations of the steroidal aglycones of saponins



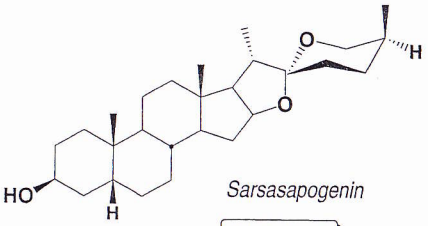
Tigogenin

25 *R*, H-5 α



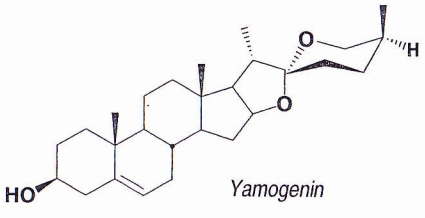
Smilagenin

25 *R*, H-5 β



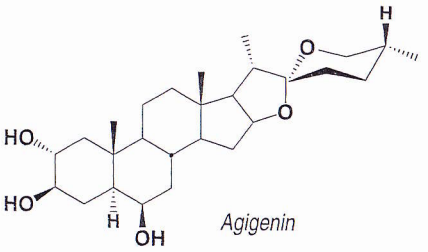
Sarsasapogenin

25 *S*, H-5 β

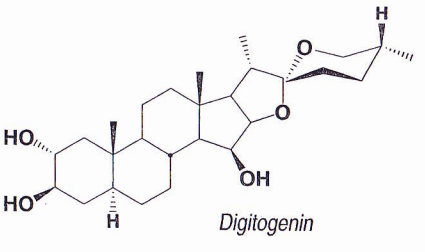


Yamogenin

25 *S*, Δ-5,6



Agigenin



Digitogenin

The structural variations are limited:

- Although the hexacyclic skeleton includes many asymmetric carbon atoms, only the configuration at C-25 can vary, giving rise to two series: neosapogenins (25-*S*, axial methyl group) or isoneosapogenin (25-*R*, equatorial methyl group). The B/C and C/D rings are always *trans* fused, and the configuration of C-20 and C-22 is always *S* and *R*, respectively, in naturally-occurring sapogenins.

- The 5,6 double bond may be retained (e.g., diosgenin) or reduced. If the reduction product has a 5α-hydrogen, the A and B rings are *trans* fused (e.g., tigogenin, digitogenin); with a 5β-hydrogen, the A and B rings are *cis* fused (e.g., smilagenin, sarsasapogenin).

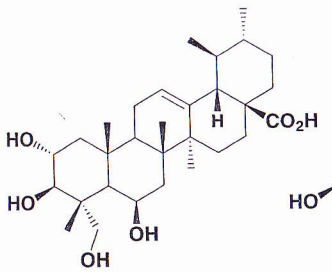
- There is always a hydroxyl group at C-3, and other carbons may also be oxidized; for example, the following positions may be hydroxylated: C-1, C-2, C-5, or C-6, and less often, C-17 or C-24 in the Liliaceae (agigenin, convallagenin, chlorogenin, cepagenin), at C-2, or C-15, or both in the Scrophulariaceae (gitogenin, digitogenin), or at C-12 in the Agavaceae, in which this oxidation most often results in a carbonyl function (e.g., hecogenin, manogenin).

- In very rare cases, there is lactonization (spirostan-26-ones, *Solanum*), demethylation (18-*nor*spirostanols, *Trillium*), or sulfatation of a hydroxyl function of the aglycone.

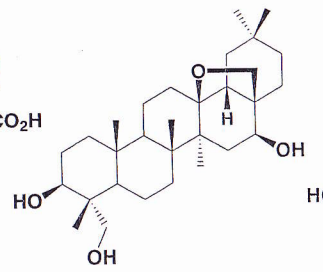
• *Triterpenoid Aglycones*

The triterpenoid aglycones of saponins, like the majority of triterpenoids, arise from the cyclization of (3*S*)-2,3-epoxy-2,3-dihydrosqualene. This cyclization first leads to dammaranes—tetracyclic compounds occurring as glycosides in drugs such as ginseng—or, when a different conformation of the precursor is involved, it leads to cucurbitanes: these are also tetracyclic and have a rather narrow distribution (chiefly in the Cucurbitaceae). Far more frequently, the tetracyclic dammarane-type compound is only an intermediate which evolves towards pentacyclic skeleta: oleananes, ursanes, and lupanes, which can, in turn, undergo rearrangements as described in the previous chapter (see p. 665 and 669: friedelanes, taraxastanes, glutinanes, and so forth).

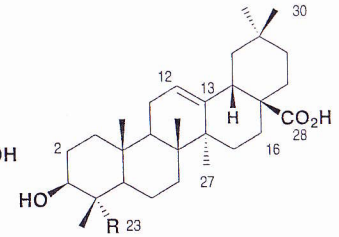
The triterpenoid sapogenins that are by far the most common are in fact pentacyclic compounds: the oleananes (also known as β-amyrin derivatives), ursanes (also known as α-amyrin derivatives), and lupanes represent the three most common skeletons.



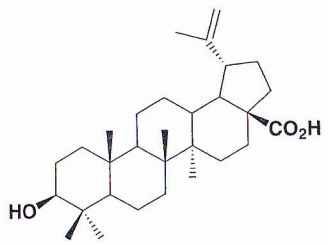
Madecassic acid



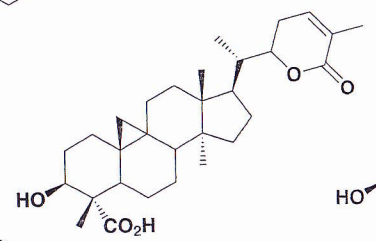
Saikogenin F



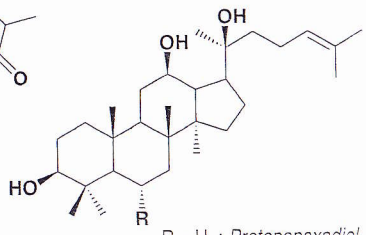
R = CH_3 : *Oleanolic acid*
R = CH_2OH : *Hederagenin*
R = CH_2OH, OH 2β, 16α : *Polygalacic acid*
R = CHO : *Gypsogenin*
R = COOH , OH 2β : *Medicagenic acid*
R = COOH, OH 2β, 27 : *Presenegin*



Betulinic acid



Abrusogenin



R = H : *Protopanaxadiol*
R = OH : *Protopanaxatriol*

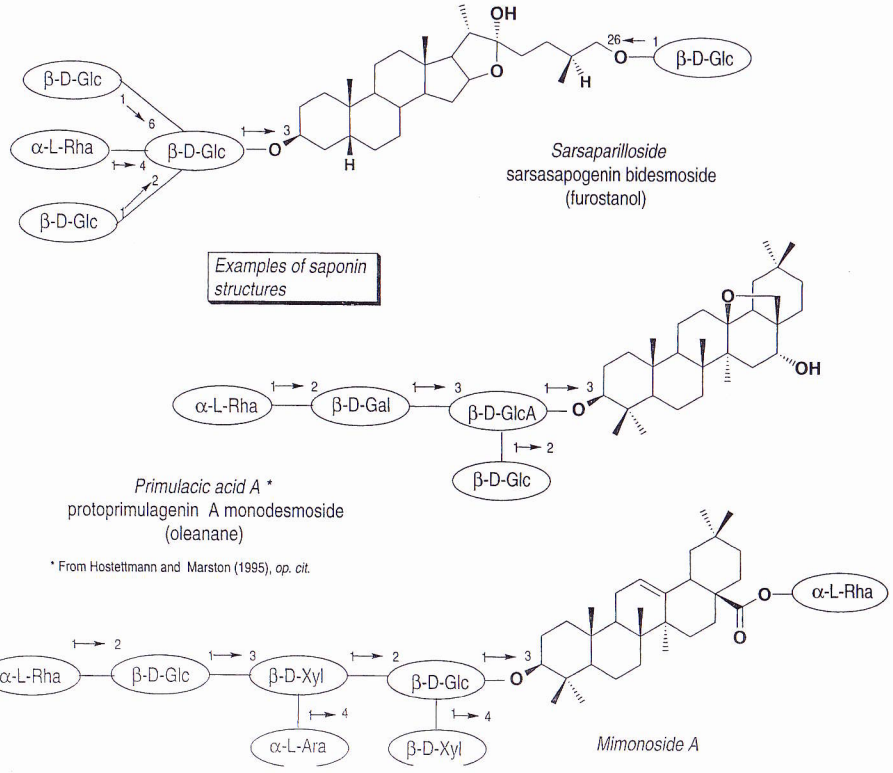
Examples of triterpenoid aglycones

The structural elements that characterize this series are the following:

- the common occurrence of a C-12–C-13 double bond;
- the common oxidation of the C-23 and C-28, but also of the C-30 methyl group (hydroxymethyl, aldehyde, or carboxyl);
- the oxidation of a more or less large number of ring carbon atoms in the following positions: 2, 7, 11, 15, 16, 21, or 22. The oxidation of one of these hydroxyl groups to a ketone is not rare (especially at C-11) and polyfunctionalization may lead, by intramolecular etherification or lactonization, to the formation of additional rings. For example, 13β,28-oxides occur that are often isolated as an artefact, a 12-en-28-ol.
- sometimes the aglycone is partially esterified, generally by low molecular weight aliphatic acids (see aescin in the seed of the common horse chestnut, theasaponin, gymnemic acids). Some aglycones are derived from lanostane, cycloartane (*Passiflora*, *Abrus*), or a nortriterpene.

B. Structure of the Glycosides

The constituent saccharides of saponins are commonplace: D-glucose (Glc), D-galactose (Gal), L-arabinose (Ara), L-rhamnose (Rha), D-xylose (Xyl), D-fucose



(Fuc), and, at least in triterpenoid saponins, D-glucuronic acid (GlcA). Amino sugars are found only in exceptional cases.

Although monosides do occur, most often, the sugar moiety of the glycoside consists of one or two linear or branched oligosaccharides (or one mono- and one oligosaccharide). The molecule can comprise up to 11 monosaccharides (most often 3-5). The mono- and oligosaccharides can be linked to the aglycone by either an ether or an ester bond. The compound is referred to as a mono- or bidesmoside, depending on whether its structure comprises one or two saccharide moieties.

Monodesmosides. The etherification (i.e., the formation of the glycosidic linkage) generally involves the reducing function of the oligosaccharide and the secondary hydroxyl group normally present at C-3, in steroids as well as in triterpenoids: the result is a *monodesmoside*.

Bidesmosides. Fairly often, the molecule also includes, in addition to the saccharide at C-3, a second sugar moiety, linked to the aglycone by an ester bond at the 28-position of the triterpenoid aglycone: this is a *bidesmoside*. In the case of steroidal saponins, the second sugar moiety, when there is one, is linked to the C-26 hydroxyl group by an ether bond (furostanol glycosides). Bidesmosides are by far the most common saponins. Their hydrolysis to monodesmosides is facile.

In some cases, the sugars and oligosaccharides linked to the aglycone are acylated by small aliphatic acids (C_2 to C_6) or by cinnamic acids (e.g., senegins and onjisaponins of *Polygala* spp., solidagosaponins). These esters, together with the glycosides of acylated aglycones described above, are sometimes referred to as saponin esters.

A few tridesmosides are known, as well as bidesmosides in which the two sugar moieties are bonded through the double esterification of a diacid (tubeimoside 1). In rare cases, a monodesmoside-type compound is not a glycoside (ether), but an ester: it is referred to as a monodesmoside acylglycoside (e.g., asiaticoside). Other esters comprise an acidic triterpene, a saccharide (at C-28), and a hexahydroxydiphenic acid (at C-3). Are these compounds, which have been isolated from a *Castanopsis*, saponins? They are probably closer to saponins than to hydrolyzable tannins. Yet other triterpenoid esters of saccharides are esterified, also at C-3, by a seco-iridoid or by sulfuric acid.

3. EXTRACTION, CHARACTERIZATION, AND QUANTITATION

The extraction, and most of all, the separation of saponins are delicate. These compounds often occur in plants in substantial quantities, but as complex mixtures: because of their high polarity, their relative fragility, and the minute structural differences between high molecular weight constituents, obtaining an intact and pure saponin is often a lengthy and arduous task. In addition, they are very hard to crystallize,

Extraction. Saponins are soluble in water, therefore they can be extracted with it, generally at the boiling point. Although an aqueous medium is a good choice for subsequent freeze-drying, it also promotes the hydrolysis of the bidesmosides. Therefore, it is often better to use alcohols (methanol, ethanol) or hydroalcoholic solutions after partial lipid removal with petroleum ether. To inactivate the esterases that commonly occur in the plant material, preliminary treatment (dilute HCl) is advisable. By adjusting the proportions of water and methanol, one can hope to obtain mono- and bidesmosides specifically. Since polar solvents extract too many compounds, a partition between water and *n*-butanol is frequently carried out after the initial extraction; *n*-butanol solubilizes the saponins, which are then precipitated by adding a solvent such as diethyl ether. Dialysis or size exclusion chromatography can be used to enrich the extract in saponins.

Separation. The separation of saponins is based on chromatographic techniques (open column chromatography, low and medium pressure liquid chromatography, HPLC, centrifugal TLC, flash-chromatography) on conventional supports (silica gel, alumina, cross-linked dextran gels), and also on functionalized supports, on highly porous polymers, and on resins. Most often, a *succession* of chromatographic separations on different supports is necessary. Counter current chromatographic techniques are also useful, especially DCCC (= *droplet counter current chromatography,* a technique in which products are partitioned between a stationary phase [liquid filling a tube] and a mobile phase [droplets of a non-miscible liquid moving along the tube]). Ion exchange resins are useful for saponins with an acidic aglycone, as long as they are not sensitive to changes in pH.

It is rarely possible to obtain a saponin in one step: a sequence of chromatographic separations on different supports is almost always required.

Characterization: values. Saponins have the ability to rupture erythrocytes, and several authors have proposed to detect the presence of saponins in drugs, and to estimate their concentration, by measuring the hemolytic activity. In general, the change in absorbance of the supernatant of an erythrocyte suspension is measured after hemolysis by a saponin or a saponin-containing drug. This principle was the basis of the European Pharmacopoeial assay (1st Ed., 1971), which defined the hemolytic value as 30,000 x *a*/*b*, with *a* and *b* representing the required quantities (in g) of standard and substance being tested, respectively, to obtain complete hemolysis. The value for the standard—a saponin mixture extracted from *Gypsophila paniculata* L.—is 30,000 by definition. One unit is equal to the quantity (in mL) of bovine blood (1/50 dilution) which is totally hydrolyzed by 1 g of substance. There are two drawbacks to this method: 1. surface-active substances other than saponins can interfere; 2. some saponins are not very or not at all hemolytic (glycyrrhizin, sarsaparilloside).

In France, the decree or *arrêté* of November 7, 1996 (article 3) reiterated the requirement to determine the foam value (Fr. Ph., 10th Ed., V.4.A), in other words: "the dilution of a decoction of the drug which, in the prescribed conditions, produces

prolonged boiling (30 minutes) of 1 g of drug in 100 mL of water. A series of calibrated tubes contains increasing dilutions of this decoction. The tubes are agitated: the foam value is the drug dilution in the tube that gives 1 cm of foam after 15 minutes at rest.

Characterization: color reactions. Color reactions can be used to characterize saponins (and sapogenins) in order to verify the identity of drugs and, if need be, to monitor separation steps. No color reaction is particularly specific to these compounds. However, the following reactions are available (non-exhaustive list):

- with acetic anhydride in the presence of sulfuric acid (Liebermann reaction). The resulting colors differ depending on whether the aglycone is a triterpene (pink to red) or a steroid (blue-green);
- with vanillin, anisaldehyde, and other aromatic aldehydes in the presence of a strong mineral acid. The products are deeply colored, probably as a result of the reaction between the aldehydes and the aglycone degradation products;
- with antimony trichloride, in the presence of acetic anhydride.

Characterization: chromatographic methods. TLC analysis is a preferred method, especially for routine quality control of saponin-containing drugs. The TLC plates are visualized by using the color reactions described in the above paragraph (or other ones, e.g., cerium [IV] sulfate). One example of TLC solvent is chloroform-methanol-water (65:35:10 v/v/v). HPLC is used more and more. It is particularly well suited for purity checks and quantitations (see below). To establish the composition of little known or unknown drugs, LC-MS is a very promising approach.

Quantitation. Quantitation can be done by colorimetry, spectrophotometry after separation of the constituents by TLC followed by elution of the spots, or, more commonly, by HPLC. The latter is a good method despite the difficulty in detecting these substances, which only rarely absorb beyond 210 nm (solutions to this problem are to attach a chromophore to the molecule, or to work at $\lambda<210$ nm in the appropriate solvents).

Structure determination. The structure determination of saponins has greatly benefitted from advances in mass spectrometry and NMR spectroscopy: soft ionization techniques (for example fast atom bombardment of the compound prepared in a matrix, i.e., FAB-MS) allow the determination of the molecular weight, and of the nature of the sugars and their linkages. ^{13}C-NMR also provides data on the aglycone, the linkages, and the number of anomeric carbons (therefore the number of sugars). In some cases, two-dimensional NMR (= 2D-NMR), which is the analysis of correlation data obtained by applying specific pulse sequences, and homo- and heteronuclear decoupling experiments have allowed the elucidation of complex structures without resorting to hydrolysis. The latter remains widely used, however, together with non-destructive spectral methods: alkaline hydrolysis of bidesmosides, acidic hydrolysis (HCl) [illegible]

a chromatographic analysis (TLC, HPLC) of the sugars, hydrolysis after permethylation, and so forth (see specialized publications).

4. BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES

Saponins commonly have hemolytic properties that are generally attributed to their interaction with the sterols of the erythrocyte membrane. This interaction induces an increase in membrane permeability and ion movements: sodium and water enter the cell, potassium exits the cell, the cell membrane ruptures, and hemoglobin is released. Monodesmosides are far more hemolytic than bidesmosides, and the activity decreases as the length of the sugar moiety increases.

It is reasonable to assume that, *in vivo*, a fair number of saponins ensure the defense of the plant against microbial or fungal attack: in the case of ivy, it has been shown that the leaves contain an enzyme capable of hydrolyzing hederasaponin C—an inactive bidesmoside —to α-hederin, a monodesmoside with a strong antibiotic activity. It is known that the resistance of oats to infestation by certain fungi is linked to the presence of avenacins, which are esters of the monodesmosides.

The activity against fungi has also been well established *in vitro* toward phytopathogenic species (alfalfa saponins), as well as toward various *Candida* and dermatophytes (ivy and woundwort saponins). Except for a few cases, this activity is due to the monodesmosides: it reaches a maximum when the saponin structure includes four or five monosaccharides. It probably results from the reaction of the saponin with the membrane sterols of the micro-organism.

Saponins are virtually devoid of antibacterial activity, but some are active *in vitro* against viruses (e.g., glycyrrhizin, saponins of *Anagallis arvensis* L. and of marigold, cyclamin).

More than a few saponins are cytotoxic (α-hederin, astragaloside) or act as anticancer agents *in vivo*, for example tubeimoside 1 from *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae) and the saponins from *Crocosmia* sp. (Iridaceae). Others inhibit the formation of induced tumors (induced by benzanthracene/TPA or by the Epstein-Barr virus/TPA).

Many saponins have a strong spermicidal activity (several years ago, vaginal creams underwent trials). As expected, there is a correlation between the spermicidal and the hemolytic activity.

When taken orally by warmblooded species, saponins are most often only weakly toxic *, probably because they are not absorbed much; things are very different when they are administered parenterally.

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* Saponins are considered responsible (or co-responsible) for the hepatogenic photosensitization caused in sheep by various species in the genera *Agave, Bracchiaria, Narthecium, Panicum*, or *Tribulus*. The aglycones (diosgenin, yamogenin) undergo isomerization and conjugation as glucuronides, then crystallize and obstruct the biliary ducts: as a result, a chlorophyll metabolite with photodynamic sensitizing (and maybe hepatotoxic) properties, phylloerythrin, is retained. See Flaøyen, A. (1996). Do Steroidal Saponins have a Role in Hepatogenous Photosensitization

The toxicity of saponins for coldblooded animals has been known since antiquity: it explains the use of some drugs to catch fish (e.g., *Serjania* [Sapindaceae], *Balanites* [Zygophyllaceae], *Schima* [Theaceae]). Doses of about 1-5 ppm are generally sufficient to make branchial capillaries burst, and disrupt respiration and osmotic equilibrium.

For some years now, the molluscicidal activity of saponins has been a focus of attention. This activity is often substantial (it is frequently on the order of 1 mg/L) and only exists for the monodesmosides. Certain substances are particularly toxic for *Biomphalaria* and *Bulinus* species that are obligatory hosts in schistosome cycles: extracts of the fruit of *Phytolacca dodecandra* L'Hérit. (Phytolaccaceae) and of the fruits of various Fabaceae (*Swartzia* sp., *Tetrapleura* sp., and others) have in fact been tested in Africa for the potential disinfection of infested waters, in conjunction with chemotherapy of schistosomiasis. What remains to be established—among other requirements—is, hopefully, the minimal toxicity to fishes and the short- and long-term safety for humans.

Several drugs known and used for their anti-inflammatory and antiedema effects owe these properties to saponins: this is true for licorice root and horse chestnut seed (see below), and also for some drugs in traditional Chinese medicine (*Bupleurum* * spp.) and for saponins such as those of *Solidago virgaurea* L. (p. 253), *Camellia sinensis* (L.) Kuntze (p. 1075), or *Sanicula europaea. L.* (Apiaceae). The action can have various origins (e.g., inhibition of the degradation of corticoids, interference with the metabolism of inflammation mediators).

Many saponin-containing drugs are traditionally used for their antitussive and expectorant properties, or both. Ivy wood, the subterranean parts of the milkwort, licorice, and the primrose continue to be used to this end, even though the mechanism of this activity is still not well understood: local irritation of mucosal membranes? It is also an irritating action that is invoked to try to explain the activity of some of the drugs on the renal excretion of water (but many of the drugs also contain flavonoids, potassium, and other potentially active compounds).

Among the potential applications of saponins, note the following:

- the analgesic properties of the saponins of *Platycodon grandiflorum* DC. (Campanulaceae) or of various *Dianthus* (Caryophyllaceae);

* BUPLEURUM, *B. chinense* DC., *B. scorzonerifolium* Willd., *B. falcatum* L., but not *B. longiradiatum* Turcz., which is toxic (œnanthotoxin). *Bupleurum* is a leading drug in oriental medicine, in China (*chaihu*) as well as in Japan (*saiko*). *Bupleurum* root is a sedative, analgesic, and antipyretic, and is used to treat fevers and infectious hepatitis. It is an ingredient of a classical combination used in Kampo medicine—*sho-saiko-to*—which apparently delays the onset of hepatocellular carcinoma in cirrhosis patients. See Oka *et al.* (1995). Prospective Study of Chemoprevention of Hepatocellular Carcinoma with Sho-saiko-to, *Cancer*, **76**, 743-749.

Bupleurum root contains many saponins. Saikosaponin A and D are the most important ones from a pharmacological standpoint. They are fuco-glycosides of saikogenin F and G, which are C-13, C-28 ethers of 3β,16β,23- and 3β,16α,23-trihydroxylated oleananes, respectively. These saponins are anti-inflammatory, induce a corticosteroid-type activity, lower blood

- the influence of alfalfa, soybean, and quillaja saponins on the intestinal absorption of cholesterol: they decrease it by forming non-resorbable complexes and maybe by acting directly on the synthesis of biliary acids from cholesterol;
- the immunoregulating activity: *in vitro* mitogenic activity of the saponins of *Mimosa tenuiflora*, potential benefits of quillajasaponins as adjuncts to vaccines;
- the cell protective activity of ginsenosides or saponins of *Bupleurum* against the effects of hepatotoxic agents (CCl_4, galactosamine, cell cultures).

A comment is in order after this incomplete enumeration: a number of these observations were made *in vitro* or under specific conditions (e.g., for the ichtyotoxins or the molluscicidal agents). What happens *in vivo*? Most of the saponins that have undergone animal experimentation are absorbed very little in the intestine as glycosides, therefore it is unlikely that these saponins are active unless it is their aglycones that have a pharmacological activity. At least the low intestinal resorption has one advantage: it makes the toxicity of these compounds negligible, even though they are commonly found in the diet.

Besides the question—which has not been studied much—of saponin pharmacokinetics, is that of whether their activity on surface tension affects the absorption of other compounds, whether those are total drug constituents (synergies in phytotherapy) or medicines (potential drug interactions); again, the available data are incomplete.

Beyond their pharmacological potential, saponins are also of interest because some of them have sweetening properties, which are intense in some cases. Licorice and glycyrrhizin have been known as sweeteners for a long time—and widely used in beverages and candies—but others are less well known: mogrosides and siamenoside of the fruits—used in Japan—of *Siraitia grosvenorii* (Swingle) C. Jeffrey (Cucurbitaceae), abrusosides of jequirity leaf (= Indian licorice = *Abrus precatorius* L. [Fabaceae]), periandrines of Brazilian licorice root (*Periandra dulcis* Mart. [Fabaceae]), cyclocaryosides and pterocaryosides of *Pterocarya paliurus* Batal. leaf (Juglandaceae), and osladine of (North American) licorice fern rhizome (*Polypodium glycyrrhiza* D. Eaton, [Polypodiaceae]). Like glycyrrhizin, these compounds have an after-taste that makes them difficult to use as sugar substitutes. Their structures differ widely and raise interesting questions about structure-activity relationships. Also of interest are the curious properties of the gymnemic acids of *Gymnema* *, and of the

* GYMNEMA. *Gymnema sylvestre* (Retz.) R. Br. ex Schultes (Asclepiadaceae) is a tropical vine (India, China) whose leaves are considered antidiabetic in Ayurvedic medicine (experiments in rodents and observations in humans tend to confirm this). These leaves can suppress the sweet taste of sucrose or saccharin. This peculiar activity is due to a complex mixture of saponins that are the glycosides of a C-4 and C-16 stereoisomer of escine, namely gymnagenin. The leaf also contains a 35 amino acid-polypeptide (gurmarin), which interferes with the sweet perception in rats but not in humans. Gymnema has not been evaluated thoroughly and it is not used in therapy. However, it is often incorporated—particularly in Japan and in the United States—in products (infusions, capsules) presented as aids in weight control programs. See: Suttisri, R., Lee, I.-S. and Kinghorn, A.D. (1995). Plant-derived Triterpenoid Sweetness

dammaranes of common jujube leaf and Japanese raisin tree leaf (*Ziziphus jujuba* Miller, *Hovenia dulcis* Thunb., [Rhamnaceae]): these compounds neutralize the perception of the sweet taste.

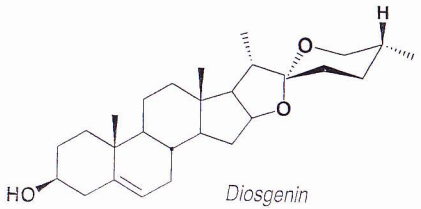
5. STARTING MATERIALS FOR STEROID HORMONE SEMISYNTHESIS

The first hormones used in therapy were extracted from animal organs (ovaries, testicles) or from urine, but their very low concentrations required long and costly procedures. Soon, the interest turned to bile acids, and in 1939, at Pennsylvania State University, a plentiful precursor was characterized: diosgenin from a Mexican yam. The simultaneous optimization of a degradation method for this precursor launched industrial production. The potential of this type of compound was magnified in 1949 when the specific and microbiological hydroxylation of progesterone was achieved. Since then, the catalog of available steroids has expanded and the needs have grown. Currently, the majority of the steroids produced by the pharmaceutical industry and used as contraceptives or in therapy (anti-inflammatory agents, androgens, estrogens, progestins) are obtained by semisynthesis from natural substances: saponins, phytosterols, cholesterol, and bile acids. Total synthesis is long, onerous, and to be reserved for specific series.

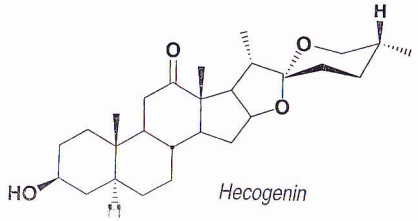
Although the diosgenin produced in Mexico has long been the chief starting material exploited to produce steroids, wild fluctuations in prices have caused a decrease in its consumption in favor of other sources, including total synthesis. Other diosgenin producers appeared on the market (China) and changed the balance between the different possible sources. We shall indicate below what sources can be exploited, without trying to specify their relative importance, but we shall emphasize that the sterols of unsaponifiable matter (e.g., stigmasterol of soybean oil) appear to be very competitive. Yet what is true for a major North American company may not be true in China: the former exploits stigmasterol, the latter, *Dioscorea* or the tigogenin of some *Agave* spp. (Agavaceae).

A. Sapogenins

Sapogenins were the first substances to be exploited. Diosgenin, which is still used, hecogenin, smilagenin, and sarsasapogenin are the most interesting ones.



Diosgenin



Hecogenin

Sources of Diosgenin

- **YAMS,**
  Dioscorea spp., Dioscoreaceae

The *Dioscorea* genus comprises about 600 species, most of which are tropical. These are perennial herbaceous plants with voluble stems, tuberized roots, which are sometimes enormous, and cordate acuminate leaves. The flowers are small, unisexual, and trimerous.

Most species have a tubercle rich in starch: *D. batatas* Decne, *D. esculenta* (Lour.) Burkill, *D. alata* L. and other species are the yams, which are widely consumed (cooked) in the tropical areas of the globe. Some of the species contain alkaloids arising from the metabolism of nicotinic acid. Many contain variable quantities of steroidal saponins. Only those species that have a saponin concentration greater than 2% have industrial potential. Central American species can be used (*D. composita* Hemsl., *D. floribunda* M. Martens & Galeotti, *D. mexicana* Gill., *D. spiculiflora* Hemsl.), as well as Indian species (*D. deltoidea* Wall.), and Chinese species (*D. zingiberensis* C.H. Wright, *D. pantaica* Prai & Burk.).

Gathering wild tubercles is possible, but there are strict regulations and the quantities are insufficient. Cultivating yams is not particularly difficult: it requires a clean and well drained soil, and, given the twining character of these species, the appropriate supports (bamboo stakes, wire cages). The tubercles grow large enough to be harvested in two or three years. They are collected after the leaves drop. The average yield in the case of *D. floribunda* is 16-18 metric tons of tubercles or approximately 500 kg of diosgenin per hectare (≈ 7.3 metric tons or 202 kg of diosgenin per acre).

Extraction of Diosgenin

In the tubercles, diosgenin occurs as dioscin, closely related glycosides (gracillin), or both. Dioscin comprises two molecules of L-rhamnose and one molecule of D-glucose; gracillin comprises two molecules of D-glucose and one molecule of L-rhamnose. Some species contain glycosides with slightly different aglycones (e.g., colletisides, which are glycosides of yamogenin or neodiosgenin).

The extraction procedure normally begins with treatment with a mineral acid to hydrolyze the glycosides. After filtering, the insoluble fraction is neutralized, washed, and treated with an apolar solvent (e.g., petroleum ether or toluene), which extracts diosgenin. In another procedure, the acidic hydrolysis is preceded by a 48- to 72-hour fermentation of the fresh tubercles followed by drying. During the acidic hydrolysis, the conditions must be established to avoid the formation of a 3,5-diene by dehydration.

Other Potential Sources of Diosgenin

Several plants species could, under the proper economic conditions, become sources of starting materials of use in the steroid industry.

This is true for a Zingiberaceae from India, namely *Costus speciosus* (Koenig) Smith, and for the fruits of a Zygophyllaceae (*Balanites ægyptica* [L.] Delile), and especially for the fenugreek (*Trigonella fœnum-grœcum* L., Fabaceae), which, in addition to the substantial concentration of steroidal saponins in its seeds, presents the advantage of a short cultivation cycle permitting annual and combined crops (for example, fenugreek and corn). It also contains other constituents (proteins, lipids) which could be exploited, as long as they are not degraded by the process of recovering the saponin (hydrolyzing extraction of the defatted seeds).

Sources of Hecogenin:

- AGAVE,
  Agave sisalana Perrine, *A. fourcroydes* Lemaire, Agavaceae

Hecogenin is an aglycone which differs from diosgenin by the absence of C-5 unsaturation and the presence of an oxo group at C-12. It is in fact this oxidation of the C ring which opened the route to steroids substituted at C-11 and contributed for a time to the focus on this aglycone. The substance occurs, as a glycoside, in the leaves of agaves, which have fleshy and often prickly leaves gathered into a bunch at the base of the plant, and grow a large floral stalk after a few years. In general, the plant does not survive after floration. Agaves are chiefly cultivated in east Africa (Tanzania, Kenya) for the production of fiber (sisal). The "latex" recovered from the leaves during the preparation of sisal undergoes prolonged fermentation, followed by a treatment under pressure which completes the hydrolysis. The insoluble slurry is filtered and dried: it contains hecogenin and the other aglycones commonly found in this type of starting material (tigogenin). Hecogenin seems to have lost its economical interest in western countries.

Sources of Smilagenin and Sarsasapogenin

These genins are constituents of the saponins found (1-3%) in the roots of various *Smilax* (Liliaceae) species. The main species with economical potential come from Central America (*S. aristolochiaefolia* Miller, *S. regelii* Killib & Morton) or from the north of South America (*S. febrifuga* Kunth.). Saponins of the same type are found in various *Yucca* species, which are Liliaceae indigenous to Central America and to the south of the United States, but are not interesting from an economic standpoint.

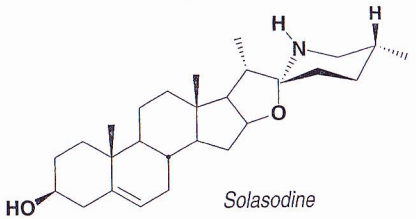
B. Other Starting Materials

Sterols. The chief source of phytosterols currently exploited is the unsaponifiable matter of soybean oil (*Glycine soja* Siebold & Zucc., Fabaceae). This by-product contains stigmasterol and sitosterol. Following extraction and purification, these sterols are converted microbiologically to steroids of pharmaceutical interest: the elimination of the side chain leads directly to androstane derivatives.

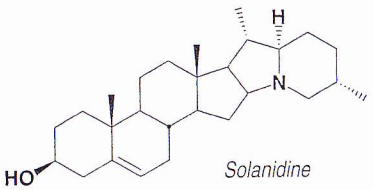
Other sources of sterols can be exploited as needed: cottonseed oil (sitosterol is by far the chief constituent), tall oil (the residue from the alkaline treatment of conifer woods to make wood pulp in the paper industry), and the waxy materials from the sugar cane.

Steroidal Alkaloids of the Spirosolane Type. The steroidal glycoalkaloids are characteristic of the Solanaceae, and particularly of the genus *Solanum*. They fall into two groups. The first group is that of the spirosolanes and is structurally quite close to the saponins of the diosgenin type: a nucleus with 27 carbon atoms arranged in six-membered rings, and spiro-aminoketal pattern (the oxygen atom is replaced by a nitrogen atom; it is not uncommon for the same plant to contain both spirostane- and spirosolane-type saponins). Solasodine (the aglycone of solasonine) is the nitrogen-containing equivalent of diosgenin. In the second group of alkaloids, the solanidanes, the nitrogen atom is part of an indolizidine nucleus (e.g., solanidine, the aglycone of solanine).

Like saponins, these compounds can be an interesting starting material for the semisynthesis of steroids. The species of interest are in the genus *Solanum*: *S. aviculare* Forst. (leaf), *S. laciniatum* Ait., and *S. khasianum* Clarke (fruit). The extraction of this type of compound takes into account their glycosidic character (alcohol extraction) and their alkaloid-like character (precipitation of the aglycone by alkalinization).



Solasodine

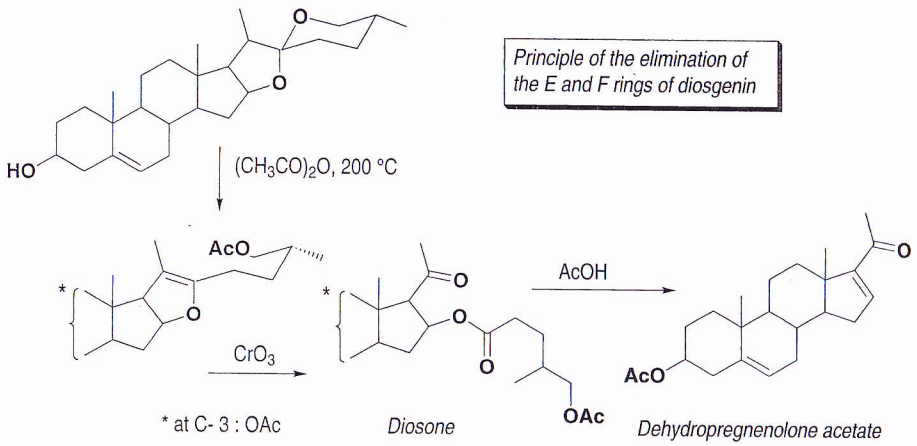


Solanidine

Other Steroidal Compounds: Starting Materials of Animal Origin. Two sources are available: the bile from slaughter house animals for C_{24} bile acids (cholic acid, deoxycholic acid) and the fat from the same animals for cholesterol.

C. Conversion of Sapogenins and of Sterols to Steroids of Pharmaceutical Interest

This facet of the chemistry of steroids does not pertain to phytochemistry: it is normally covered in therapeutic chemistry or organic chemistry texts. The figure below and the one on p. 689 summarize the main lines towards the obtention of a C_{21} skeleton and the key intermediates in the synthesis of these compounds. The use of micro-organisms shortens the routes, provides mild experimental conditions, and ensures strict stereospecificity, thereby greatly facilitating the access to all of these molecules.



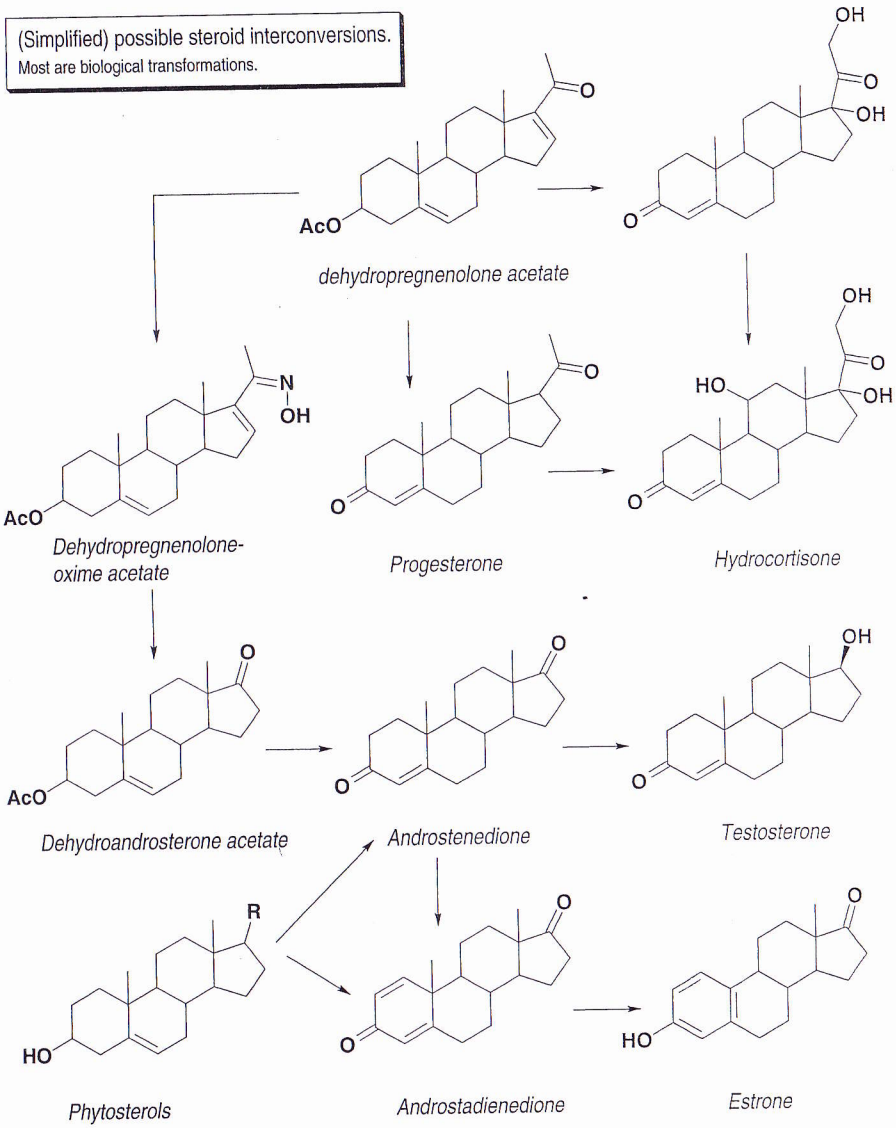
6. CHIEF SAPONIN-CONTAINING DRUGS

A. Saponin-containing Drugs that are Chiefly Anti-inflammatory

- **LICORICE,**
  Glycyrrhiza glabra L., Fabaceae

A drug used in the orient for its sweetening power, as well as for its medicinal virtues, and recommended by the Greeks to treat ulcers, this *radix dulcis* was prescribed by Arab physicians to treat cough and to relieve the side effects of laxatives. The dried root and stolons of licorice, whole or cut, and peeled or not (Eur. Ph., 3rd Ed.), currently have many uses, chiefly in pharmacy and food technology.

The Plant. Licorice is a perennial plant with erect and grooved stems (1-1.5 m), and with alternate, compound, imparipinnate leaves with 7-17 entire folioles. The inflorescences are erect racemes composed of more or less dark lilac-colored flowers. The fruit is a small flattened pod (1.5-2.5 cm) constricted between the seeds



The Drug. The different varieties of licorice, Spanish (*typica* Reg. and Herd.), Russian (*glandulifera* Wald. and Kit.), and Persian (i.e., Iranian, *violacea* Boissier), are obtained from wild plants and from "semiwild" plants cultivated in the Near East (Iraq, Syria), in Afghanistan, and other countries. Spain continues to produce this drug and cultivation trials are in progress in France. France imports roots (from Turkey, Iran, the countries of the former USSR, China, and Pakistan, in proportions that vary from year to year) and licorice extracts (China, United States, Iraq). Licorice from Asia is often produced by *G. uralensis* Fischer (*gancao*).

Several commercial products are available: licorice root in straight pieces (the form most often sold in French pharmacies), sorts, crude root for extraction, and

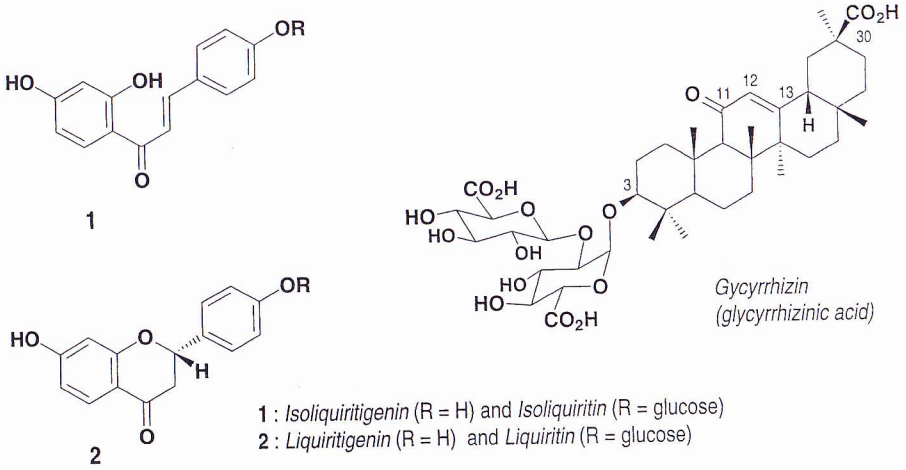
GLYCYRRHIZA GLABRA L.

peeled licorice. The crude extract of licorice (stick or block licorice) is obtained by decoction of the washed roots, filtration, and concentration under reduced pressure.

The licorice root and stolons have a typical odor and taste. The root is generally not very ramified. The stolons can reach a length of several meters and are commonly cut into 10-15-cm (1-2-cm diameter) fragments. A thin layer of cork surrounds an internal layer of bark which is striated radially, and a lignified pale yellow cylinder with radial features. The stolons differ from the roots by the presence of a central medulla. The microscopic examination of the cut and of the powder reveals the presence of bundles of partially lignified, yellow, and elongated phloem fibers (700-1,200 x 10-20 μm), with thick walls and punctiform lumen, alongside rows of cells containing calcium oxalate prisms (10-35 x 2-5 μm). There are xylem vessels with yellow lignified pitted walls, and a large number of round or oval starch grains (2-20 μm).

Chemical Composition. Along with 25-30% starch, 3-10% glucose and sucrose, coumarins, triterpenoids, sterols, and other compounds, the drug contains flavonoids and saponins, to which the pharmacological activity is attributed.

The saponins are mainly represented by glycyrrhizin (also known as glycyrrhizic acid): 3-5% of the weight of the dried drug (this figure varies depending on the source of the specimen; some authors report up to 12 and over 14%, but the result depends on the quantitation method). This compound is a monodesmoside which, upon hydrolysis, releases two molecules of D-glucuronic acid and one molecule of glycyrrhetic acid. The latter is a carboxylic acid with an oleanane skeleton, characterized by the presence of an enone (12-en-11-one).



1 : *Isoliquiritigenin* (R = H) and *Isoliquiritin* (R = glucose)
2 : *Liquiritigenin* (R = H) and *Liquiritin* (R = glucose)

Numerous flavonoids and isoflavonoids have been isolated: flavanones (e.g., liquiritin, glabrol), chalcones (e.g., isoliquiritin, licochalcones), isoflavones (formononetin, glabrone), isoflavonols, isoflavenes, and coumestanes. The chief constituents (liquiritin and isoliquiritin) are partially hydrolyzed upon drying.

Tests. The assay includes a TLC analysis to characterize glycyrrhetic acid (after refluxing the drug in HCl and redissolving the residue in diethyl ether). Isoliquiritigenin is characterized at the same time. Quantitatively, tests include total ash (<10% for the unpeeled drug, <6% for the peeled drug), ash insoluble in hydrochloric acid (<2% and <0.5%, respectively), and a quantitation of glycyrrhizic acid (= glycyrrhizin). The former method, which required the isolation of the aglycone by preparative TLC, has been replaced (addendum 1998) by HPLC quantitation in an aqueous extract obtained in the presence of ammonia. The official drug must contain not less than 4% glycyrrhizic acid. (See also French standard NF 32-177 [1990]).

Pharmacological Properties. Traditionally, licorice is considered an antitussive. Some authors think that licorice lozenges calm the cough reflex by stimulating the secretion of saliva (and deglutition).

Licorice extract has gastric antiulcer activity. This activity is due to glycyrrhizin and its aglycone, but for some authors, licorice sticks or blocks retain their antiulcer activity after glycyrrhizin removal, hence the hypothesis that (spasmolytic? secretion-inhibiting?) flavonoids are involved. The mechanism of action remains ill-understood: perhaps the anti-inflammatory effect, the inhibition of acidic secretion, and other factors intervene (but not, as it was believed for a long time, the inhibition of the enzymes of prostaglandin E2 and F2α metabolism). Semisynthetic substances such as carbenexolone (= glycyrrhetic acid hemisuccinate) were designed after glycyrrhetic acid. The antiulcer activity of carbenexolone is thought to be linked to an increase in mucus secretion and viscosity; unfortunately there are side effects (hypokalemia, hypertension, edema).

The anti-inflammatory activity of glycyrrhetic acid has been demonstrated in several experimental models. It is well accepted that the triterpene acts indirectly by potentiating corticoids: It inhibits the deactivation of cortisol by 11β-hydroxysteroid dehydrogenase (urinary cortisol levels increase significantly upon licorice poisoning). It has also been shown that *in vitro*, glycyrrhetic acid inhibits Δ4(5 β)-reductase, which is the enzyme responsible for the hepatic inactivation of steroid hormones with a 4-en-3-one function, resulting in a greater availability of these hormones. Glycyrrhetic acid also inhibits 3β-hydroxydehydrogenase.

The abusive consumption of licorice or licorice-based products can lead to edema, hypokalemia, hypertension, abnormalities in muscle contractility, and alterations in cardiac rhythm *. These symptoms, which result from an action on the renin-angiotensin-aldosterone system, are reminiscent of hyperaldosteronism (Conn's syndrome). They are linked to a mineralocorticoid-type activity for which glycyrrhetic acid is responsible: retention of sodium, chlorides, and water, increased excretion of potassium, and decreased diuresis. As described above, the competitive inhibition of the enzymes involved in steroid degradation explains this activity: the

* At high risk are consumers of beverages based on licorice concentrates (diabetics, athletes, some patients in alcoholism treatment programs) and licorice candy lovers. Hypertension and renal insufficiency facilitate the intoxication.

mineralocorticoid receptors are stimulated by the cortisol which accumulates due to the inhibition of 11β-hydroxydehydrogenase by glycyrrhetic acid.

Glycyrrhizin, which continues to be the subject of many publications, has an activity against many viruses, at least *in vitro* or in animal models. It also has a weak antibacterial activity, as well as antihepatotoxic, immunostimulating, and healing activities. It is devoid of teratogenic, mutagenic, or carcinogenic activities, and it counteracts the action of mutagens such as benzo[a]pyrene. Finally, glycyrrhizin is a sweetener 50 times more potent than sucrose.

Uses. Glycyrrhetic acid (= glycyrrhetinic acid = enoxolone [INN]) is mainly used topically for its anti-inflammatory properties: symptomatic treatment of moderate inflammation without secondary infection (atopic eczema and seborrhea of the face, sunburns, diaper rash, pruritus vulvae, insect bites). It is an ingredient of combinations designed for topical treatment of cutaneous irritations, periodontitis, and inflammation of the mouth and throat (combined with formaldehyde, lysozyme, biclothymol, propanocaine, or lidocaine).

Licorice root is mainly used to prepare extracts (sticks, blocks, fluidextract) used in pharmacy as a flavor and for their own activity: the proprietary products that contain them are proposed for the symptomatic treatment of the epigastralgia linked to gastric and duodenal ulcers and to gastritis.

The drug may be used crude: it is a common flavor in mixtures designed for infusion, and it is included as an antispasmodic in laxative herb teas. Phytopharmaceuticals based on licorice may claim the following indications: traditionally used to treat the symptoms of digestive ailments such as epigastric bloating, impaired digestion, eructation, and flatulence; traditionally used for the symptomatic treatment of cough, and locally, as an antalgic in disorders of the oral cavity, pharynx, or both. The 1998 French Explanatory Note has the following warnings: do not use in patients with hypertension, except as prescribed by a physician; do not combine with corticoid treatment; the maximum dose is 8 g/day for the infusion, 3 mg of glycyrrhizin/kg/day for the extract, and 5 g/day for the powder. The prescriber must take into account other sources (licorice contained in beverages or candy *). The German Commission E monograph emphasizes that clinical trials have demonstrated the healing effect of glycyrrhizin and glycyrrhetic acid on gastric ulcers, and that the secretolytic and expectorant activities have been shown in animals. It specifies the recommended daily doses (5-15 g of drug, i.e., 200-600 mg of glycyrrhizin; *succus liquiritiae*: 0.5-3 g depending on the indication). The uses, contraindications, drug interactions, and side effects listed in the monograph are reflected in the requirements for the package insert: indications (to facilitate the dissolution and elimination of bronchial secretions, for the

* The glycyrrhizin concentration varies from 5 to 40 g/kg in licorice candy (which amounts to 90 to 480 mg per box depending on the brand). In liquid concentrates, it is 23 g/L, and in beverages designed to be diluted, it is 0.5 g/L (which corresponds to a final concentration of 65 to 100 mg/L in the prepared beverage).

adjunctive treatment of chronic gastritis); contraindications (hepatitis, cirrhosis, hypertension, hypokaliemia); side effects and drug interactions (none when the drug is used properly). The required warnings are about the risk of edema and hypertension; that prolonged use may potentiate the effects of cardiac glycosides; that adjusting the dose of antihypertensive drugs may be difficult; and to avoid concurrent use of potassium-sparing diuretics (spironolactone, triamterene, amiloride). Treatment duration: 4-6 weeks maximum; a diet rich in potassium is recommended (e.g., bananas, dried apricots).

Licorice is widely used in the food technology industry which takes advantage, among other things, of its sweetening properties and its role as flavor enhancer. It is an ingredient of many beverages, including anise-flavored cocktails with or without alcohol, sodas, and dark beers. The product labels should include warnings against abuse (maximum recommended dose of glycyrrhizin: 125 mg/day [100 mg/day for German Commission E]). Other large consumers include the confectionery and tobacco industries: 90% of the licorice imported by the United States is believed to be used by the latter.

• Common Horse Chestnut, *Æsculus hippocastanum* L., Hippocastanaceae

The fresh seeds of this ornamental species have been used in phlebology and proctology since the end of the nineteenth century. They are the subject of a monograph in the latest edition of the French Pharmacopoeia.

The Plant, the Drug. This tall tree (20-30 m) is characterized by big sticky buds and, on long petioles, compound palmate leaves with 5-7 folioles. The flowers, irregular and fragrant, have white petals spotted with pink, and are gathered in racemes of cymes. The fruit is a spiny loculicidal capsule, often containing one seed. The seed is globulous or ovoid (diameter 2-4 cm), and covered with a shiny brown tegument marked by a large whitish spot corresponding to the hilum. The seed tegument is creamy white in the unripe horse chestnut and takes on a mahogany color during ripening. The cotyledons are fleshy, oily, and rich in starch, and are often fused, with the fusion line being more or less visible. The taste is acrid and bitter.

Chemical Composition

The **seed cotyledons** are very rich in starch (40-50%) and other sugars, and contain lipids (6-8%), flavonol glycosides, cyclitols, and saponins. The latter represent up to 10% of the weight of the drug. The "total" saponins, referred to as aescin, are a mixture of several glycosides derived from two triterpenoid aglycones from the olean-12-ene series: protoaescigenin and barringtogenol C (aescines $I_{a,b}$, $II_{a,b}$, III, etc.). Both aglycones are polyhydroxylated (at C-3, C-16, C-21, C-22, C-28, and in the case of protoaescigenin, at C-24) and their

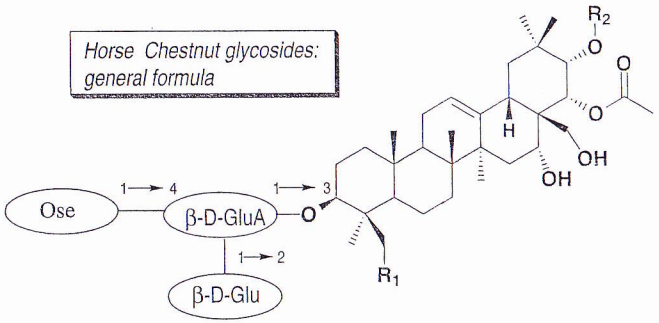
groups at C-21 and C-22 are esterified by low-molecular weight aliphatic acids (acetic acid, tiglic acid, angelic acid). The glycosidic linkage is established between the hydroxyl group at C-3 of the aglycone and the D-glucuronic acid of a trisaccharide that varies.

Ose = *glucose, galactose, xylose*

R_1 = H *(protoescigenine)* or OH *(barringtogénol)*

R_2 = *angeloyl, tigloyl, α-methylbutyryl, isobutyryl*

Horse Chestnut glycosides: general formula



The **seed tegument**, which was formerly eliminated from the commercial drug, contains proanthocyanidins which are oligomers of (–)-epicatechol: procyanidin B-2 is the chief representative, and occurs alongside other dimers linked by one bridge (B-5) or two bridges (A-2, A-4 [4β –>6], A-6, A-7 [4β –>8]), and differing from one another by their second bridge, 2β –>5 or 2β –>7. The drug also contains simple trimers (with one bridge: C-1), trimers built upon a type A unit with two bridges (4β –>8, 2β –>7, aesculitannins A-G), and tetramers (aesculitannins E-G).

The **trunk bark** is particularly rich in tannins and contains 2-3% coumarins (aesculoside, see p. 269).

Tests. The horse chestnut is identified by its macroscopic characteristics and by verifying the presence of flavonoids (cyanidin reaction). The assay per se includes, among others, the TLC analysis of a 70% ethanol extract to characterize aescin (visualization by spraying with anisaldehyde), total ash (<4%), and saponin quantitation by colorimetry ($FeCl_3$ in acetone in the presence of sulfuric acid) after extraction with 70% ethanol: the horse chestnut contains no less than 3%, expressed as aescin.

Pharmacological Properties. The anti-inflammatory, anti-edema, and anti-exudative properties of the horse chestnut extract and of aescin are clearly demonstrated by experiments in several inflammation models such as the edema of the rat's foot induced by various phlogogenic agents. In laboratory experiments, the activity of the horse chestnut extract on vascular tone—which it increases, for example on the isolated dog saphena— is accompanied by a free radical scavenging activity and an activity on the capillaries: increase in resistance and decrease in permeability (itself induced, for example, by histamine or serotonine in rats). The

inflammatory process, and could be linked to its action on capillaries and veins, as well as an interference with lysosomal enzymes (in vitro, it counteracts hyaluronidase but not elastase). A corticoid-type activity has also been considered and it is known that the adrenals must be intact for the activity to manifest itself. Other authors think that the origin of the action of aescin on venous tone is its interference with prostaglandin production. In the case of total extracts, the role of proanthocyanidins must also be taken into account, given their known effects on capillaries, and their known antioxidant and enzyme inhibiting activities (elastase, collagenase, hyaluronidase).

In the late 1990s, several clinical trials were designed to assess the efficacy of horse chestnut extracts, therefore it is practically no longer disputed (but the real benefit of the treatment remains controversial). One placebo-controlled clinical trial showed that high doses of the extract (150 mg/day x 2 months) in combination with permethol had a significant effect on the functional symptoms of venous insufficiency (pain, heaviness, cramps, paresthesia). In 1996, the results of a randomized trial on 240 patients *vs.* placebo and leg compression stocking were published; they demonstrated the efficacy of an extract titrated for aescin (100 mg/day of aescin) in significantly reducing the edema resulting from chronic venous insufficiency. However, there were comments about this study, particularly about the limits of the evaluation method (plethysmography).

Uses. Aescin is proposed as an antiedema agent—particularly in topical preparations—to treat the symptoms of venous and lymphatic vessel insufficiency. It is also an ingredient of anti-inflammatory and anesthetic topical preparations for the treatment of aphthas and ulcerations of the oral mucosa. A combination of horse chestnut extract and permethol is marketed in France for the following full-fledged "indication": to improve the leg symptoms of venous and lymphatic insufficiency (fullness in the legs, pain, restless legs syndrome).

The 1998 French Explanatory Note allows horse chestnut-based medicines to claim two indications by the oral and topical route: traditionally used to treat the functional symptoms of capillary fragility disorders of the skin (ecchymosis, petechiae), the subjective symptoms of venous insufficiency (fullness in the legs), and the symptoms of hemorrhoids. For the latter, the treatment must be of short duration and if the symptoms persists, a proctological examination is in order.

These phytomedicines most often consist of combinations of the drug or its extract with other plants or their extracts (cypress, witch hazel, goldenseal, black haw, butcher's broom, sweet clover). Some medicines contain horse chestnut extract and a flavonoid (troxerutin) or a coumarin (methylesculetin).

The German Commission E does not recommend using the horse chestnut leaf, but it approves the use of the seed (extract titrated to contain 16-20% aescin) for the pathological manifestations of chronic venous insufficiency (pain, heaviness, cramps, edema, itching).

B. Saponin-containing Drugs of Use in Phlebology and Proctology

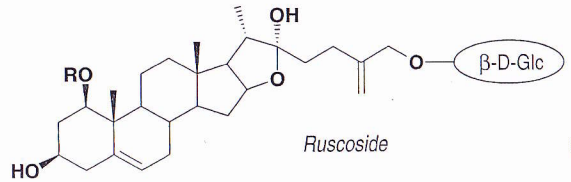
• Butcher's Broom, *Ruscus aculeatus* L., Liliaceae

The rhizome and roots of butcher's broom, together with asparagus, wild celery, fennel, and parsley, were formerly an ingredient of the traditional French five-root syrup or *sirop des cinq racines*, and are now widely used to obtain saponins as well as vascular protective and vascular tonic extracts. The 10th edition of the French Pharmacopoeia specifies that the dried subterranean parts contain not less than 2.5% total saponins, expressed as ruscogenins.

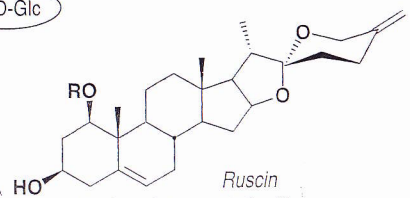
The Plant, the Drug. Butcher's broom is a lignified plant, perennial by a rhizome, with hardy stems gathered in erect tufts. The cladodes or false leaves end in a sharp point. The flower is greenish and inserted in the center of a small bract, on the cladode. The fruit is a scarlet berry which imparts to the branches some ornamental value. The plant grows wild in woods and underwoods in all of Europe.

The drug consists of knotty, articulated, yellowish fragments about 5 mm thick with a surface showing thin separate rings of about 1-3 mm in diameter. The rhizome bears numerous long and sinuate roots.

Chemical Composition. Alongside sterols, fatty acids, and sugars, the rhizome of butcher's broom contains a very small amount of essential oil, benzofuranoid derivatives, and flavonoids. The compounds to which the pharmacological activity is attributed are steroidal saponins which may represent up to 6% of the weight of the dried drug. The chief glycosides are derivatives of ruscogenin (= 1β-hydroxy-diosgenin) and of neoruscogenin (= 25(27)-dehydroruscogenin): in fact, the drug contains all four glycosides corresponding to the spirostane and furostane forms of these two aglycones, ruscoside, and ruscin, as well as their partial hydrolysis products. They occur alongside sulfated derivatives: the C-1 sulfate of ruscogenin and the C-1 sulfate of a furost-5-ene trihydroxylated at C-1, C-3, and C-22. Aculeoside A, a true diglycoside (diether at C-1 and C-24) of a neoruscogenin dihydroxylated at C-23 and C-24, has also been characterized.



Ruscoside



Ruscin

Tests. The drug is identified by its morphology and by the microscopic characteristics of the powder (sclerenchymatous cells, calcium oxalate raphides). In addition, ruscogenin is characterized by TLC analysis after reflux in the presence of hydrochloric acid and extraction (CH_2Cl_2). The saponins are extracted with methanol, the medium rendered aqueous, the saponins back extracted (butanol), and the extraction residue redissolved in a sulfuric acid solution to quantitate the saponins by absorbance measurement. The saponin concentration is not less than 2.5% expressed as ruscogenins.

Pharmacological Properties and Uses. Various properties are traditionally attributed to butcher's broom, particularly a diuretic activity, which remains to be demonstrated. Experiments on isolated organs and in animals show that the extract of butcher's broom prevents the dilation of overloaded venous vessels. The activity of the saponins, which are efficiently absorbed when administered orally, is thought to be linked in part to their stimulation of the post-junctional α-adrenergic receptors of the smooth muscle cells of the vascular wall, and in part to their direct action on the venous wall fibers. Veinlet contraction can be obtained by local application; the effect is temperature-dependent. Several clinical observations tend to prove the vascular protective and venous tonic properties of high doses of butcher's broom-based preparations, as well as their usefulness in the treatment of the functional symptoms of venous insufficiency and of the acute attack of piles.

The ruscogenins, alone or in combination (trimebutine, retinol), are ingredients of suppositories and creams indicated for the symptomatic treatment of painful and pruriginous anal symptoms, anal fissures, and especially the acute attack of piles. The treatment must be of short duration and if the symptoms persist, a proctological examination must be conducted. Forms for oral administration associate several ruscogenins (or a butcher's broom extract) to other "venous tonics" (sweet clover, esculin, hesperidin methyl chalcone, black currant extract), and/or ascorbic acid; indications: to treat the symptoms of chronic venous insufficiency (fullness in the legs, heaviness, restless legs syndrome) and for some of the available forms, to treat metrorrhagia linked to contraception (mini-pill or *intra-uterine device* = IUD); used to treat the functional symptoms of the acute attack of piles.

In France, the indication officially recognized (orally and topically, French Expl. Note, 1998) for butcher's broom-based medicines is the following: "traditionally used for the subjective symptoms of venous insufficiency such as fullness in the legs; to treat the symptoms of hemorrhoids".

• FIGWORT, *Ranunculus ficaria* L. = *Ficaria ranunculoides* Roth., Ranunculaceae

There are uses in pharmacy for the dried tuberized root of this small perennial herb with oval, cordate, and shiny leaves, whose flowers have 6-12 petals with a bright yellow claw, and blossom as early as March. The drug consists of fleshy tubercles shaped like baseball bats; it is rich in starch (

eccentric and linear hilum), and, to be official, it must contain not less than 20% "crude saponins" determined by simple gravimetry after extraction with 80% ethanol. The assay also includes the TLC analysis of the saponins (two major spots) and their aglycones (only one major spot, oleanolic acid).

Structurally, the figwort saponins are glycosides of hederagenin and oleanolic acid. According to various books, the drug is believed to contain protoanemonin, which is irritating for the skin and mucosal membranes.

Figwort root is traditionally used—only by topical administration—to treat the subjective symptoms of venous insufficiency such as fullness in the legs and to treat the symptoms of hemorrhoids [French Expl. Note, 1998]. Figwort root or its extract may be used in combinations (e.g., aqueous extract, combined with horse chestnut bark and marigold capitulum extracts, to treat the symptoms of hemorrhoids).

C. Saponin-containing Drugs Useful for Treating Cough

• SENEGA (SNAKEROOT), *Polygala senega* L., Polygalaceae

According to the latest edition of the European Pharmacopoeia, the drug (senega root) consists of "the dried and usually fragmented root and root-crown of *Polygala senega* or of certain other closely related species or of a mixture of these *Polygala* species". In addition, the 1998 French Explanatory Note, lists the subterranean parts and specifies that "the species containing podophyllotoxin must be excluded" (e.g., *P. polygama* Walt. *).

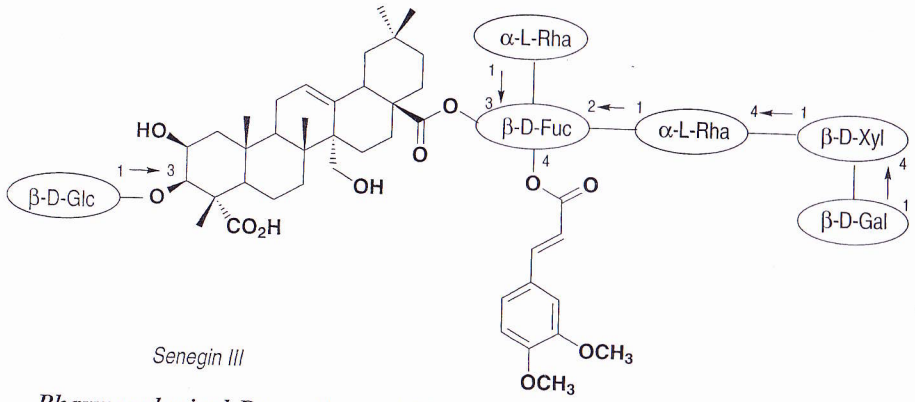
The Plant, the Drug. The senega is a small (20-30 cm) herbaceous perennial plant bearing sessile lanceolate leaves, and tight spikes of irregular white flowers with petaloid sepals of uneven size. The species grows wild in eastern Canada and in the northeast United States. The species has become rare, but others may be used instead, for example, *P. latifolia* Torr. and Gray, cultivated in Japan.

The drug consists of a knotty brownish-gray crown with a root attached (10 cm x 1-8 mm in diameter). This root is more or less twisted, and has a longitudinal, decurrent, prominent, and more or less distinct ridge, shaped like an elongated helix; sometimes there are secondary roots (e.g., in the Japanese species and varieties). The microscopic examination of the cut confirms the identification by showing phelloderm with cells containing oil droplets, a phloem particularly well developed in the ridge area, and abnormal secondary structures forming one or two large

* Note that these species are not named, and that the French Pharmacopoeia has no requirement for verifying the absence of this type of compound. This type of lignan has indeed been detected in Polygalaceae: Hokanson, G.C. (1979). The Lignans of *Polygala polygama*

cuneiform rays in the phloem and wood. TLC analysis of the saponins completes the identification (extraction in 70% ethanol, visualization with phosphomolybdic acid).

Chemical Composition. The drug is known to contain lipids, methyl salicylate, phenolic acids, oligosaccharides, and 5 to 10% saponins. These are glycosides of presenegin, which is a polyhydroxylated (2β, 3β, 27) and dicarboxylic (C-23 and C-28) olean-12-ene. The chief constituents are bidesmosides, namely senegin II, III (= onjisaponin B), and IV . They are 3-glucosides of presenegin, and differ from one another by the number of units (4, 5, or 6) in the sugar moiety which esterifies the carboxyl group at C-28.



Senegin III

Pharmacological Properties and Uses. The pharmacology of the drug has not been well studied: experiments in dogs have shown that senega snakeroot syrup is an expectorant, perhaps *via* a reflex mechanism. The drug is commonly used as senega snakeroot syrup, which is an ingredient of formulations proposed to treat non-productive coughs, with the precautions that are customary for this type of drug (preliminary diagnosis of the cause of the cough, repeated clinical examination in case of persistent cough). Senega snakeroot syrup is also an ingredient of combinations (e.g., with codeine, pholcodine, Tolu balsam, and/or plant tinctures [gum plant, Indian tobacco, henbane, aconite]).

In France, the phytomedicines based on senega snakeroot may claim the following indications (orally): symptomatic treatment of cough (1998 French Explanatory Note, with the above reservation). In Germany, the use of senega snakeroot is similar (catarrh of the respiratory tract). The Commission E monograph mentions the risk of gastrointestinal irritation in case of prolonged use.

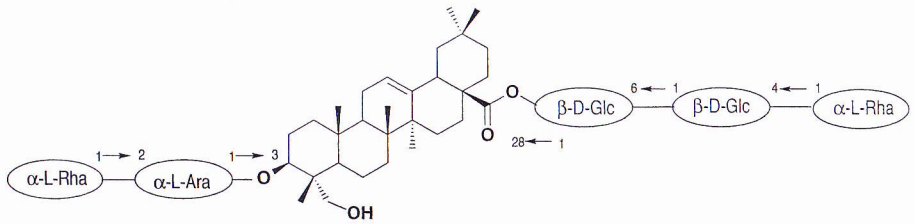
• *Polygala tenuifolia* Willd. (= *P. sibirica* L.) is an Asian species used as an expectorant in oriental medicine (*yuanzhi*, official in the People's Republic of China), and has a similar composition: the saponins that it contains (the onjisaponins) are glycosides of presenegin, some of which are referred to as senegins (synonymous terms). The European senegas, whose chemical composition is little known, contain bidesmosides of a similar prosapogenin, namely tenuifolin (from *P. chamaebuxus* L., *P. alpestris* Rchb. [*P. alpina*]).

• Common Ivy, *Hedera helix* L., Araliaceae

This species, prized since antiquity for many healing virtues, remains in use in contemporary phytotherapy (wood) and cosmetology (leaves). The dried leaf is the subject of a monograph in the 10th edition of the French Pharmacopoeia.

The Plant, the Drug. Ivy is a very common plant that grows at altitudes up to 1,200 m in woods, hedges, and cool rocky areas. It is a shade- and half-shade-loving plant that climbs up trees and walls. It has creeping and climbing stems which emit roots and grow hooks. The cauline leaves are divided into 3 to 5 triangular lobes (8-10 x 10-12 cm), whereas those of the floriferous heliophilous stems are oval and entire (6-8 x 4-6 cm). The flowers are gathered in terminal umbels, and the fruits are globulous blackish berries with circular features near the top.

Chemical Composition. The composition of the ivy wood does not seem to have been a topic of detailed investigations. The leaves produce sterols, a small quantity of steam distillable products (germacrene B, elemene), caffeic esters of quinic acid, flavonoids (rutin), and like other Araliaceae, polyalkynes: falcarinol, falcarinone, 11-dehydrofalcarinol. The concentration of the saponins ranges from 5 to 8%. These—the hederasaponins B to I—are bidesmosides of oleanolic acid, hederagenin (hydroxylated at C-23), or bayogenin (dihydroxylated at C-2β and C-23). Hederasaponin C (= hederacoside C) is by far the chief constituent, and its level ranges, depending on the season, from 5 to 7% (the French Pharmacopoeia specifies that the three chief saponins are hederasaponin B and C and "*saponine k10*"). The berries also contain saponins.



Hederacoside C

α-Hederin

Hederagenin

Tests. Ivy can be identified by its macroscopic characteristics and by the microscopic examination of the powder: broken thick-walled trichomes and secretory ducts surrounded by thick-walled protective cells. The drug identity is

TLC of an 80% methanol extract (visualization by spraying a solution of sulfuric acid in methanol and heating to 100 °C) and an HPLC quantitation of the saponins in the same extract. This technique allows the determination of the concentration of hederasaponin C: it must be not less than 2.5%.

Biological Properties. The ivy wood extract, traditionally considered an expectorant, prevents the acetylcholine-induced bronchospasm in the guinea pig. An ivy leaf extract (30% ethanol) was spasmolytic in vitro (guinea pig ileum stimulated by acetylcholine) and it was shown by fractionation that α-hederin was the main constituent responsible for the activity.

The antifungal properties of the extract titrated for hederasaponin C have been demonstrated *in vivo* (in mice infested with *Candida albicans*, 50 mg/kg); the same can be said of the toxicity to flukes (in sheep infested with *Dicrocœlium*, 800 mg/kg). Toxicity toward *Amoeba*, *Trichomonas*, and *Leishmania* (*L. infantum, L. tropica*) has been reported, *in vitro*, for the sodium salts of the monodesmosides. The ivy leaf extract is also cytotoxic and antibacterial. The saponins from the berries are toxic to molluscs.

Uses. Ivy-based preparations are mostly used in cosmetic products: creams, lotions, shampoos, and "anticellulite" preparations. In France, phytopharmaceuticals based on ivy wood are traditionally used orally for the symptomatic treatment of cough and to treat acute benign bronchial disease [French Expl. Note, 1998]. The leaves are traditionally used topically as an adjunct in weight loss programs and in the emollient and itch-relieving treatment of skin disorders, and as a trophic protective agent for cracks, bruises, frostbite, and insect bites. The German Commission E monograph describes ivy leaf as an expectorant and spasmolytic, and an irritant for the skin and mucosas; it is used to treat chronic bronchial inflammation and catarrh of the respiratory tract.

Ivy Toxicity. In most cases, the accidental ingestion of ivy berries causes no symptoms. Gastrointestinal distress is possible. The bitter and acrid taste of the ripe berry normally deters the (young) consumer.

Ivy and ivy-based products can cause dermatitis, erythema with vesicles, and cutaneous erosion, all of which subside more or less rapidly. The reaction, enhanced by humidity and sunlight, is due to falcarinol.

• PRIMROSE, *Primula veris* L. = *P. officinalis* (L.) Hill., Primulaceae

The drug consists of the dried flower (Fr. Ph., 10h Ed.). This species indigenous to western Europe is characterized by almost oval leaves which abruptly narrow into a wide petiole, by a floral stalk covered with down, and by bright yellow flowers with a tubular swollen calyx with obtuse teeth and a small concave corolla, with five orange spots at the top of the [illegible]

differs by its flowers, which have a wide, planar, pale yellow corolla with no spots and a calyx with sharp teeth.

The flower contains flavonoids (gossypetin) and nearly 2% saponins in the calyx. The subterranean parts contain 5 to 10% saponins represented, in *P. veris*, by primulic acid (= primulacic acid A), the tetrasaccharide of an internal ether (28, 13) derived from a 13β,16α,28-trihydroxylated oleanolic acid (protoprimulagenin A) and by compounds of very similar structure: 16-acetylpriverogenin A and 22-acetylpriverogenin B. The two species have very similar compositions (qualitative differences; *P. elatior* also contains glycosides of echinocystic acid and of 28-dehydroprimulagenin A).

Formerly considered to be substitutes for senega snakeroot, the primrose root and flower are used, in France, orally for the symptomatic treatment of cough, and locally in mouthwashes, as an adjunct in the emollient and itch-relieving treatment of skin disorders, and as a trophic protective agent for cracks, abrasions, frostbite, chaps, and insect bites [French Expl. Note, 1998].

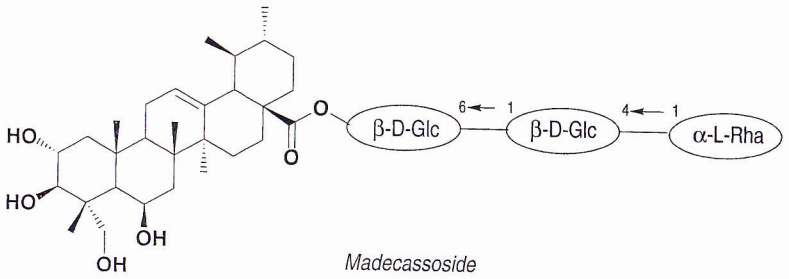
The German Commission E monograph authorizes only one indication on package inserts: adjunctive treatment to promote secretion and relieve irritation in case of catarrh of the upper respiratory tract. The risk of allergic reaction upon contact with the skin must be mentioned (see p. 414). Commission E recognizes that the root has secretolytic and expectorant properties identical to those of the flower, hence its use for bronchial catarrh.

D. Saponin-containing Drugs of Use in Dermatology

• HYDROCOTYLE, *Centella asiatica* (L.), Apiaceae

Also known as Gotu kola or Indian pennywort, this perennial herb is widely distributed in India, and in the Indian Ocean, from Madagascar to Indonesia. It is easy to identify by its small rounded leaves, more or less cordate, borne at the nodes of a long running stem, and by umbels with very small flowers. The drug contains a trace of essential oil (β-caryophyllene, α-humulene, germacrene-D, trans-β-farnesene, α-copaene), sterols, flavonol glycosides, polyalkynes, and saponins: asiaticoside (0.3%) and madecassoside (1.5-2%). These are not glycosides, but esters in the 28-position of a trisaccharide (α-L-Rha 1—>4 β-D-Glc 1—>6 β-D-Glc 1—>) and of triterpenoid acids derived from ursane: asiatic acid (2α,3β,24-trihydroxy-urs-12-en-28-oic acid) and madecassic acid (= 6β-hydroxyasiatic acid = 2α,3β,6β,24-tetra-hydroxy-urs-12-en-28-oic acid). The minor compounds formerly mentioned in some Indian specimens have not been found again in more recent analyses, no matter what their geographical origin.

The drug has been used in Ayurvedic medicine since remote times to treat skin diseases and nervous disorders (epilepsy, hysteria). It is official in the People's Republic of China (*jixuecao*) where it is prescribed orally for numerous indications



Madecassoside

Hydrocotyle preparations are thought to accelerate the healing of superficial wounds. This activity is difficult to evaluate clinically. It is confirmed by experiments in rodents and consistent with the convergent observations made in humans. It is attributed to asiaticoside and derivatives of the same type, which are thought to stimulate the synthesis of collagen and mucopolysaccharides: indeed, small doses of asiaticoside, as well as asiatic and madecassic acid, tested in vitro on cultured human fibroblasts, specifically increase collagen production. Other authors believe that only asiatic acid stimulates collagen production, and that the other constituents act only on proline synthesis.

Centella extracts are used topically as a complementary treatment of legs ulcers of venous origin (at the granulation stage), in the adjunct treatment of limited surgical wounds and minor burns, and as complementary treatment of fibrous hypertrophic scars. Orally, it is indicated to relieve the symptoms of venous and lymphatic vessel insufficiency (fullness in the legs), and used for insufficient healing (atonic wounds) or excessive healing (hypertrophic scars, cheloids). Allergic reactions are observed in rare cases.

An abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM* can be used for hydrocotyle-based medicines that have one of the following indications [topically, French Expl. Note, 1998]: traditionally used 1. to treat the functional symptoms of cutaneous capillary fragility such as ecchymosis and petechiae; 2. for the subjective symptoms of venous insufficiency such as tiredness or fullness in the legs; 3. for hemorrhoidal symptoms; 4. as an adjunct in the emollient and antipruriginous treatment of skin disorders, as a trophic protective agent for cracks, bruises, frostbite, and insect bites; 5. for sunburns, superficial and limited burns, and diaper rash.

• URBAN GINSENG, *Mimosa tenuiflora* (Willd.) Poiret, Mimosaceae

The bark of this Latino-American species, also known as *tepescohuite*, is used in Mexico, based on traditions inherited from the Mayas, to treat various skin disorders. The bark powder, sprinkled onto burns, is said to be an analgesic and to help tissue repair, as reported after accidents (explosions, earthquakes).

M. tenuiflora is a small tree ubiquitous in Central America and in the north of South America, characterized by bipinnate leaves, dense spikes of white flowers, and

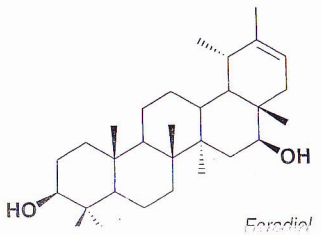
a lanceolate inerm pod constricted between the seeds. The bark powder has a weak smell and a floury taste, and contains many starch grains, phloem fibers, sclerified cells surrounded by others containing crystals, and suber cells with reddish-brown contents.

Chemical analysis has shown that the bark contains *N,N*-dimethyltryptamine, phytosterol glycosides, and mono- and bidesmosides of oleanolic acid and macherinic acid (= 21β-hydroxy-oleanolic acid), namely mimonosides A-C. *In vitro*, the mimonosides have cell protective activity on murine and human fibroblasts, activating and prolonging cell multiplication for about ten days. This might explain, at least partially, the effects for which the drug is recognized. There is also a synergy with various immuno-stimulants (concanavalin A, lipopolysaccharides).

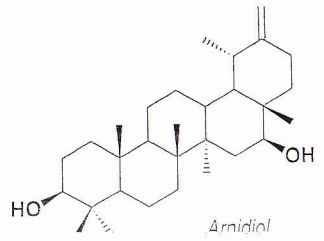
• MARIGOLD, *Calendula officinalis* L., Asteraceae

The Plant, the Drug. The marigold (Fr. Ph., 10th Ed.) is a small perennial herbaceous cultivated plant, with hardy and angular stems, and with sessile leaves. The inflorescences are big capitulums (3-8 cm) surrounded by two rows of hairy bracts. The tubulous and hermaphrodite disk-flowers are generally darker orange-yellow than the female ray-flowers, which have tridentate ligulas. The fruit is a curved akene, which is spiny on its convex side. The microscopic examination reveals, at the base of the ligulate flowers, long pluricellular biseriate trichomes and finely echinulate pollen grains with three pores. The drug assay consists mainly of a TLC analysis (flavonoids and phenolic acids).

Chemical Composition. The composition of marigold—the flowers or capitulums—is fairly well known: flavonoids (0.3-1.5% 3-mono- and -oligo-saccharides of isorhamnetin and quercetin), carotenes (lycopene), xanthophyll, essential oil (2-3 mL/kg) with oxygenated sesquiterpenoid derivatives (cadinols), and polysaccharides. Sesquiterpenoid lactones have not been found; instead loliolide and ionones were characterized. The triterpenoid compounds are particularly abundant: mono-, di-, and trihydroxylated derivatives, free and esterified, of lup-20(29)-ene, olean-12-ene, tarax-20(30)-ene, tarax-20(21)-ene (=ψ), and ursa-12-ene, such as α- and β-amyrin, arnidiol, faradiol, ursadiol, calenduladiol, and heliantriols. Several saponins have also been isolated and identified: saponins A-D, D2, and F. These are bidesmosides (28-glucose esters) and monodesmosides of oleanolic acid: the



Faradiol



Arnidiol

glycosidic linkage is formed with a D-glucuronic acid, which may be unique or may be the first unit of a di- or trisaccharide. The saponin concentration ranges from 2 to 10% depending on the variety and the harvest time.

Pharmacological Properties. The ethanol extract (80%) obtained from dried marigold, as well as the homeopathic mother tincture, possess *in vitro* antibacterial properties. In topical application, marigold preparations have an anti-inflammatory effect which has been demonstrated on several animal models. They are thought to promote healing.

Research conducted on an experimental model (croton oil-induced mouse ear inflammation) with a supercritical carbon dioxide extract reveals that it is the lipophilic fraction, devoid of saponins and polysaccharides, that is anti-inflammatory. Further fractionation, monitored by checking the fractions for biological activity, shows that the activity is due to triterpenes (mono-ols and diol esters), mostly faradiol monoesters. Free faradiol, prepared by hydrolysis, clearly has the same degree of activity as indomethacin on the same model.

Uses. Because of the non-trivial acute toxicity of the extracts, marigold capitulum-based preparations must be reserved for local use: traditionally used to treat minor wounds after thorough cleansing (with soap and water) and removal of contaminants; as an adjunct in the emollient and antipruriginous treatment of skin disorders; as a trophic protective agent for cracks, abrasions, chaps, and insect bites; for sunburns, superficial and limited burns, and diaper rashes; and as an antalgic in diseases of the mouth, pharynx, or both (collutoria, lozenges) [French Expl. Note, 1998]. The German Commission E monograph recognizes that marigold has healing properties and describes that anti-inflammatory effects have been observed after local application. Authorized indications for package inserts are inflammation of the skin and mucosal membranes, bruises, burns, and scratches (infusion on a compress or as a gargle). The drug is widely used in cosmetology as an emollient, vulnerary, and moisturizing agent (in lotions, creams, soaps, and after-sun lotions).

E. Saponin-containing Drugs: "Adaptogens"

• GINSENG, *Panax ginseng* C.A. Meyer, Araliaceae

Ginsengs are plants which, in oriental medicine, have enjoyed a strong reputation since ancient times for being tonic, regenerating, and rejuvenating, even though their pharmacological activity is difficult to pin down. The activity that is claimed explains the genus name *Panax*, formed from the Greek *pan* (all) and *akos* (remedy). This panacea (*panakeia*) *(p. 708) was believed to be the universal remedy.

The Plant, the Drug. The wild "Korean" ginseng (*shanshen*), official in the People's Republic of China, has become hard to find. It has essentially been replaced

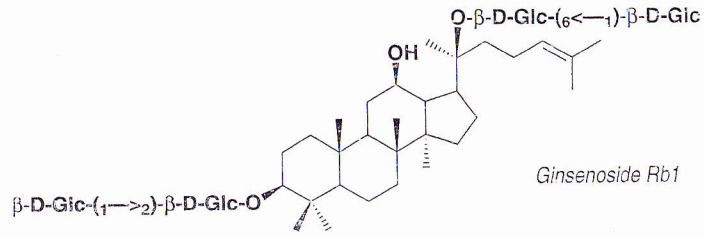
by cultivated ginseng or "true" ginseng, which is not the only one in use. Other species are also used:

- American ginseng cultivated in North America (*P. quinquefolium* L.);
- *San-chi* ginseng, *P. notoginseng* Burkill F.H. Chen, official in the People's Republic of China where it is reputed as a tonic and hemostatic;
- Japanese ginseng (*chikusetsu-ninjin*, *zhijieshen*), *P. pseudoginseng* Wall. subsp. *japonicus* (C.A. Meyer) C.Ho & Tseng (= *P. japonicus* C.A. Meyer), cultivated in China, Vietnam, and Japan, considered to be an antipyretic, stomachic, and expectorant;
- the *bipinnatifidus* (Seem) Li and *angustifolius* (Burkill) Li varieties of *P. pseudoginseng*, and more.

Ginseng is a small herbaceous plant with palmatilobate leaves, umbels of white flowers, and red berries. It grows wild, but has been overexploited, in mountain areas, from Nepal to Manchuria, and from eastern Siberia to Korea.

The drug consists of the dried root, which has been listed since 1989 in the French Pharmacopoeia (10h Ed.). This root is fusiform or cylindrical, fragile and of low density, more or less ramified, sometimes arched and even folded over. Certain specimens are very suggestively anthropomorphic: they are the most prized and their shape probably explains, in part, the attention devoted to ginseng, and especially its reputation—unfounded—for enhancing "strength in the elderly". Ginseng is cultivated in Asia (Korea, China), but the market is also supplied in great part by North American cultures (in 1990, 1.5 million pounds were produced in Wisconsin), and exported essentially towards China, Taiwan, Hong-Kong, and Singapore. Traditionally, "white" ginseng is the washed root without secondary roots, sundried or oven dried, and most often peeled; "red" ginseng owes its reddish-brown color to a preliminary steaming; in the United States, the root is washed and then dried with hot air for 12-14 days.

Chemical Composition. Numerous compounds have been characterized in the root: polysaccharides, glycopeptides (panaxanes), vitamins, sterols, amino acids and peptides, essential oil (5 mL/kg, characterized by high levels of sesquiterpenoid hydrocarbons), and polyalkynes (panaxynol, panaxytriol). The numerous saponins isolated from the drug (about 20) are—except for one—glycosides of tetracyclic aglycones of the dammarane series, more specifically a 3β,12β,20(*S*) trihydroxylated type (protopanaxadiol) and a 3β,6α,12β,20(*S*) tetrahydroxylated type (protopana-



Ginsenoside Rb1

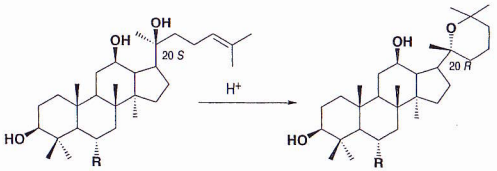
xatriol**). The differences between the saponins (ginsenosides*** Ra-1-2, Rb-1-3, Rc-f, Rg-1-2, Rh-1) reside in the mono-, di-, or trisaccharide nature of the two sugar sequences at C-3 and C-20, or at C-6 and C-20. In exceptional cases, all three hydroxyl groups at C-3, C-6, and C-12 of protopanaxatriol can be engaged in glycosidic bonds (e.g., ginsenoside 20-gluco-Rf). Malonyl-ginsenosides have also been characterized (in white ginseng).

All of the ginsengs do not have the same composition: most of them contain some of the ginsenosides found in the Korean ginseng, but in different proportions, and they also contain specific saponins, which are glycosides of protopanaxadiol and protopanaxatriol: pseudoginsenoside F11 and quinquenoside R1 in the American ginseng, and gypenoside XVII and notoginsenosides R1-4 in San-chi ginseng. The difference is greater in the case of the Japanese ginseng: half of the "chikusetsusaponins" are bidesmosides (at C-3 and C-28) of oleanolic acid. As far as Korean ginseng is concerned, it is thought to have an intermediate composition between that of the Korean and Japanese ginseng, but different from that of the Japanese ginseng varieties growing in Yunnan! Finally, if we mention that the composition of red ginseng differs slightly from that of white ginseng, that the quantitative and qualitative composition of the rootlets and secondary roots differs from that of the main root, and that it varies depending on the age of the specimen, its source, and other factors, it should be obvious that it is difficult to speak generally of the composition of ginseng—not to mention that the commercial drugs are often grossly falsified. The saponin concentration of the root of Korean ginseng can range from 1 to 3%, and saponins Rb1, Rb2 (diols), Rg1 (triol), and R0 (oleanane) are generally the most abundant.

Tests. The drug must be identified by its macro- and microscopic characteristics, as well as by showing the presence of triterpenes and amino acids, and it must pass different tests: TLC analysis of an alcoholic extract, fraction extractible in 70% alcohol (20-30%), and reducing sugars after hydrolysis (<10%). The saponin concentration, determined by colorimetry ($SbCl_3$) after extraction (methanol:water 80/20 v/v) and selective partition of the glycosides into a butanol phase, must be not less than 2%. In addition, HPLC allows an estimate of the relative proportions of the chief ginsenosides.

* Delaveau reminds us that Panacea, who was Hygeia's ° sister, was also the daughter of Asclepius, the god of medicine. The pharmacist's "caduceus" shows the serpent of Epidaurus drinking from Hygeia's cup. It is the same serpent that wraps around Asclepius's staff to form the emblem of physicians. As for the true caduceus, Hermes's emblem with two serpents, that's another story... (see Delaveau, P. [1992]. La mémoire des mots en médecine, pharmacie et sciences, Louis Pariente, p. 294 sqq. (° from Hygeia arose hygiene).

** The apparent discrepancy between the customary nomenclature and the number of hydroxyl groups is explained by the fact that upon hydrolysis of the saponins, panaxadiol and panaxatriol are produced, and although they include a fourth, tertiary alcohol function at C-20, it is blocked into a tetrahydropyran ring.



R = H : *Protopanaxadiol*
R = OH : *Protopanaxatriol*

R = H : *Panaxadiol*
R = OH : *Panaxatriol*

*** Russian workers initially named it "[illegible]" based on [illegible]

Pharmacological Properties and Uses. Reviewing a plethora of literature (several thousand references) which is heterogeneous and of uneven scientific quality is no easy task. The biochemical, endocrinologic, and immunologic effects of the pure saponins have been studied *in vitro* and in animals: they are often opposite. Ginseng root is thought to be a CNS stimulant, to increase resistance to fatigue and stress, to improve memory, and to have an anabolic effect. These are the indications given by results of animal experiments with high doses, and most often by the parenteral route. As in the case of other so-called medicinal plants, to extrapolate these effects to an effect in humans is a difficult step, especially since these properties have not been confirmed by rigorous clinical trials: the numerous "observations in humans" that have been published do not shed much light on the issue, because the clinical definition of the pathological condition being treated is often unclear, data interpretation often subjective, and the placebo effect substantial.

Referred to as an "adaptogen" (i.e., a stimulant of the body' s "nonspecific resistance"), ginseng is traditionally used in France, despite the lack of truly demonstrated, specific activity, alone or in "cocktails" of assorted vitamins and stimulants, for the symptomatic treatment of functional asthenia [French Expl. Note, 1998]. The 1991 German Commission E monograph describes its use to fight the feeling of fatigue, lack of energy, lack of ability to concentrate, and during convalescence.

Although no acute toxicity has been observed for this drug, various side effects have been described (including a ginseng abuse syndrome). The truth is that the published studies are not reliable: most of them are about nonstandardized forms of ginseng, and unspecified doses and administration routes; worse, in most publications the drug identity is not known with any degree of certainty—and ginseng is one of the drugs most commonly adulterated (including by the addition of medicines such as phenylbutazone or aminopyrine). The drug interactions that are sometimes described are difficult to interpret. Some authors suspect that long-term use can lead to effects mimicking those of corticoids. This is probably the piece of data which led the writers of the 1998 French Explanatory Note (and those of the Commission E monograph) to specify that the posology of ginseng must not exceed 2 g of powder per day, and that the treatment must be limited to a maximum of three months. Some authors recommend against the use of ginseng in pregnant women and against simultaneous use with MOA inhibitors.

• SIBERIAN GINSENG, *Eleutherococcus senticosus* Maxim., Araliaceae

The "dried subterranean parts, entire or fragmented" of this species were included [illegible] 1996 (addendum number 34, August 5 decree or arrêté)

E. senticosus (= *Acanthopanax senticosus* [Rupr. and Maxim.] Harms) or Siberian ginseng is a thorny bush common in western Siberia, from the Amur to Sakhalin Island and to Korea and Shanxi Province. Russian workers have proposed its roots as a substitute for ginseng, under the evocative name of "Siberian ginseng". The microscopic examination of the root powder is completed by a TLC analysis of a methanolic extract to characterize eleutherosides B and E. The French Pharmacopoeia does not require the quantitation of any particular constituent.

Chemically, the Siberian ginseng root contains polysaccharides, phenolics (coumarins, lignans, phenylpropanoic acids) and eleutherosides. This (inadvertently?) ill-chosen term suggest a homogeneous and novel class of compounds, which is not the case: although some of them are specific and triterpenoid in nature (eleutherosides I-M), the others are commonplace and belong in miscellaneous series, since they include isofraxoside (eleutheroside B_1), glycosides of syringaresinol (eleutherosides D-E) and of sinapyl alcohol, and the methyl ester of galactose (eleutheroside C); some constituents are not even glycosides (daucosterol (eleutheroside A), sesamin (eleutheroside B_4)).

Like ginseng, Siberian ginseng is an "adaptogen" which "normalizes pathological conditions". As above, the results of studies in humans remain debatable because they cannot be verified (e.g., estimate of tone in car rally drivers). The drug may claim the following indication: traditionally used to treat functional asthenia. The German Commission E monograph lists uses identical to those of ginseng and hypertension as a contraindication.

F. Detergent Saponin-containing Drugs

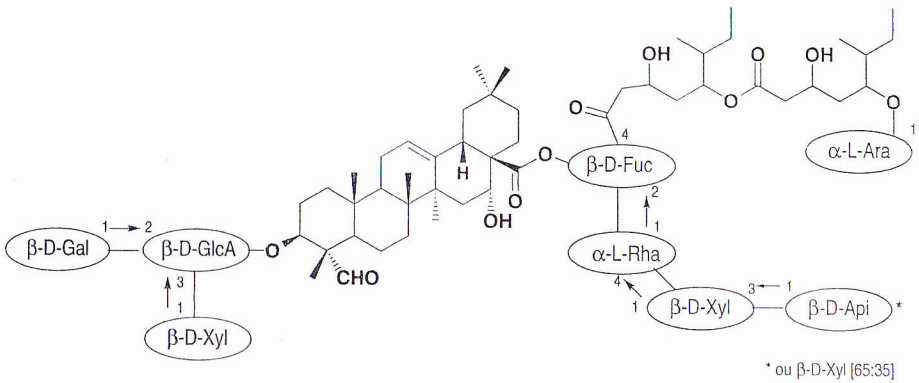
- **QUILLAJA,**
  Quillaja saponaria Molina, Caryophyllaceae

After falling into (official) oblivion for half a century, the drug reappeared in the French Pharmacopoeia (10th Ed.) in 1989.

The Plant, the Drug. The drug is improperly referred to as Panama "wood". It consists of the dried bark of the trunk, without suber, of *Q. saponaria* and the French Pharmacopoeia specifies that another species may be used: *Q. smegmadermos* DC. Morphologically, the drug consists of very long (10-15 x 0.3-1 cm cross section and up to 1 m in length) flattened fragments. Examination of the break with a magnifier reveals shiny crystals. These are voluminous prisms of calcium oxalate (50-170 µm), found again in the powder under the microscope, mixed with very long sclerified and twisted fibers. The official drug must have a foam value not lower than 3,000, must contain not more than 5% suber, and must undergo TLC analysis (characterization of the aglycones after hydrolysis).

Chemical Composition. The foaming properties of the decoctions of the drug are due to 9-10% of "quillaic

of very complex structure. For example, compound QS-III ($C_{104}H_{168}O_{55}$, $M^{+\cdot}$ 2296) is a bidesmoside comprising ten monosaccharides, two molecules of 3,5-dihydroxy-6-methyloctanoic acid, and a polyfunctional oleanene, namely quillaic acid.



Saponin QS-21 A

Biological Properties. Quillaja extracts, titrated for their saponins and administered in the long term, lower blood cholesterol in animals: the saponins form an insoluble complex with cholesterol, which inhibits its intestinal absorption. The saponins have also been tested in pharmaceutical technology for their ability to promote the penetration of medicinal peptides across mucosal membranes.

Panama wood saponins potentiate the response against the antigens of various infectious agents. Therefore, they have potential as adjuvants in the formulation of vaccines, particularly for veterinary use (e.g., potentiation of the immunogenic power of the surface proteins of *Borrelia burgdorferi*, the spirochaeta responsible for Lyme disease). They have further potential applications in that the recombinant viral proteins obtained by molecular biology, which are generally weakly immunogenic, require formulation with an adjuvant (aluminum hydroxide). There is ongoing research on immunostimulating complexes (ISCOMS), which are cage-shaped nanoparticles formed by the antigen, a *Quillaya* saponin, cholesterol, and phospholipids. These complexes involve relatively less saponin for an equal efficacy as an adjuvant, which decreases their toxicity.

Uses. At present, quillaja is a detergent used as an ingredient in shampoos and in other hygiene and cosmetology products. In the pharmaceutical industry, it is sometimes used in topical preparations designed for the emollient and itch-relieving treatment of skin disorders, and as a trophic protective agent for cracks, bruises, frostbite, and insect bites [French Expl. Note, 1998].

Is the drug toxic? That is what is implied by several classic textbooks. Note, however, that long-term administration of an aqueous extract to rats (two years) did not result in any harmful effects. In addition, although some quillaja saponins are

QUILLAJA SAPONARIA Mol

saponin [mixture]), their acute toxicity by the oral route is low (LD_{50} = 1.625 g/kg, mouse), and so is their long-term toxicity (no effect for a dose of 0.7 g/kg/day x 84 weeks, mouse). In some countries, quillaja is an authorized additive (ADI for the extract: 0-5 mg/kg [FAO-WHO]).

• SOAPWORT, *Saponaria officinalis* L., Caryophyllaceae

An herbaceous plant with round stems, with opposite, decussate, sessile leaves, and with pale pink flowers with a very elongated calyx striated in red, the soapwort is listed in the 10th edition of the French Pharmacopoeia. The drug consists of the stems and leaves, with or without flowers, or the rootstock (dried rhizome and root).

The drugs, identified by their morphology and their microscopic characteristics, have a foam value greater than 1,000 (aerial parts) or greater than 2,000 (rootstock). The rootstock assay includes, among others, a TLC analysis of the aglycones (visualization with vanillin in the presence of sulfuric acid) following the sulfuric acid hydrolysis of the saponins; the aerial parts assay includes a TLC analysis after 60% alcohol maceration designed to show the presence of flavonoids (spraying with aminoethanol diphenylborate and PEG 400) and to detect potential falsification by *Silene vulgaris* (Moench) Garcke (= *S. inflata* L.). The saponins are thought to be mostly glycosides of quillaic acid.

The soapwort enjoys a reputation for being a diuretic and a "depurative", and is not listed among the drugs eligible for the abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM*. The German Commission E monograph lists uses only for the root: expectorant by irritation of the gastric mucosa, used for catarrh of the respiratory tract.

• GYPSOPHILAS, *Gypsophila* spp., Caryophyllaceae

Traditionally, because of their high concentration of surface-active saponins (often over 10%), the subterranean parts of various species in this genus are used as sources of commercial saponins (soap root, Spanish soapwort, Egyptian soapwort; *G. paniculata* L., *G. arrostii* Guss., *G. struthium* Loefl.). The main use for these gypsophilas is the formulation of cosmetic products. The chemical composition of the *G. paniculata* "saponin" is not completely known; it contains mostly bidesmosides of oleanolic acid with an aldehyde function at C-23: gypsogenin (gypsoside A, saponaside D [= saponaroside D]) and quillaic acid (= 16α-hydroxy-gypsogenin). Because of its particularly high surface activity, gypsophila saponin is, according to Hostettmann and Marston [1995], incorporated into formulations for fire extinguishers * (p. 714).

G. Other Saponin-containing Drugs

The activity of some drugs reputed to be diuretics, but of limited interest, such as the asparagus (Liliaceae, Fr. Ph., 10th Ed.) or the rupture wort ** (*Herniaria glabra* L., *H. hirsuta* L., Caryophyllaceae) is due to saponins—according to some authors, who make this claim without proof. The same may be true for some drugs that were fads in the nineteenth century, but that are of limited use today, such as the sarsaparilla*** (*Smilax* sp., Liliaceae, Central America, Far East).

Also on the market are some yucca preparations (*Yucca* spp., Liliaceae) and some alfalfa preparations, which, in animals, display blood cholesterol lowering activities clearly linked to the presence of saponins: the use of alfalfa probably has some drawbacks. Many other drugs contain saponins, but the role of the saponins in the activity that is attributed to the drugs is difficult to assess: see, among others, European goldenrod (p. 253) and mullein (p. 113).

In the case of *Chrysanthellum*, there are no data to help attribute the pharmacological properties claimed by some authors to any particular class of metabolites: thus the choice to mention this drug here is completely arbitrary.

* In Japan, extracts of the pericarp of the fruit of *Sapindus mukorossi* Gaertner (Sapindaceae) are used in fire extinguishers. These extracts are antifungals and antibacterials, and they are approved by the Japanese ministry of health as ingredients in cosmetic formulations. The saponins from this drug are mostly glycosides of hederagenin. Among the other species currently exploited for their surface-active properties are the following: *Yucca schidigera* Roezl ex Ortgies (palmilla, Agavaceae) and *Chorogalum pomeridianum* (DC.) Kunth (California soap plant, Liliaceae).

** According to the German Commission E, *H. glabra* is a weak spasmolytic. It is used for many disorders (kidney problems, arthritis, rheumatism, respiratory difficulties). Since the drug efficacy has not been demonstrated, Commission E does not recommend its use. The plant is rich in triterpenoid derivatives which are oligosaccharide-containing esters at C-28 of medicagenic, 16-hydroxymedicagenic, and gypsogenic acid. See Freiler, M., Reznicek, G., Schubert-Zsilavecz, M., Reiner, J., Haslinger, E., Jurenitsch, J. and Kubelka, W. (1996). Struktur der Triterpensaponine aus *Herniaria glabra*, *Sci. Pharm.*, **64**, 359-365.

*** In the United Kingdom, the 1990 edition of the BHP devotes a monograph to the dried root of sarsaparilla (but it no longer appears in the BP). The monograph describes the commercial drug as being obtained mainly from American species—*S. aristolochiaefolia* Miller, *S. febrifuga* Kunth, *S. ornata* Hook. F., and *S. regelii* Killib & Morton—and as an anti-inflammatory. The various species within the genus contain glycosides of sarsasapogenin and of smilagenin, such as parillin (perhaps as bidesmosides of furostanols, e.g., sarsaparilloside). The pharmacology of these species has practically not been studied. Saponins such as parillin are potent antifungals and antibacterials *in vitro*. In the nineteenth century, sarsaparillas were used as antisyphilitics and to treat various skin disorders, including leprosy.

• *Chrysanthellum indicum* DC, subsp. *afroamericanum* B.L. Turner, Asteraceae

This plant, indigenous to Peru and Bolivia, grows wild in Africa, on the edges of rural roads, on neglected lands, and around human settlements, especially in high plateau areas. The species is characterized by bi- and tripinnatisect leaves, yellow flowers gathered into small capitulums (8-10 mm) of the radial type with one or two linear bracts, and ovoid akenes, more or less compressed and with a membranous wing. It is sometimes falsified by closely related species such as *C. americanum* (L.) Vatke, which has more or less lobate leaves, or by other Asteraceae, such as *Parthenium integrilolium* L., hence the importance of a detailed morphological examination and a TLC analysis (to verify the presence of flavonoids).

The drug consists of the entire plant. It is known to contain flavonoids of limited distribution: eriodictyol 7-*O*-glucoside, iso-okanin 7-*O*-glucoside, maritimein (an aurone), marein (a chalcone), and saponins, namely chrysanthellins A and B. The structural elucidation of these molecules has shown that they are bidesmosides, and that their aglycones—echinocystic acid and caulophyllogenin—are hydroxylated derivatives of olean-12-enoic acid.

The drug is described, based on little experimental data, as a hepatoprotective and anti-edema agent, and, based on observations arising from urban medicine, as an antilithic and a blood lipid lowering agent. The drug does not appear in the French Pharmacopoeia or on the list of plants eligible for the abridged application dossier for a French government marketing authorization or *dossier abrégé d'AMM*, but it is sometimes used in phytotherapy (as an infusion, nebulisate, or powder) to treat biliary secretion insufficiency, disorders of the lipoprotein metabolism, and to relieve the symptoms of venous insufficiency. Some cosmetic ingredient manufacturers present *Chrysanthellum* as an anti-aging product.

• Alfalfa, *Medicago sativa* L., Fabaceae

Alfalfa is better known as fodder than for its (potential) medicinal virtues. All of the organs of the main species, of the closely related species, and of their hybrids contain saponins, which are glycosides of soyasapogenols, and bi- and tridesmosides of acidic oleanenes (medicagenic and 16α-hydroxymedicagenic acid, hederagenin) esterified by an oligosaccharide at the C-28 carboxyl group and glycosylated at C-3. Alfalfa also contains phenolics (coumestrol) as well as L-canavanine, which is especially concentrated in the seeds (0.8-1.5% compared to 0.1% in the leaves). Like many other saponins, those of alfalfa lower blood cholesterol (in rabbits and monkeys); they are thought to enhance the fecal excretion of cholesterol (in mice). There are no data to confirm an activity supposedly beneficial for arthritis (on the contrary, arthritis pain may be a side effect of alfalfa ingestion). The drug is seldom used in France. The few clinical observations made after seed ingestion have limited relevance, therefore the therapeutic efficacy remains to be demonstrated.

Alfalfa use can induce or reactivate systemic lupus erythematosus-like manifestations: pancytopenia and arthritis pain, among others, have been observed in humans. These symptoms have an auto-immune origin and could be linked to L-canavanine. High doses may cause gastrointestinal symptoms.

On an industrial scale, alfalfa leaf juice is obtained by expression, then a protein concentrate is recovered by coagulation for use in chicken feed. Its high content in xanthophylls helps to color chicken flesh and eggs. Current research is in progress to produce "white" proteins that would have nutritional as well as functional benefits (emulsifiers).

Ultimately, the consumer must beware of products labeled "alfa" or "alfalfa". Alfa is *Stipa tenacissima* L. (Poaceae), a starting material in the paper industry, and in French, alfalfa is sometimes used to designate another Poaceae, *Phleum pratense* L.

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