Saponins. Classification, methods of analysis. Medicinal plants and raw materials containing saponins.

Saponins are glycoside compounds often referred to as a ‘natural detergent’ because of their foamy texture. They get their name from the soap wort plant (Saponaria), the root of which was used historically as a soap (Latin *sapo* – soap). Foremost among this is the strong tendency to froth formation when shaken with water.

The other properties are hemolytic activity, sneezing effect, toxicity, complex formation with cholesterol and antibiotic properties. Saponins have long been known to have strong biological activity. When studying the effect that saponins have on plants, it has been discovered that saponins are the plants active immune system.
They are found in many plants, they consist of a polycyclic aglycone that is either a choline steroid or tritetpenoid attached via C3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called sarsaponins. The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water soluble side chain.

Plant materials containing saponins have long been used in many parts of the world for their detergent properties. For example, in Europe the root of *Saponaria officinalis (Caryophyllaceae) and in South America* the bark of *Quillaja saponaria (Rosaceae). Such plants contain a high* percentage of glycosides known as saponins (Latin *sapo, soap) which* are characterized by their property of producing a frothing aqueous solution. They also have haemolytic properties, and when injected into the blood stream, are highly toxic.

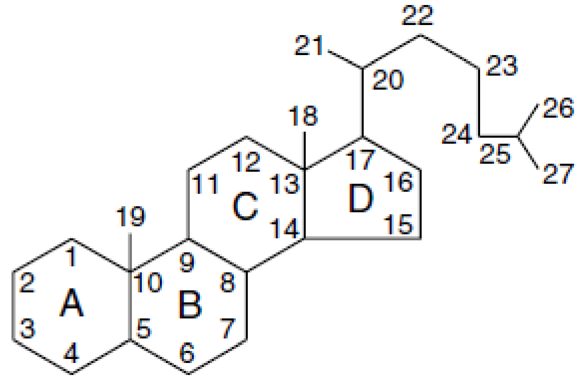
The fact that a plant contains haemolytic substances is not proof that it contains saponins, and in the species examined by Wall (1961) only about half of those containing haemolytic substances actually contained saponins. When taken by mouth, saponins are comparatively harmless. Sarsaparilla, for example, is rich in saponins but is widely used in the preparation of non-alcoholic beverages.

Saponins have a high molecular weight and a high polarity and their isolation in a state of purity presents some difficulties. Often they occur as complex mixtures with the components differing only slightly from one another in the nature of the sugars present, or in the structure of the aglycone. Various chromatographic techniques have been employed for their isolation. As glycosides they are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uronic acids.

According to the structure of the aglycone or sapogenin, two kinds of saponin are recognized—the steroidal (commonly tetracyclic triterpenoids) and the pentacyclic triterpenoid types (see formulae below).

Both of these have a glycosidal linkage at C-3 and have a common biogenetic origin via mevalonic acid and isoprenoid units.

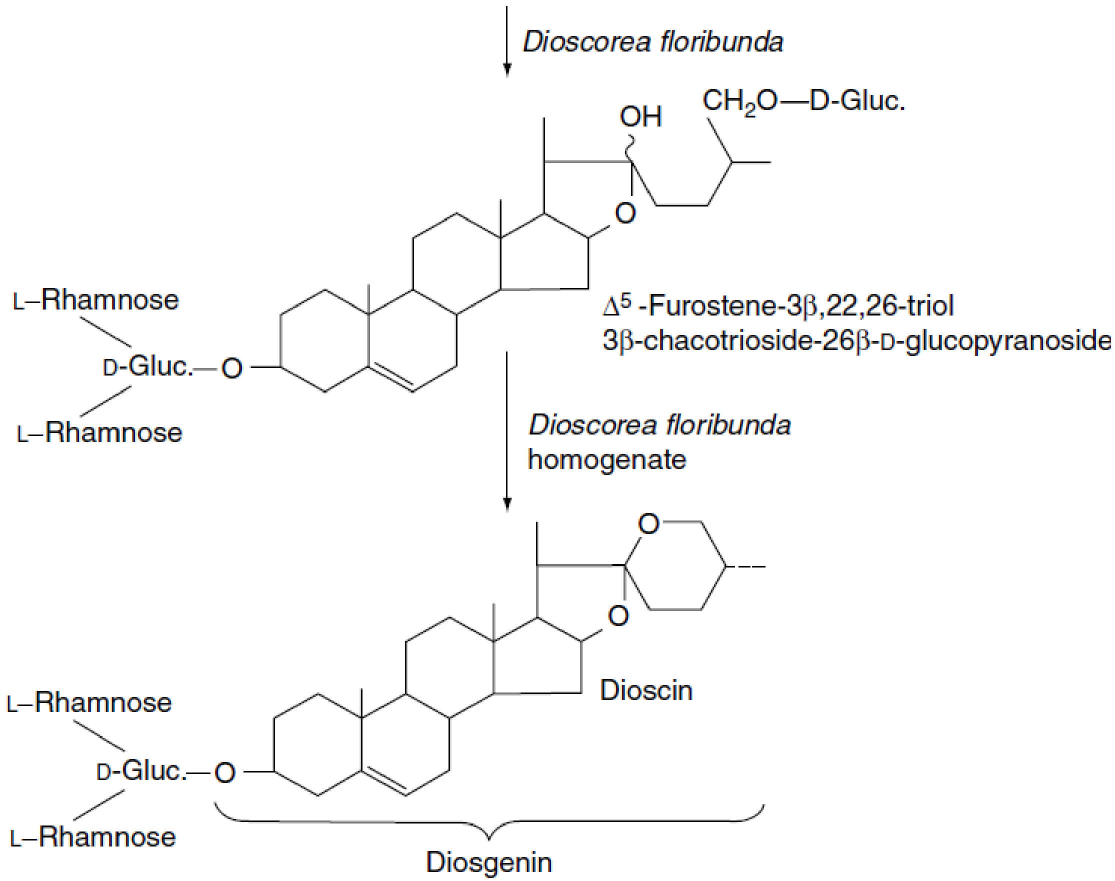
A distinct subgroup of the steroidal saponins is that of the steroidal alkaloids which characterize many members of the Solanaceae. They possess a heterocyclic nitrogen-containing ring, giving the compounds basic properties



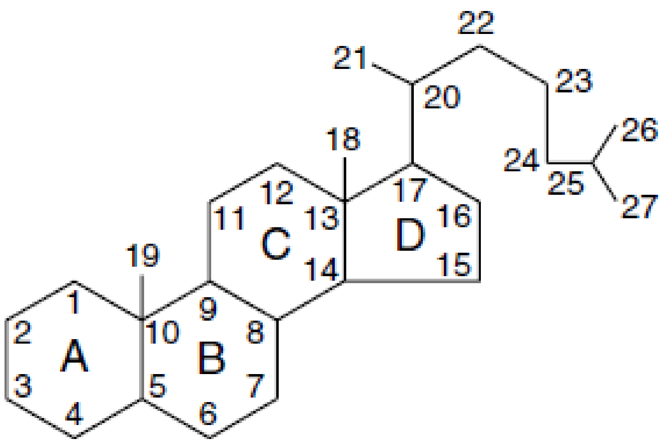
Steroid skeleton

The steroidal saponins are less widely distributed in nature than the pentacyclic triterpenoid type. Phytochemical surveys have shown their presence in many monocotyledonous families, particularly the Dioscoreaceae (e.g. *Dioscorea spp.), Agavaceae (e.g. Agave and Yucca spp.) and Smilacaceae (Smilax spp.). In the dicotyledons the* occurrence of diosgenin in fenugreek (Leguminosae) and of steroidal alkaloids in *Solanum (Solanaceae) is of potential importance. Some* species of *Strophanthus and Digitalis contain both steroidal saponins* and cardiac glycosides (q.v.).

Steroidal saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. Some are used as starting materials for the synthesis of these compounds. Diosgenin is the principal sapogenin used by industry but most yams, from which it is isolated, contain a mixture of sapogenins in the glycosidic form.



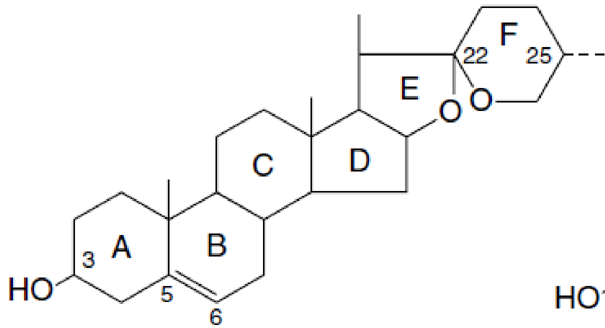
As with cardiac glycosides, the stereochemistry of the molecule is of some importance, although not so much so for cortisone manufacture. Natural sapogenins differ only in their configuration at carbon atoms 3, 5 and 25, and in the spirostane series the orientation at C-22 need not be specified (cf. steroidal alkaloids). Mixtures of the C-25 epimers—for example, diosgenin (Δ5,25α-spirosten-3β-ol) and yamogenin (Δ5,25β-spirosten-3β-ol)—are of normal occurrence and their ratio, one to the other, is dependent upon factors such as morphological part and stage of development
of the plant. In some instances in the plant, the side-chain which forms ring F of the sapogenin is kept open by glycoside formation as in the bisdesmosidic saponin sarsaparilloside of *Smilax aristolochiaefolia.*



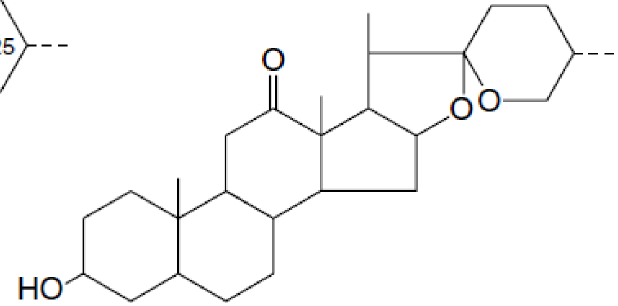
Steroid skeleton

Natural Steroids for the Production of Pharmaceuticals

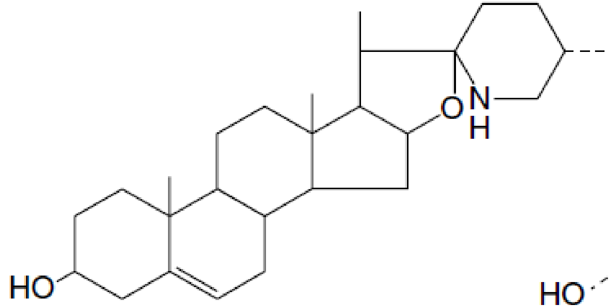
Although total synthesis of some medicinal steroids is employed commercially, there is also a great demand for natural products which will serve as starting materials for their partial synthesis.The range of steroids required medicinally, cortisone and its derivatives are 11-oxosteroids, whereas the sex hormones, including the oral contraceptives, and the diuretic steroids have no oxygen substitution in the C-ring. There are some of the more important natural derivatives which are available in sufficient quantity for synthetic purposes. Hecogenin with C-ring substitution provides a practical starting material for the synthesis of the corticosteroids, whereas diosgenin is suitable for the manufacture of oral contraceptives and the sex hormones. Diosgenin, however, can also be used for corticosteroid synthesis by the employment, at a suitable stage in the synthesis, of a microbiological fermentation to introduce oxygen into the 11α-position of the pregnene nucleus.



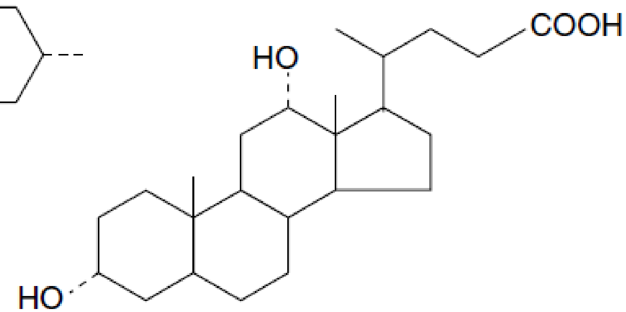
Diosgenin (Δ⁵ 25α-spirosten-3β-ol)
(various spp. of *Dioscorea*,
Fenugreek)



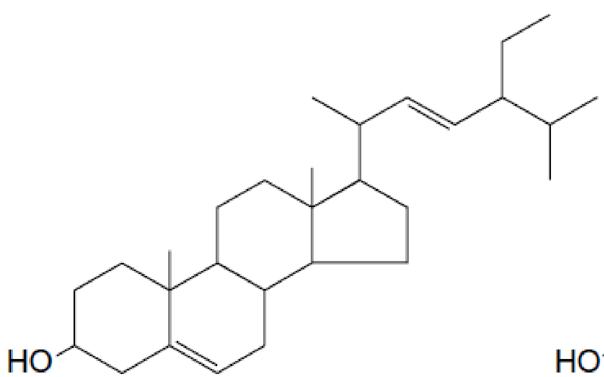
Hecogenin (*Sisal* spp.)



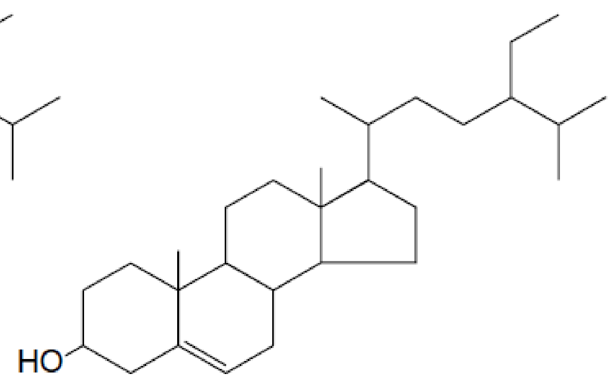
Solasodine (*Solanum* spp.)



Deoxycholic acid (ox-bile)



Stigmasterol (soya)

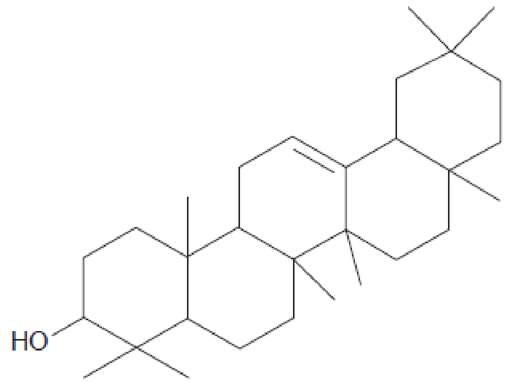


Sitosterol (soya)

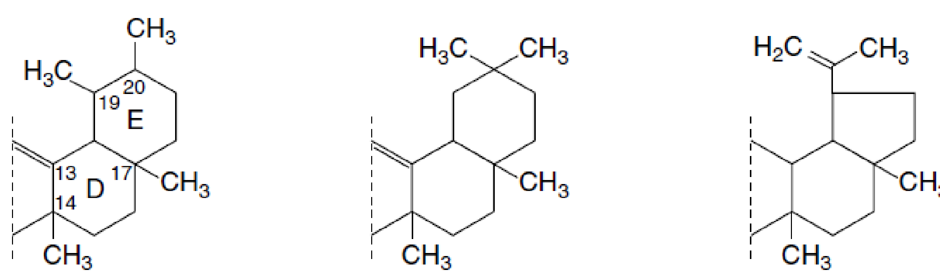
Unlike the steroidal saponins, the pentacyclic triterpenoid saponins are rare in monocotyledons. They are abundant in many dicotyledonous families, particularly the Caryophyllaceae, Sapindaceae, Polygalaceae and Sapotaceae. Among the many other dicotyledonous families in which they have been found are the Phytolaccaceae, Chenopodiaceae, Ranunculaceae, Berberidaceae, Papaveraceae, Linaceae, Zygophyllaceae, Rutaceae, Myrtaceae, Cucurbitaceae, Araliaceae, Umbelliferae, Primulaceae, Oleaceae, Lobeliaceae, Campanulaceae, Rubiaceae and Asteraceae. Altogether some 80 families are involved.

In these saponins the sapogenin is attached to a chain of sugar or uronic acid units, or both, often in the 3-position.

Triterpenoid saponins may be classified into three groups represented by α-amyrin, β-amyrin and lupeol. The related triterpenoid acids are formed from these by replacement of a methyl group by a carboxyl group in positions 4, 17 or 20



Pentacyclic triterpenoids



α-Amyrin β-Amyrin Lupeol

Plant materials often contain these saponins in considerable amounts. Thus, primula root contains about 5–10%; liquorice root about 2–12% of glycyrrhizic acid (and a correspondingly larger amount of glycyrrhizin, the potassium calcium salt); quillaia bark up to about 10% of the mixture known as ‘commercial saponin’; the seeds of the horsechestnut up to 13% of aescin. As some plants contain more than one saponin and purification is often difficult. Oleanolic acid also occurs as a saponin in sugar beet, thyme, *Guaiacum spp. (also in the nor-form), and in the free state in* olive leaves and clove buds.

Physical and chemical properties of saponins

Saponins are rarely crystalline and generally amorphous powder with high molecular weight. They carry many asymmetric centres and are optically active. They are generally soluble in water and form colloidal solutions. These are also soluble in ethyl and methyl alcohol and are usually insoluble in organic solvents like petroleum ether, chloroform and acetone etc. They are bitter in taste and nonalkaline in nature, produce sneezing and have the property of lowering surface tension. They are hydrolysed by acids, alkalies to yield aglycone called sapogenin and one or more molecule of same or different sugars or their oxidation products. They can also be hydrolysed by enzymes, soil bacteria, and by photolysis. In mild conditions using very dilute acids (0.01–0.1 N), organic acids give rise to partially hydrolysed saponins called prosapogenin.

Saponins are extremely toxic to fishes but do not render them inedible, as saponins are not poisonous to man when taken orally. Very dilute solution of saponins hemolyses red blood corpuscles. The hemolysis take place due to the formation of complex with the cholesterol of erythrocyte membrane causing its destruction, this is a chief property of saponin, very rarely shown by any other plants product.

Saponins accelerate the germination and growth of the seeds. Saponins show fungicidal, bactericidal activity, antiviral activity, antibiotic property, inflammation inhibition activity, spermicidal, antifertility, molluscicidal, etc.

Saponins have been reported to possess blood purifying and abortion causing properties, anthelmintic effect, sedative property and antispasmodic effects.

Saponin Isolation Methods

1. The plant material is extracted either with water or methanol, or aqueous methanol under reflux.

2. The extract is concentrated, followed by precipitation with:

- ether, or acetone;
- lead acetate in case of **acidic saponins;**
- or basic lead acetate in case of **neutral saponins** followed by decomposition with acid.

3. The individual saponin glycosides are separated by using different chromatographic methods.

Qualitative Tests for Saponin Glycosides

Qualitative reactions on saponins are divided into three groups:

1. Reactions, which are based on physical properties of saponins — Froth test.

2. Reactions, which are based on biological properties — Haemolysis test.

3. Reactions, which are based on chemical properties of saponins:

a) a reaction with 10% of basic and neutral acetate of lead; in the presence of triterpenoidal saponins residue with the neutral acetate of lead is formed and in the presence of steroidal ones — with the basic acetate of lead;

b) a reaction with 10% solution of copper sulphate, a residue of brick-red colour is formed;

c) a reaction with hydrate of barium oxide: a residue of white colour is formed;

d) a reaction with 1% alcohol cholesterol solution — a residue is formed.

To the colour reactions on saponins belong the following reactions:

a) a reaction of Liebermann-Burchard: a dry residue of the extract is dissolved in acetic acid and the mixture of acetic anhydride and concentrated sulphuric acid is added. After that the colouring changes from pink through green to blue;

b) a reaction with chlorohydrate and concentrated sulphuric acid; a yellow ring is formed, which becomes purple-red, then purple;

c) a reaction of Lafon (with concentrated sulphuric acid, ethyl alcohol and 10% solution of sulphuric acidic), bluish-green colouring is formed;

d) a reaction with 1% of sodium nitrite and concentrated sulphuric acid; blood-red colouring is formed.

For qualitative identification of saponins paper chromatography is used. TLC is also used.

Medicinal plants and raw materials containing saponins.

LIQUORICE ROOTS – GLYCYRRHIZAE RADICES

Liquorice consists of subterranean peeled and unpeeled stolons, roots and subterranean stems of *Glycyrrhiza glabra* Linn, and other species of *Glycytrhiza, belonging to family Fabaceae*.

Glycyrrhiza glabra var. typica Reg. et Herd., a plant about 1.5 m
high bearing typical papilionaceous flowers of a purplish-blue colour.
The underground portion consists of long roots and thin rhizomes or
stolons. The principal root divides just below the crown into several
branches which penetrate the soil to a depth of 1 m or more. A considerable
number of stolons are also given off, which attain a length
of 2 m but run nearer the surface than the roots. The plant is grown in
Spain, Italy, England, France, Germany and the USA.

History. Liquorice is referred to by Theophrastus.

The Roman writers referred to it as *Radix dulcis, but it does not appear to have been* cultivated in Italy until about the thirteenth century. Its cultivation in England, now commercially ceased, has been traced back as far as the sixteenth century.

Harvesting generally occurs in the autumn of the fourth year. The soil is carefully removed from the space between the rows to a depth of 2 or 3 feet as required, thus exposing the roots and rhizomes at the side, the whole being then removed bodily. The earth from the next space is then removed and thrown into the trench thus formed and these operations are repeated continuously. Every portion of the subterranean part of the plant is carefully saved; the drug consists of both runners and roots, the former constituting the major part.

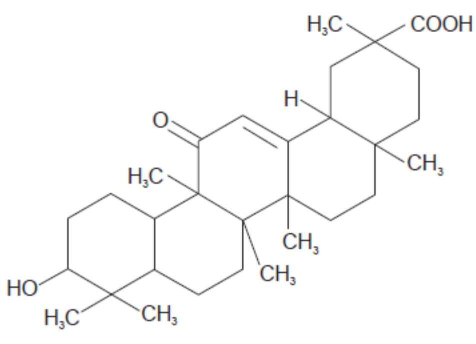
The roots are properly washed, trimmed and sorted, and either sold in their entire state or cut into shorter lengths and dried, in the latter case the cortical layer being sometimes removed by scraping. The older or ‘hard’ runners are sorted out and sold separately; the young, called ‘soft,’ are reserved for propagation.

Characteristics

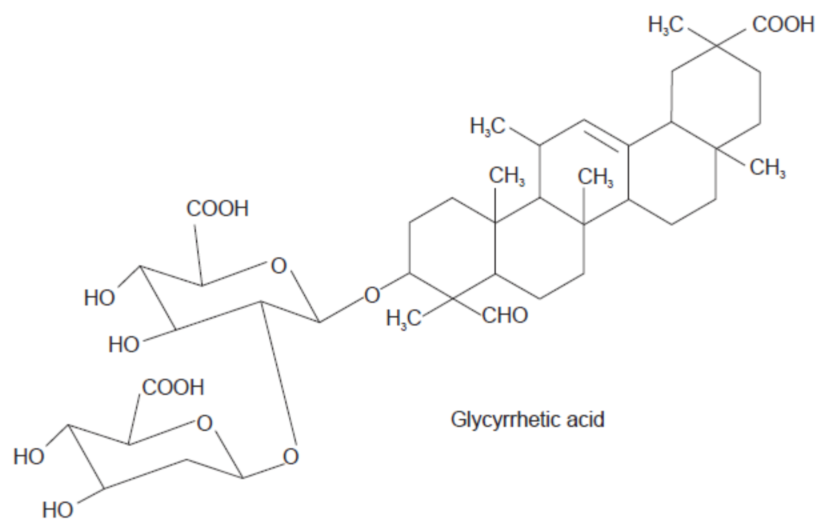
Liquorice root is in long, straight, nearly cylindrical, unpeeled pieces, several feet in length, varying in thickness from ¼ inch to about 1 inch, longitudinally wrinkled, externally greyish brown to dark brown, warty; internally tawny yellow; pliable, tough; texture coarsely fibrous; bark rather thick; wood porous, but dense, in narrow wedges; taste sweet, very slightly acrid. The underground stem which is often present has a similar appearance, but contains thin pith. When peeled, the pieces of root (including runners) are shorter, a pale yellow, slightly fibrous externally, and exhibit no trace of the small dark buds seen on the unpeeled runners here and there. Otherwise it resembles the unpeeled.

Chemical Constituents

The chief constituent of liquorice root is Glycyrrhizin (6–8%), obtainable in the form of a sweet, which is 50 times sweeter than sucrose, white crystalline powder, consisting of the calcium and potassium salts of glycynhizic acid. Glycyrrhizic acid on hydrolysis yields glycyrrhetic or glycyrrhetinic acid.
Glycyrrhizinic acid is a triterpenoid saponin having α-amyrine structure. It shows especially in alkaline solution frothing but it has very weak haemolytic property.
The yellow colour of the drug is due to chalcone glycoside isoliquiritin. The drug also contains sugar, starch (29%), gum, protein, fat (0.8%), resin, asparagin (2–4%), a trace of tannin in the outer bark of the root, yellow colouring matter, and 0.03% of volatile oil.



Glycyrrhetinic acid



Glycyrrhetic acid

Uses

Glycyrrhiza is widely used as a sweetening agent and in bronchial problems such as catarrah, bronchitis, cold, flu and coughs. It reduces irritation of the throat and yet has an expectorant action. It produces its demulcent and expectorant effects. It is used in relieving stress. It is a potent healing agent for tuberculosis, where its effects have been compared to hydrocortisone. Glycyrrhiza is also effective in helping to reduce fevers (glycyrretinic acid has an effect like aspirin), and it may have an antibacterial action as well. It is used in the treatment of chronic inflammations such as arthritis and rheumatic diseases, chronic skin conditions, and autoimmune diseases in general. It should be used in moderation and should not be prescribed for pregnant women or people with high blood pressure, kidney disease or taking digoxin-based medication. Prolonged usage raises the blood pressure and causes water retention. Externally, the root is used in the treatment of herpes, eczema and shingles.

CALTROPS HERB – TRIBULI TERRESTRIS HERBA

Medicinal raw material consists the dried herb of *Tribulus terrestris Linn., belonging to* family Zygophyllaceae.

Tribulus terrestris is an annual plant widely distributed around the world. It has taproot. The stems radiate from the crown to a diameter of about 10 cm to over 1 m, often branching. They are usually prostrate, forming flat patches, though they may grow more upwards in shade or among taller plants. Stems branch from the crown and are densely hairy. Leaves are opposite and pinnately compound. Densely hairy leaflets are opposite and up to 3 mm long.

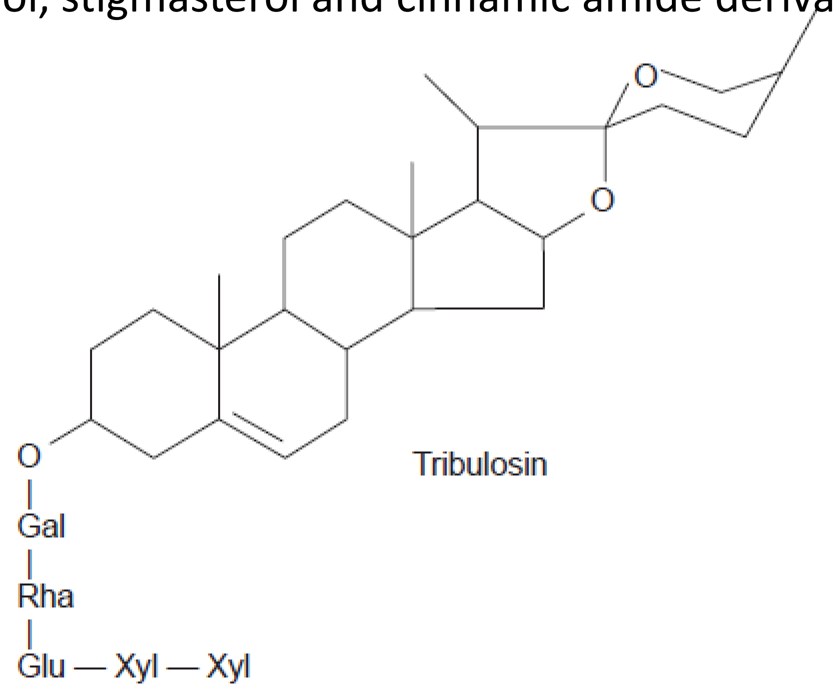
The flowers are 4–10 mm (0.16–0.39 in) wide, with five lemon-yellow petals, five sepals, and ten stamens. In Southern California, it blooms from April through October, where it is highly invasive in waste places and disturbed sites.

After the flower blooms, a fruit develops that easily falls apart into five burs. The burs are hard and bear two to four sharp spines, 10 mm in long and 4–6 mm in broad point-to-point. These burs strikingly resemble goats' or bulls' heads, characteristics which give the bur its common names in some regions. The "horns" are sharp enough to puncture bicycle tires and other air-filled tires.

Chemical Constituents

The dried herb of *T. terstris consist of steroidal saponins as* the major constituents. It includes terestrosins A, B, C, D and E, desgalactotigonin, F-gitonin, esglucolanatigonin and gitonin. The hydrolysed extract consists of sapogenins such as diosgenin, chlorogenin, hecogenin and neotigogenin.

Certain other steroidal such as terestroside F, tribulosin, trillin, gracillin, dioscin have also been isolated from the aerial parts of the herb. The flavonoid derivatives reported from the fruits includes tribuloside and number of other glycosides of quercetin, kaempferol and isorhamnetin. It also consists of common phytosterols, such as, β-sitosterol, stigmasterol and cinnamic amide derivative, terestiamide.



Tribulosin

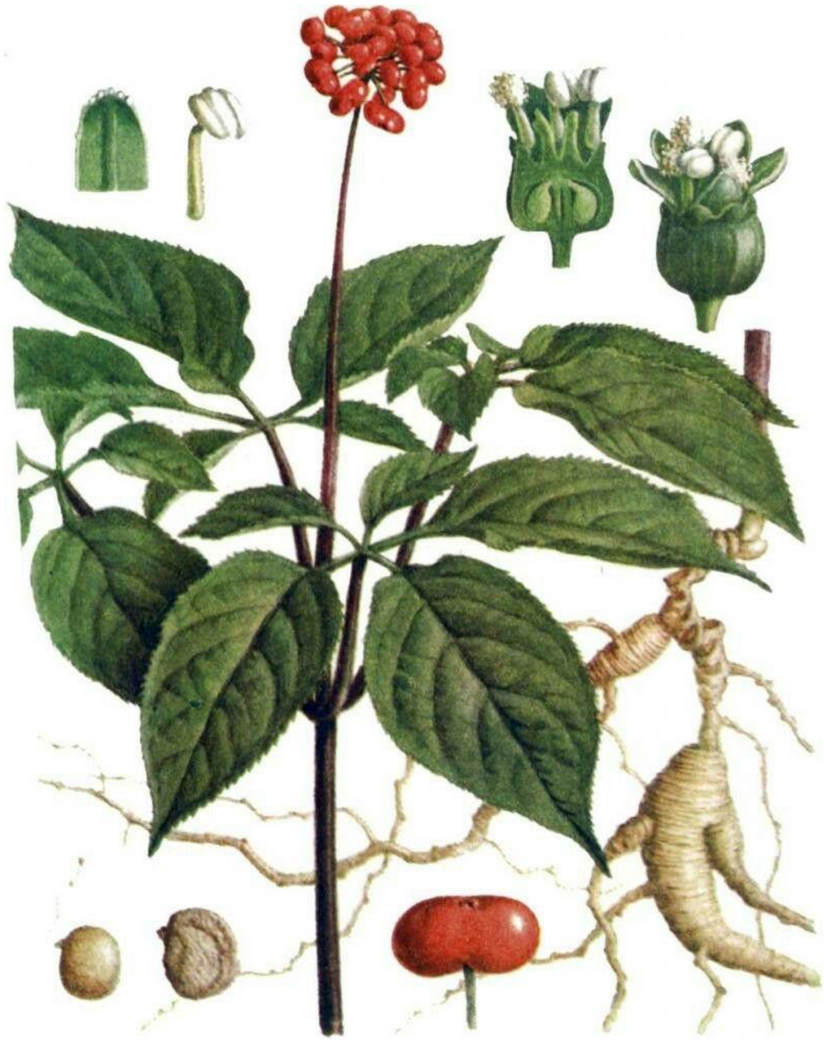
Uses

The medicinal raw material has cooling, antiinflammatory, antiarthritic, diuretic, tonic, aphrodisiac properties. It is used in building immune system, in painful micturition, calculus affections and impotency.

GINSENG ROOTS - PANAX GINSENG RADICES

It consists of dried roots of *Panax ginseng* C.A. Mey and other species of Panax like *Panax japonicus* (Japanese Ginseng), *Panax pseudoginseng* (Himalayan Ginseng), *Panax quinquefolius* (American Ginseng), *Panax trifolius* (Dwarf Ginseng) and *Panax vietnamensis* (Vietnamese Ginseng), belonging to family Araliaceae.

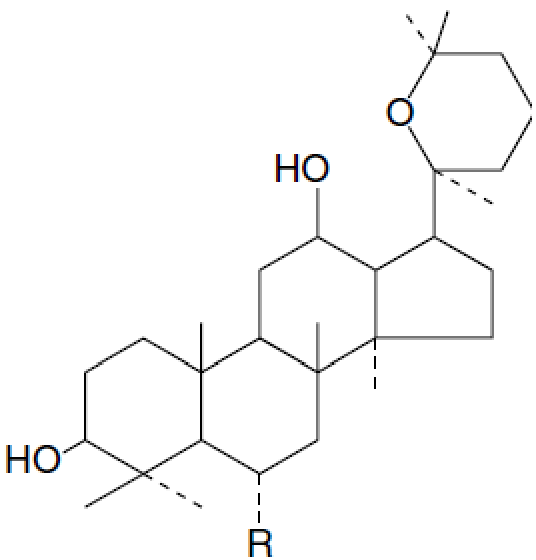
For some 2000 years the roots of *Panax ginseng* C. A. Meyer (Araliaceae) have held an honoured place in Chinese medicine. Today it is a product of world-wide usage. Production is principally confined to China, Korea and Siberia, although it is cultivated commercially on a small scale in Holland, England, Germany and France (Champagne district).



Panax ginseng is a herbaceous perennial growing from 30 to 60 cm tall. Plants have a spindle or cylinder-shaped taproot usually with 1 or 2 main branches. Plants produce 3 to 6 leaves that are palmately compound with each leaf having 3 to 5 leaflets. The margins of the leaflets are densely serrulate. The flowers are born in a solitary inflorescence that is a terminal umbel with 30 to 50 flowers. The peduncles of the flowers are 15 to 30 cm long. The flower ovary is 2-carpellate and each carpel has two distinct styles. Mature fruits are 4-5 x 6-7 millimeters in size, red in color, and round with flattened ends. The white seeds are kidney-shaped.

Chemical Constituents

Several saponin glycosides belonging to triterpenoid group, ginsenoside, chikusetsusaponin, panxoside. More than 13 ginsenosides have been identified. Ginsenosides consists of aglycone dammarol where as panaxosides have oleanolic acid as aglycone. It also contains large amount of starch, gum, some resin and a very small amount of volatile oil.



Panaxadiol, R = H
Panaxatriol, R = OH

Uses

The root is adaptogen, alterative, carminative, demulcent, emetic, expectorant, stimulant and tonic. The saponin glycosides, also known as ginsenosides or Panaxosides, are thought responsible for Panax ginseng's effects. Ginsenosides have both stimulatory and inhibitory effects on the CNS, alter cardiovascular tone, increase humoral and cellular-dependent immunity, and may inhibit the growth of cancer in vitro. It encourages the secretion of hormones, improves stamina, lowers blood sugar and cholesterol levels. It is used internally in the treatment of debility associated with old age or illness, lack of appetite, insomnia, stress, shock and chronic illness. Ginseng is not normally prescribed for pregnant women, or for patients under the age of 40, or those with depression, acute anxiety or acute inflammatory disease. It is normally only taken for a period of 3 weeks. Excess can cause headaches, restlessness, raised blood pressure and other side effects, especially if it is taken with caffeine, alcohol, turnips and bitter or spicy foods.


Thank you
for
your attention