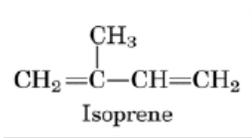


Terpenes and their Derivatives:

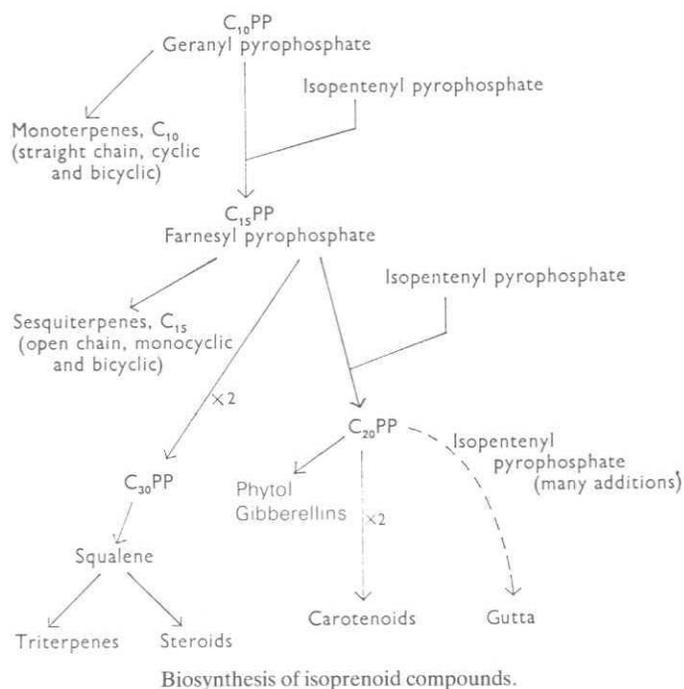
The terpenes have been long associated with the term Essential Oils comprising resins, steroids and rubber. In fact, they are hydrocarbons that usually contain one or more C=C double bonds, while the terpenoids are oxygen-containing analogues of the terpenes. They are thoroughly distributed in the plant kingdom, especially in those plants that have abundant chlorophyll.

Among these are compounds which fall in the general class of terpenes, compounds made up of 5-carbon units, often called isoprene units, put together in a regular pattern, usually head-to-tail in terpenes up to 25 carbons.



Terpenes containing 30 carbons or more are usually formed by the fusion of two smaller terpene precursors such that the head-to-tail "rule" appears to be violated. In overall, terpenes hold potential interest practical applications especially in the fragrance and flavour industries, as well as in the pharmaceutical and chemical industries.

Biosynthesis of Terpenes:



Preliminary stages in the biosynthesis of isoprenoid compounds

Classification of Terpenes:

- Hemiterpenes C_5H_8
- Monoterpenes $\text{C}_{10}\text{H}_{16}$
- Sesquiterpenes $\text{C}_{15}\text{H}_{24}$
- Diterpenes $\text{C}_{20}\text{H}_{32}$
- Sesterterpenes $\text{C}_{25}\text{H}_{40}$

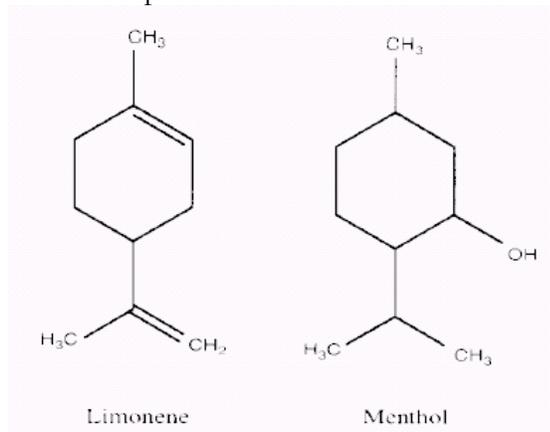
- Triterpenes $C_{30}H_{48}$
- Tetraterpenes $C_{40}H_{64}$
- Polyterpenes $(C_5H_8)_n$

- **Hemiterpenes $[C_5H_8]$:**

Hemiterpenes do not appear to accumulate in plant tissues, but are instead found to be associated with other compounds like alkaloids, coumarins and flavonoids.

- **Monoterpenes $[C_{10}H_{16}]$:**

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C_{10}). But, some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids.



The branched-chain C_{10} hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally occurring monoterpenes identified. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – monocyclic; Camphor – bicyclic; Pinenes (α and β) – Pine genera) as well. Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium* (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses.

Essential Oils:

Monoterpenes together with sesquiterpenes and diterpenes form the majority of **essential oils** and are distributed in more than 2, 000 plant species belonging to some 60 families (*Rutaceae*; *Myrtaceae*; *Umbelliferae*; *Labiatae*; *Compositae*; *Pinaceae*).

They are distributed in all plant parts: flowers (Bergamot); leaves (Citronella, Laurel); roots (Vetiver); rhizome (Ginger); wood (Sandalwood); bark (Cinnamon tree); fruit and seeds (Nutmeg). These essential oils accumulate in cell cytosol, vacuoles of epidermal cells, mesophyll cells of petals, and glandular cells.

Like all secondary metabolites, the essential oils are known to have several important functions *viz*: Protection against predators (Microorganisms; Fungi; Insects; Herbivores); Attraction of pollinators and dispersal of diaspores; Inhibitors of germination and growth, etc.

Pharmacological Properties of Essential Oils:

1. Antiseptics

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antibio-resistant strains. Moreover, they are also known to be active against fungi and yeasts (*Candida*).

The most common sources of essential oils used as antiseptics are: Cinnamon, Thyme; Clover; Eucalyptus; *Culin* savory; Lavender. Citral, geraniol, linalol and thymol are 5.2, 7.1, 5 and 20 times more potent than phenol.

2. Expectorants and diuretics

When used externally, essential oils like (L'essence de térébenthine) increase microcirculation and provide a slight local anaesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains.

Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect.

3. Spasmolytic and sedative

Essential oils from the *Umbellifereae* family, *Mentha* species and verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia.

4. Others

Cholagogue; anti-inflammatory; cicatrising...

Derivatives of Monoterpenes:

1. Iridoids

The iridoids are cyclopentan-*[c]*-pyran monoterpenoids and constitute a group of which the number of known members is constantly increasing (El-Naggar and Beal list 280 iridoids).

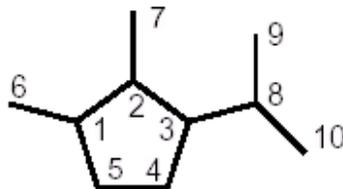


Figure 1: Iridane skeleton (1-isopropyl-2, 3-di-methylcyclopentane)

The name derives from *Iridomyrmex*, a genus of ants that produces these compounds as a defensive secretion. Most occur as glycosides (Aucubin – a common iridoid-glycoside); some occur free and as bis compounds. There are many seco-iridoids in which the pyran ring is open (Gentiopicroside – a seco-iridoid of gentian) and in a few the pyran ring oxygen is replaced by nitrogen (Actinidine – a simple monoterpene alkaloid).

2. Tetrahydrocannabinol (*Cannabis sativa*)

Tetrahydrocannabinol has psychotropic properties.

LOR OBJECT 259 – Marijuana (Cannabis) as Medicine

Principal Essential Oil Producing Plants:

1. *Mentha × piperita* L. (Fam. Labiatae)

Plants in the mint family have been used for medicinal purposes since before 2000 BC, but menthol was not isolated until 1771. Menthol, a monoterpene isolated from various mints, is a topical pain reliever and antipuretic (relieves itching).

Oil of Peppermint:

Peppermint leaf is the dried leaves of *Mentha × piperita* L and contains less than 1.2% of volatile oil. The latter is obtained by steam distillation of flowering tops. The official oil is required to contain from 4.5 – 10% of esters calculated as methyl acetate, not less than 44% of free alcohols calculated as menthol and 15 – 32% of ketones calculated as menthone. Variations in the different components can affect the quality of the oil and gas chromatography can be used to analyse the different components (30 – 40 peaks) and hence quality of the oil.

Japanese peppermint oil is derived from *Mentha canadensis* var. *piperascens* and it contains 70 – 90% menthol, for the extraction of which it is largely used.

Dried peppermint leaf is used in herb teas, either on its own or in mixtures. Peppermint tea is regarded as a stimulant, a cure for flatulence and has antiseptic properties. It is used in treating indigestion, sore throats, colds and toothache, among others.

Oil of Spearmint:

Spearmint or ordinary garden mint consists of the leaf and flower top of *Mentha spicata* L. (*M. viridis* Linn.) and *Mentha × cardiaca*. The oil is prepared by steam distillation and contains less than 55% of carvone.

Oil of spearmint contains (-)-carvone, (-)-limonene, phellandrene and esters. S- (+)-carvone (caraway-like flavour) and R- (-)-carvone (spearmint-like flavour) are important aroma compounds for use in foods and beverages. As with peppermint, oil production is influenced by age of plant, time of collection, chemical varieties and hybridisation.

Spearmint is commonly used both fresh and dried as a culinary herb and is widely appreciated as a jelly or sauce with certain meats, potato and peas.

Mentha arvensis:

Mentha arvensis has a sweet aroma very similar to spearmint. It is reputed to be rust resistant, and is therefore chosen in preference to *M. spicata* for Crop & Food Research trials.

2. Clove Oil

Cloves are the dried flower buds of *Syzygium aromaticum*. They have been used as a stimulant aromatic, as a spice and for the preparation of volatile oil for a long time. Cloves are cultivated in Zanzibar and in the neighbouring island of Pemba. But the industry has now deteriorated and the principal producers are now Madagascar, Brazil and Penang. Smaller quantities are grown in Malacca, Seychelles, Mauritius and the West Indies. Cloves contain about 14 – 21% of volatile oil, 10 – 13% of tannin, various triterpene acids and esters and the glucosides of sitosterol, stigmasterol and campesterol.

Clove oil contains 84 – 95% of phenols (eugenol; ~ 0.3% acetyleneol), sesquiterpenes (α - and β -caryphyllenes), and small quantities of esters, ketones and alcohols. Medicinal oil has a phenol content of about 85 – 95%. The oils that have a relatively low phenol content are known as 'opt' and are the ones mainly used in pharmacy, while the

'strong' oils are used in the manufacture of vanillin. Oil of clove is used as a flavouring agent, stimulant, aromatic and antiseptic.

Clove stem oil is produced in Zanzibar and in Madagascar. It is used mainly in the flavouring and perfumery industries. Clove leaf oil is distilled in Madagascar and in Indonesia and is used for the isolation of eugenol.

3. Eucalyptus Oil

The genus *Eucalyptus* is a very large one, but only a certain number of species produce oil suitable for medicinal use. Sustainable oils are derived from *Eucalyptus polybractea*, *Eucalyptus smithii*, *Eucalyptus australiana* and *Eucalyptus globulus*. In the case of the latter, the oil is used in pharmacy, dentistry and against respiratory disorders. 'Citron-scented' eucalyptus oil, derived from *Eucalyptus citriodora* is used in perfumery and contains a high proportion of the aldehyde citronellal. Moreover, Griffin *et al.* ascribe the antimicrobial action of this oil to the synergistic action of citronellal and citronellol in the ratio 90: 7.5.

Oil of eucalyptus is a colourless or pale yellow liquid. It has an aromatic and camphoraceous odour; a pungent, camphoraceous taste, which is followed by a sensation of cold. It is required to contain not less than 70% of cineole. 1, 8-Cineole and *o*-cresol form a solid complex and the crystallising temperature of this form the basis for the official assay of the oil.

Essential Oils used in Industrial Applications:

| Name | Botanical name | Important constituents |
|----------------------------|---------------------------------------|---|
| Turpentine | <i>Pinus</i> spp. | Terpenes (pinenes, camphene) |
| Juniper | <i>Juniperus communis</i> | Terpenes (pinene, camphene); sesquiterpene (cardinene); alcohols |
| Cade (Juniper Tar Oil) | <i>Juniperus oxycedrus</i> | Sesquiterpene (cardinene); phenols (guaiacol, cresol) |
| Coriander | <i>Coriandrum sativum</i> | Linalool (65 – 80% alcohols); Terpenes |
| Otto of rose | <i>Rosa</i> spp. | Geraniol, citronellol (70 – 75% alcohols); esters |
| Geranium | <i>Pelargonium</i> spp. | Geraniol; citronellol; esters |
| Indian or Turkish geranium | <i>Cymbopogon</i> spp. | Geraniol (85 – 90%) |
| Sandalwood | <i>Santalum album</i> | Santanols (sesquiterpene alcohols); esters; aldehyde |
| English lavender | <i>Lavandula intermedia</i> | Linalool; linalyl acetate (little); cineole, etc. |
| Continental lavender | <i>Lavandula officinalis</i> | Linalool; linalyl acetate (much); ethyl pentyl ketone |
| Rosemary | <i>Rosmarinus officianlis</i> | Borneol and linalool (10 – 18%); bornyl acetate, etc. (2 – 5%); Terpenes; cineole |
| Pumilio pine | <i>Pinus mugo</i> var. <i>pumilio</i> | Bornyl acetate (~ 10%); Terpenes; sesquiterpenes |
| Peppermint | <i>Mentha piperita</i> | Menthol (~45%); methyl acetate (4 – 9%) |
| Cinnamon bark | <i>Cinnamomum verum</i> Presl. | Cinnamic aldehyde (60 – 75%); eugenol; Terpenes |
| Cassia | <i>Cinnamomum cassia</i> | Cinnamic aldehyde (80%) |
| Lemon | <i>Citrus limon</i> | Citral (over 3.5%); limonene (~90%) |

| | | |
|-----------------------------|---|--|
| Lemon grass | <i>Cymbopogon</i> spp. | Citral & citronellal (75 – 85%); Terpenes |
| 'Citron-scented' eucalyptus | <i>Eucalyptus citriodora</i> | Citronellal (~70%) |
| Spearmint | <i>Mentha spicata</i> and <i>M. cardiaca</i> | Carvone (55 – 70%); limonene; esters |
| Caraway | <i>Carum carvi</i> | Carvone (60%); limonene; etc. |
| Dill | <i>Anethum graveolens</i> | Carvone (50%); limonene; etc. |
| Cinnamon leaf | <i>Cinnamomum verum</i> Presl. | Eugenol (up to 80%) |
| Clove | <i>Syzygium aromaticum</i> (L.) Merr & L.M. Perry | Eugenol (up to 85 – 90%); acetyl eugenol; methylpentyl ketone; vanillin |
| Thyme | <i>Thymus vulgaris</i> | Thymol (20 – 30%) |
| Horsemint | <i>Monarda punctata</i> | Thymol (~60%) |
| Ajowan | <i>Trachyspermum ammi</i> | Thymol (4 – 55%) |
| Anise and star-anise | <i>Pimpinella anisum</i> and <i>Illicium verum</i> | Anethole (80 – 90%); chavicol methyl ether; etc. |
| Fennel | <i>Foeniculum vulgare</i> | Anethole (60%); fenchone; a ketone (~20%) |
| Eucalyptus | <i>Eucalyptus globulus</i> | Cineole (over 70%); Terpenes; etc. |
| Cajuput | <i>Melaleuca</i> spp. | Cineole (50 – 60%); Terpenes; alcohols and esters |
| Camphor | <i>Cinnamomum camphora</i> | After removal of the ketone camphor contains safrole; Terpenes; etc. |
| Parsley | <i>Petroselinum sativum</i> | Apiole (dimethoxysafrole) |
| Indian dill | <i>Peucedanum soja</i> | Dill-apiole (dimethoxysafrole) |
| Nutmeg | <i>Myristica fragrans</i> | Myristicin (methoxysafrole) up to 4%; Terpenes (60 – 85%); alcohols; phenols |
| Chenopodium | <i>Chenopodium ambrosioides</i> var. <i>anthelmintica</i> | Ascaridole (60 – 77%), an unsaturated terpene peroxide |
| Mustard | <i>Brassica</i> spp. | |
| Wintergreen | <i>Gaultheria procumbens</i> | Methyl salicylate |
| Bitter almond | <i>Prunus communis</i> var. <i>amara</i> | Benzaldehyde & HCN |

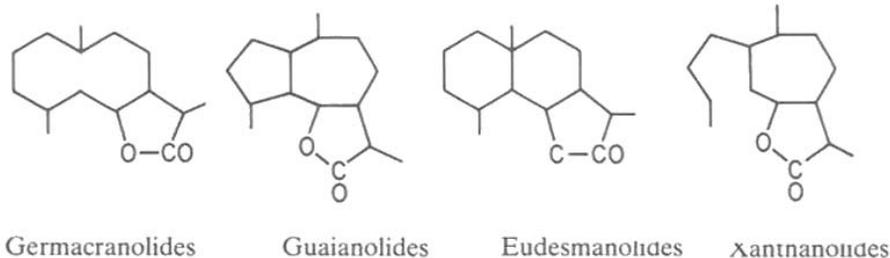
- **Sesquiterpenes [C₁₅H₂₄]:**

Sesquiterpenes are biogenetically derived from farnesyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury.

- **Sesquiterpene Lactones:**

Over 500 compounds of this group are known; they are particularly characteristics of the *Compositae* but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many anti-tumor, anti-leukaemic, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents.

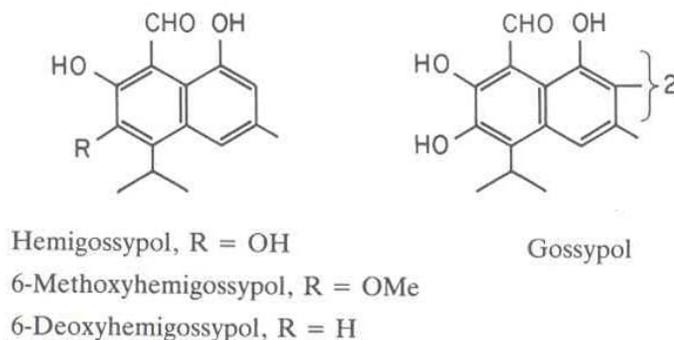
Chemically the compounds can be classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc.



A structural feature of all these compounds, which appears to be associated with much of the biological activity, is the α,β -unsaturated- γ -lactone.

Gossypol:

Hemigossypol and related aldehydes together with the dimeric gossypol are sesquiterpenes stress compounds found in the subepidermal glands, immature flower buds and seed kernels of the cotton plant (*Gossypium* spp.).



Besides having insecticidal properties, gossypol is of considerable pharmaceutical interest in that in humans, it functions as a male anti-infertility agent. In China, gossypol has been used to treat infertility in males and studies have shown an increase in sperm production.

- **Diterpenes [C₂₀H₃₂]:**

Diterpenes occur in all plant families and consist of compounds having a C₂₀ skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a sidechain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate (GGPP).

Acyclic diterpenes, other than phytol, are not commonly accumulated, although small amounts do occur in a number of plants. Some of these compounds are kairomones and pheromonal substances for insects. Macrocyclic diterpenes are commonly isolated from gymnosperms. Examples are cembrene and its relatives. Among plants, the taxanes occur only in the gymnospermous family *Taxaceae*. Furthermore, diterpenes are also the major constituents of resins (Primaric acid from *Pinus* species).

Complex derivatives of macrocyclic precursors, the co-carcinogenic diterpenes, occur in two plant families, the *Euphorbiaceae* and the *Thymelaeaceae*. Many of these compounds are extremely caustic. They are not carcinogenic, but when an animal is exposed to co-carcinogens and later to carcinogens, the activity of the latter is enhanced. The caustic properties and co-carcinogenicity are not directly linked.

Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) as well as in antispasmodics and antioxiolytics.

Diterpenic Plants used in therapeutical fields:

- ***Grindelia (Grindelia robusta Nutt.):***

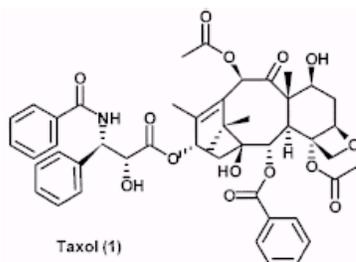
Grindelia contains 10 to 20% of active principles that are used in the formulation of expectorants; cough sedatives and antispasmodics.

Diterpenic Plants of potential interest:

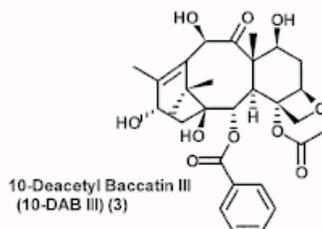
- ***(Taxus baccata L.):***

Taxol:

One of the most well known medicinally valuable terpenes is the diterpene, taxol. Taxol was first isolated from the bark of the Pacific yew, *Taxus brevifolia*, in the early 1960's, but it was not until the late 1980's that its value as an anticancer drug was determined. It acts to stabilize the mitotic apparatus in cells, causing them to act as normal cells rather than undergo rapid proliferation as they do in cancer.

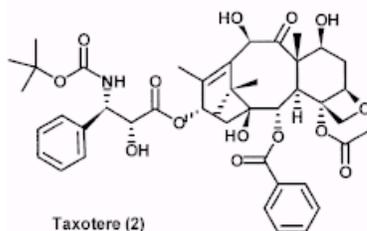


There has been a great deal of interest in taxol from the standpoint of understanding what pieces of the compound are required for activity. These structure-activity relationships have been determined by isolating or synthesizing homologues and derivatives of taxol and testing these compounds for their anti-cancer activity in various cell culture and *in vivo* screens. If the side chain ester was removed to give baccatin III, activity was lost. If different N-acyl groups were present on the side chain, activity was maintained. If the 2' - OH on the side chain was converted to another group, activity was reduced unless the group could be hydrolyzed *in vivo*. The stereochemistry at C - 7 has no effect on the activity, nor is C - 10 acylation critical. The oxetane ring must be present.



Since one of the major problems with taxol, as with many other natural products used as drugs, is solubility in aqueous systems, much effort has been devoted to finding a

more soluble form. Taxotère (docetaxel) was prepared by the French firm, Rhone-Poulenc, and has similar activity to taxol (paclitaxel), but slightly better solubility.



Other researchers have tried to add a group to the 2' – OH, which would increase the solubility and then be removed *in vivo*. These are mostly esters with a salt incorporated to increase the water solubility. Other investigators have modified the C – 13 side chain ester and added functionality at C-14 to increase the oral bioavailability. These modified taxoids are still at early stages of testing, but illustrate the principles of development of second-generation drugs.

LOR OBJECT 242 – Taxol: biosynthesis, molecular genetics and biotechnological applications

LOR OBJECT 260 – Bringing Science and Technology to bear high-value plants: Case study for Natural Product Production

LOR OBJECT 261 – Microbial gifts from rain forests

LOR OBJECT 266 – Beneficial uses of plant pathogens: anticancer and drug agents derived from plant pathogens

- ***(Coleus forskohlii* Brig.):**

Forskolin / Coleonol:

The genus *Coleus* has been long used in Ayurvedic medicine to treat various cardiac and respiratory diseases.



Chemical and pharmacological studies on *Coleus forskohlii* have shown that the active principle is forskolin. This compound is extensively used in treating hypertension as it has proved to be a good vasodilator and it is known to inhibit platelet aggregation as well.

Furthermore, studies have shown that this botanical bears promise as a safe and effective weight loss regimen. The effect of *Coleus forskohlii* is particularly valid in the absence of change in frequency and intensity of physical exercise and without diet restrictions during the course of the trial.

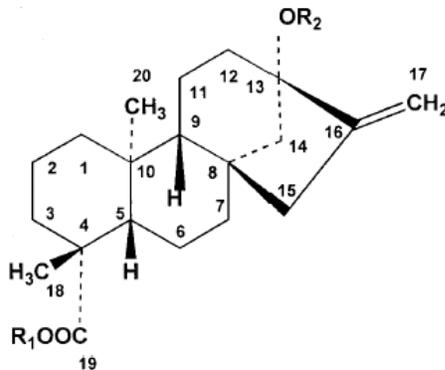
LOR OBJECT 264 – Diterpene Forskolin (*Coleus forskohlii*): A possible new compound for reduction of body weight

LOR OBJECT 268 – Passive cutaneous anaphylactic inhibitory and mast cell stabilising activity of coleonol

- ***(Stevia rebaudiana Bertonii):***

The sweetening properties of *Stevia rebaudiana* have been known for centuries, especially in its native land of Paraguay. The dried leaves of *Stevia rebaudiana* contain about 42 per cent water-soluble constituents. The sweet compounds represent about 14 per cent constituents of dried leaves and are diterpene glycosides based on the kaurene skeleton.

These are mainly comprised of stevioside; steviobioside; rebaudioside A, B, D, E; dulcoside A; and dulcoside B [1]. These *Stevia* sweeteners are similar in structure in that a steviol aglycone is connected at C-4 and C-13 to mono-, di- or trisaccharides consisting of glucose and/or rhamnose residues, as shown in the figure below.



The most abundant glycoside is stevioside followed by rebaudioside. The rest of the sweet components are present in much smaller amounts. Sweetening potencies of various diterpenes found in *Stevia rebaudiana* are listed in the table below.

Sweetening potencies of *Stevia rebaudiana* diterpene glycosides

| Diterpene | R ₁ | R ₂ | Sweetening potency |
|------------------------------|---------------------|--------------------|--------------------|
| Stevioside | G ¹ (1)* | G (2) | 250 – 300 |
| Rebaudioside A | G (1) | G (3) | 350 – 450 |
| Rebaudioside B | H | G (2) | 300 – 350 |
| Rebaudioside C (Dulcoside B) | G (1) | R ₂ (1) | 50 – 120 |
| Rebaudioside D | G (2) | G (2) | 200 – 300 |
| Rebaudioside E | G (2) | G (2) | 250 – 300 |
| Dulcoside A | H | G (2) R (1) | 50 – 120 |
| Stevioside | H | G (2) | 100 – 125 |

G = Glucosyl; R₂ = Rhamnosyl; * Number of G or R groups

The non-sweet constituents mainly are labdane diterpenes, triterpenes, sterols and flavonoids.

Stevia sweetener is a low calorie, heat stable, intensely sweet (300 times sweeter than sucrose at 0.4% concentration), proven safe by years of use in Japan, and is approved in Europe. The consumption of steviol glycosides in Japan has accounted for more than 20 per cent of low calorie sweeteners. Interest in this low calorie sweetener was renewed in North America during the 1990s because of export opportunities and positive nutritional properties. *Stevia*-based sweeteners are approved for sale in Japan, China, Taiwan, South Korea, Vietnam, Thailand, and a number of countries in South America. According to an extensive marketing study commissioned by several independent companies, the potential market for *Stevia* sweeteners in the six Asian countries listed above would be in excess of

\$1.2 billion per year by the year 2005. This assumes a modest 4 per cent to 8 per cent market penetration in different countries.

Unlike aspartame, Stevia sweeteners are heat stable to 200 ° C, are acid stable and do not ferment, making them suitable for use in a wide range of products including baked/cooked foods. In some food uses its lack of bulk make it unsuitable to replace all of the sugar in recipes, such as confectioneries, icings etc. In addition to sweetening foods stevia extracts can increase the palatability and attractiveness (enjoyment) of food through enhancement of flavours and odours. Stevia products also have beneficial uses as herbal and medicinal products and for some more unusual uses, e.g. in tobacco products. A fermented extract of stevia showed bactericidal activity against food borne bacteria including *E. coli*.

Food and Culinary Uses

- Table top sweetener – for tea, coffee etc
- Soft drinks, cordials, fruit juices
- Ice-creams, yogurts, sherbets
- Cakes, biscuits
- Pastries, pies, baking
- Jams, sauces, pickles
- Jellies, desserts
- Chewing gum
- Candies, confectioneries
- Sea-foods, vegetables
- Weight-watcher diets
- Diabetic diets
- Flavour, colour and odour enhancers
- A source of antioxidants
- Alcoholic beverage enhancer (aging agent and catalyst)

Medicinal Uses

- Toothpaste, mouthwashes – plaque retardant/caries preventor
- Skin care – eczema and acne control, rapid healing agent
- Diabetic foods and weight loss programs
- Hypertension treatment and blood pressure control
- Calcium antagonist
- Bactericidal agent
- Pill and capsule additive to improve taste

Other Uses

- Tobacco additive and flavoring
- Production of plant growth regulators (potential use)

LOR OBJECT 263 – *Stevia rebaudiana* Leaves – A Low Calorie Source of Sweeteners

• Triterpenes [C₃₀H₄₈] & Steroids:

These C₃₀ constituents are abundant in nature, particularly in resins and may occur as either esters or glycosides (often called saponins - molecules made up of sugars linked to steroids or tripterpenes - due to their ability to make aqueous solutions appear foamy).

They are derived essentially from coupling of two sesquiterpene precursors. They may be aliphatic, tetracyclic or pentacyclic. Tetracyclic ones include the limonoids, the sterols found in wood and yeast.

Arbruside E, for example, comes from a plant called *Arbrus precatorius* (jequirity), which has been used as an abortifacient and purgative. Arbruside E, however, appears to be relatively non-toxic, and is 30-100 times sweeter than sucrose, making it a potential sugar substitute.

Triterpenes of the quassinoid class, such as bruceantin, have been shown to have significant antineoplastic activity in animal systems and have been investigated for the treatment of cancers.

Steroids are modified triterpenes. They are probably most familiar from their role as hormones, *i.e.*, androgens such as testosterone and estrogens such as progesterone. Steroids, such as cortisone, are most often used as anti-inflammatory agents, but many have other uses such as in birth control pills. Prior to 1943, most steroids were obtained from natural sources. For example, progesterone could only be isolated in quantities of 20 mg from 625 kg of pig ovaries. The large numbers of commercially and medicinally valuable steroids available today have been made possible by the semi-synthetic preparation of progesterone from diosgenin. This process, known as the Marker process, was developed in the early 1940's by Russell Marker, a rather colourful character, who left his position of professor of chemistry at Penn State to pursue this project in Mexico. Marker collected Mexican yams in bandit country, processed the yams to isolate the diosgenin, and then converted the diosgenin into *ca.* 3 kg of pure progesterone at a cost of \$8 per gram.

Saponins:

Saponins are glycosides with a distinctive foaming characteristic. They are found in many plants, but get their name from the soapwort plant (*Saponaria*), the root of which was used historically as a soap (Latin *sapo* → soap). According to the structure of the aglycone or sapogenin, two kinds of saponins are recognised – the steroidal and the pentacyclic triterpenoid types. They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C₃ and an ether bond to a sugar side chain.

The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water-soluble side chain. Saponins are bitter and reduce the palatability of livestock feeds. However if they have a triterpenoid aglycone they may instead have a licorice taste as glucuronic acid replaces sugar in triterpenoids. Some saponins reduce the feed intake and growth rate of non-ruminant animals while others are not very harmful. For example, the saponins found in oats and spinach increase and accelerate the body's ability to absorb calcium and silicon, thus assisting in digestion.

Saponins are also diverse in ginseng. Many of the effects of this common drug plant are due to these compounds. The anti-inflammatory activity of English ivy, *Hedera helix*, also is due to compounds of this group. The sweet tasting compounds of licorice, *Glycyrrhiza* spp., are saponins. In large amount these compounds cause irregularities in heart action. Gymnemic acids from *Gymnema sylvestre* (Asclepiadaceae) suppress the perceived sweetness of sugar and to lesser extent amino acids. Chewing the root of this plant blocks the sweet taste of sugar, saccharin and cyclamates for hours. A recent popular herbal medicine, *Tribulus terrestris*, also contains saponins that are thought to be similar in action to certain steroidal compounds used by athletes.

Steroidal Saponins:

These 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.), *Amaryllidaceae* (e.g. *Agave* spp.) and *Liliaceae* (e.g. *Yucca* and *Trillium* spp.). In the dicotyledons, the occurrence of diosgenin in fenugreek is of potential importance.

Examples of steroidal saponins

| Steroidal saponin | Sugar components | Occurrence |
|-------------------|----------------------------------|--|
| Sarsaponin | 2 glucose; 1 rhamnose | <i>Smilax</i> spp. |
| Digitonin | 2 glucose; 2 galactose; 1 xylose | Seeds of <i>Digitalis purpurea</i> and <i>D. lantana</i> |
| Gitonin | 1 glucose; 2 galactose; 1 xylose | Seeds and leaves of <i>Digitalis purpurea</i> and seeds of <i>D. lantana</i> |
| Dioscin | 1 glucose; 2 rhamnose | <i>Dioscorea</i> spp. |

Steroidal saponins are of great importance and interest, owing to their relationships with such compounds as the sex hormones, cortisone, diuretic steroids, vitamin D and the cardiac glycosides. The C₂₇ steroidal saponins of *Dioscorea* species serve as chemical precursors for the synthesis of anti-inflammatory compounds and contraceptive drugs. Cholesterol appears to be a usual precursor for steroidal saponins.

Triterpenic Saponins:

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*, *Ranunculaceae*, *Papaveraceae*, *Linaceae*, *Rutaceae*, *Myrtaceae*, *Cucurbitaceae*, *Araliaceae*, *Oleaceae*, *Rubiaceae* and *Compositae*.

Triterpenoid saponins may be classified into three groups represented by α -amyrin, β -amyrin and lupeol and are formed by the replacement of a methyl group by a carboxyl group in position 4, 17 or 20.

Plant materials often contain these saponins in considerable amounts. Thus, primula roots contains about 5–10%; liquorice root about 2–12% of glycyrrhizic acid; quillaia bark up to about 10% of the mixture known as 'commercial saponin'; the seeds of horse chestnut up to 13% of aescin.

Pentacyclic triterpenoid saponins

| Saponin | Genin | Sugar components | Occurrence |
|--------------------------|-------------------|--|---|
| Aescin | Aescigenin | 2 glucose; 1 glucuronic acid; 1 tiglic acid | <i>Aesculus hippocastanum</i> |
| Aralin | Aralidin | 2 arabinose; 1 glucuronic acid | <i>Aralia japonica</i> |
| Calendula saponin | Oleanolic acid | ? | <i>Calendula officinalis</i> |
| Cyclamin | ? | 1 xylose; 2 glucose; 1 arabinose | <i>Cyclamen europaeum</i> |
| Glycyrrhizic acid | Glycyrrhetic acid | 2 glucuronic acid | <i>Glycyrrhiza</i> spp. |
| Guaiaic saponin | Oleanolic acid | ? | <i>Guaiaicum</i> spp. |
| Gypsophila saponin | Gypsogenin | 1 galactose; 1 xylose; 1 arabinose; 1 fucose; 1 rhamnose | <i>Gypsophila</i> spp. And other <i>Caryophyllaceae</i> |
| Hederacoside A (hederin) | Hederagenin | 1 glucose; 1 arabinose | <i>Hedera helix</i> (ivy) and other <i>Araliaceae</i> and |

| | | | |
|------------------|----------------|---|---|
| Primula saponin | Primulagenin | 1 rhamnose; 1 glucose; 1 galactose; glucuronic acid | <i>Sapindaceae</i> <i>Primula</i> spp. |
| Quillaia saponin | Quillaic acid | Glucuronic acid | <i>Quillaia saponaria</i> |
| Saikosaponin a | Saikogenin F | 1 glucose; 1 fructose | <i>Bupleurum</i> spp. |
| Thymus saponin | Oleanolic acid | ? | <i>Thymus vulgaris</i> |

Cardiotonic heterosides / Cardenolides:

Cardiac glycosides are glycosides of mostly C₂₃-steroidal compounds. They have a characteristic 5- or 6-membered lactone ring. They are called cardiac glycosides because they modify heart action. Cardenolides inhibit the Na⁺-K⁺-ATPase pump in mammals. This group of compounds is found in a large number of families, many of which are unrelated. There are about 400 known cardenolides. A number of toads and frogs make cardiac active compounds that are steroidal, but not glycosidic, in nature. Cardenolides are derived from steroidal precursors, probably cholesterol, via the intermediacy of pregnenolone or progesterone intermediates. The exact pathway does not seem to have been established with certainty. Many questions about the probable acetate/malonate origin of the five or six membered lactone ring.

Most members of the family *Asclepiadaceae* contain cardiac glycosides. Many of the insects that feed on these plants sequester the glycosides. The story of monarch butterflies and milkweeds is well known. In this case the larvae eat the plant, and sequester a different complement of cardenolides from that which occurs in the plants. The glycosides are passed on to the adult insects. The adults are marked with warning coloration (aposematic labelling) and are not eaten by most predators. However, in the area in Mexico in which the butterflies overwinter, there are bird predators that eat the adults without apparent ill effects. Naive blue jays will eat one adult, but will reject others in subsequent feeding trials.

Several plants with cardiac glycosides or cardenolides are used medicinally. Among these are *Digitalis* species (*Scrophulariaceae*) and ouabain (*Strophanthus* species) (*Apocynaceae*). These plants are often used to treat heart problems.

Biological and Pharmacological Properties of Saponins:

- Antimicrobial and antifungal
- Haemolysis properties (toxic to cold blooded animals)
- Anti-inflammatory
- Expectorant and antitussive
- Anti-haemorrhoidal
- Healing properties

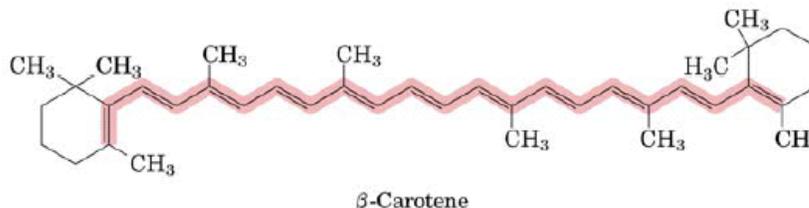
- **Tetraterpenes [C₄₀H₆₄]:**

Important among these are the C₄₀ yellow or orange-red carotenoid pigments, of which about 180 have been reported. Carotene was isolated from carrots as early as 1831. Between 1913 and 1915, the existence of a fat-soluble growth factor, now known as Vitamin

A, was proved by feeding experiments to be present in materials such as butter or cod-liver oil.

Carotenes $C_{40}H_{56}$:

Carotenes in association with chlorophyll participate in photosynthesis, but also occur in other plant organs as the carrot.



They have vitamin A activity. The colours of the red tomato and the orange are due to the carotenoids lycopene and citraurin, respectively. Because the carotenes are non-toxic, they are used as colorants in the formulation of tablets and in the food industry.

Some carotenoids are oxygenated derivatives:

| Carotenoid | Formula | Occurrence |
|-------------|----------------------|----------------------|
| Bixin | $C_{25}H_{30}O_4$ | Annatto |
| Capsanthin | $C_{40}H_{58}O_3$ | <i>Capsicum</i> spp. |
| Capsorubin | $C_{40}H_{60}O_4$ | <i>Capsicum</i> spp. |
| Crocetin | $C_{20}H_{24}O_4$ | Saffron |
| Crocin | $C_{44}H_{64}O_{24}$ | Saffron |
| Fucoxanthin | $C_{40}H_{60}O_6$ | Brown algae |

The carotenoids are probably the widely distributed group of colorants in nature. In addition to the natural carotenoids, many others have been synthesized. Both types are generally more expensive than the synthetic azodyes. The colors possible are also limited, generally to yellows and oranges. Carotenoid colors tend to be relatively stable, in that they retain their coloring ability throughout a normal shelf life, but do degrade through oxidation. Loss of color as they degrade can be offset by increasing the concentration used. Carotenoids are not corrosive and are not usually affected by reducing agents such as ascorbic acid.

Annatto provides a variety of yellow to orange colors and is used widely in foods, particularly for margarines, and milk and cheese products. It is derived from the pulp of the seeds of *B. orellana*. The colour is provided by a range of dyes including tannins and flavonoids but also some carotenoids, of which bixin and nor-bixin are the most important. It is available as refined powders in several different forms (soluble in water at pH 4.0, acid, oil, and water and oil). It is also tasteless. In Europe it is known as E.160.

Saffron was widely used for as a colorant for several substrates but its use today is largely confined to coloring food. As it is so expensive to produce it tends not to be used in the mass-production of food. The major constituent is crocin.

β -carotene is used widely for providing a uniform colour. Apocarotene gives colour to cheese and simulated food products, and canthaxanthin, which is very soluble in fats, is used in icings, meat, salad dressings and products containing tomatoes.

- **Polyterpenes $[C_5H_8]_n$:**

Polyterpenes are composed of many isoprene units. Common examples, both having macromolecules of molecular weight over 100 000, are found in indiarubber and gutta-percha. Doubtless, the rubber-like substances of many other plants have similar composition. Chemically, pure rubber is *cis*-1,4-polyisoprene $(C_5H_8)_n$, although in the natural state other materials are present. Its occurrence is confined to the dicotyledons, and the one important commercial source is *Hevea brasiliensis*. Gutta-percha is *trans*-1,4-polyisoprene, and chile, obtained from *Achras sapota*, contains a mixture of low molecular weight *cis*- and *trans*-polyisoprenes. No biological function for polyisoprenes has yet been discovered.