UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
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Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

1. Introduction

Lecturer: Oldřich Lapčík

Bioactive Natural Compounds

- Knowledge of certain scale of natural remedies, spices, perfumes and also of psychotropic substances is inherent to every civilization

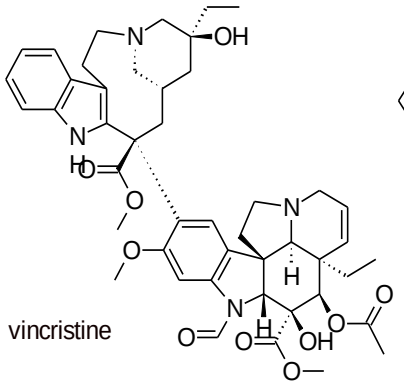
- Culture influences the way that individual species are used
  - Compare e.g. traditional and modern use of curare and physostigmin

- Spice, remedy, poison – the difference often is only a matter of dosage and the way of application
  - e.g parsley – a common vegetable/spice but also a criminal prolicidal drug

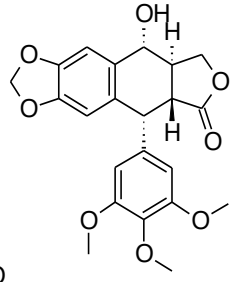
- Numerous foods contain substances displaying important biological effects, which are sometimes fully effective only after a long intake, a monotonous diet or after inappropriate food preparation
  - e.g. glucosinolates in cruciferous plants or cyanogens in cassava

Bioactive Natural Compounds

- The effects of natural compounds have been known and exploited from time immemorial
- Molecular fundament started to be recognized about two hundred years ago
- The process of discovery:
  - Description of a biological activity
  - Identification of the active compound(s)
  - Isolation of the active compound from a natural source
  - Elucidation of the structure
  - Chemical synthesis
  - Derivatisation, synthesis of analogues
  - Study of links between the structure and the activity



vincristine



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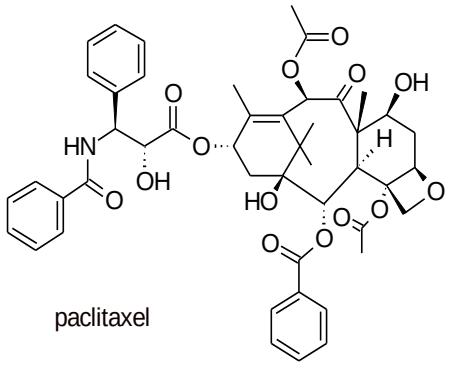
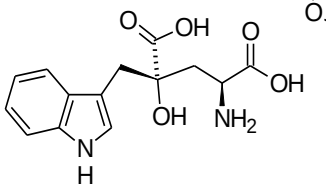
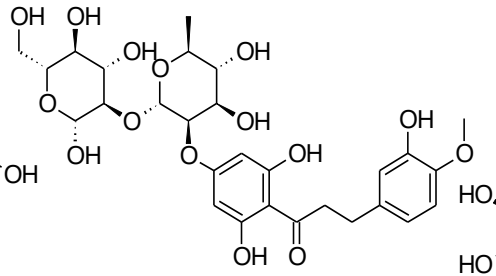


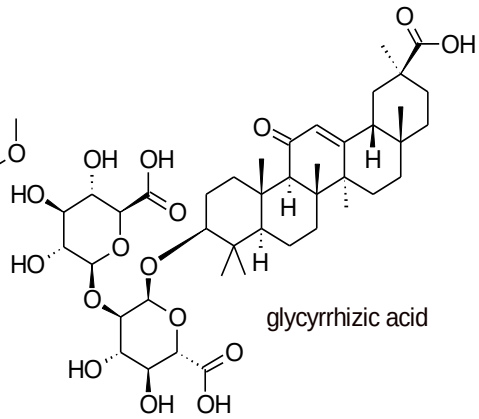
paclitaxel



2*S*,4*S* monatin



neohesperidin DHC



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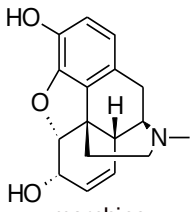
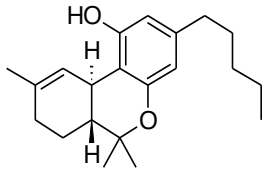
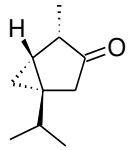


morphine



tetrahydrocannabinol



β-thujone

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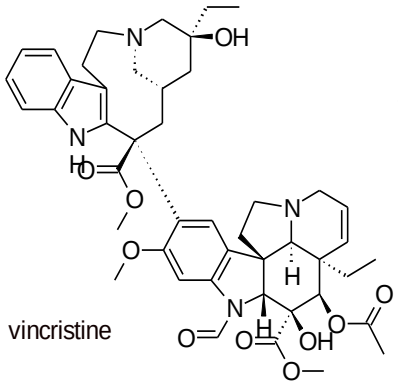
common features



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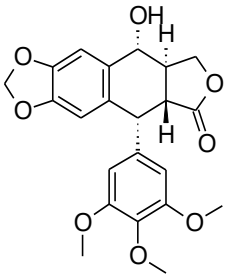

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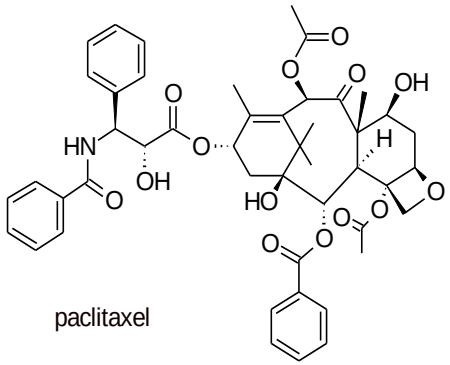
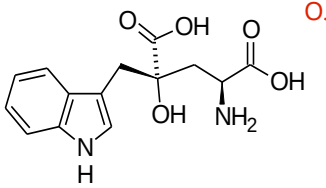


paclitaxel



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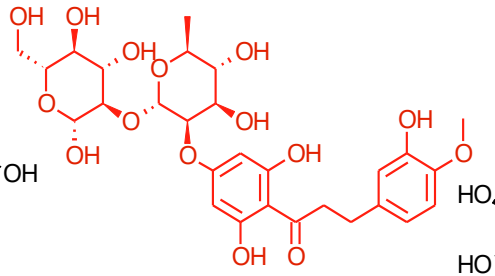
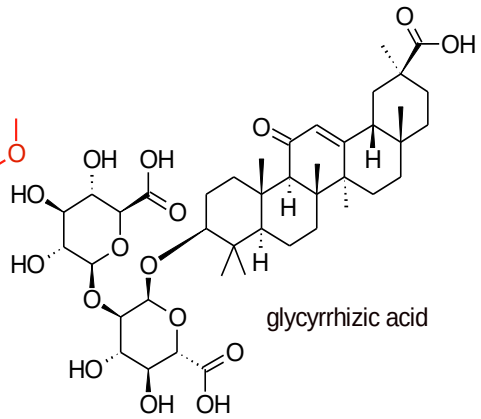


neohesperidin DHC



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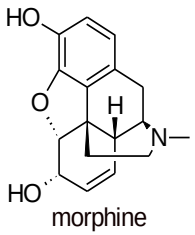
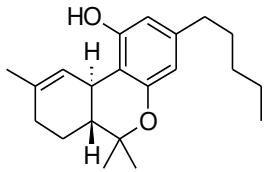


morphine



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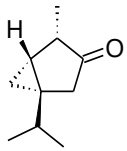
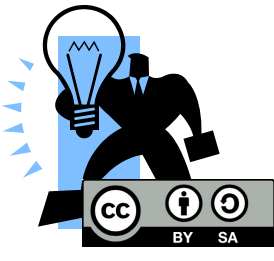
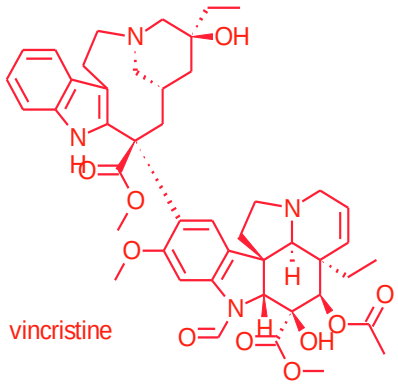


β-thujone

Natural or natural-derived compounds





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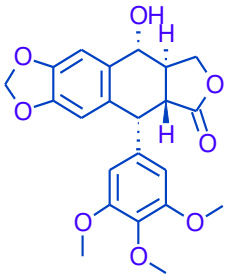

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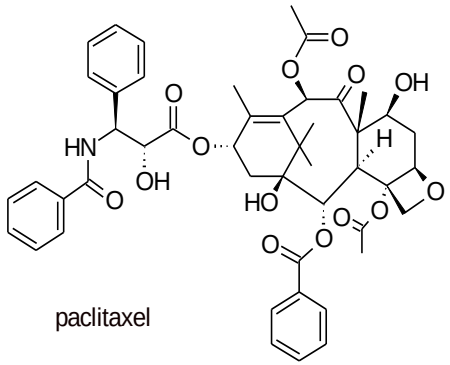

paclitaxel

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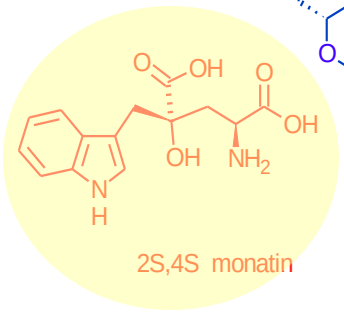
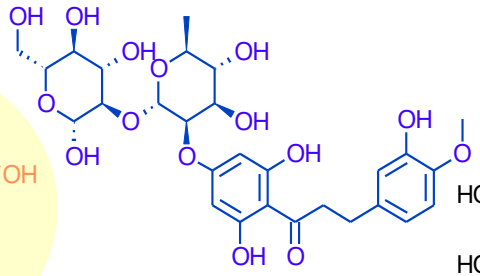


2S,4S monatin



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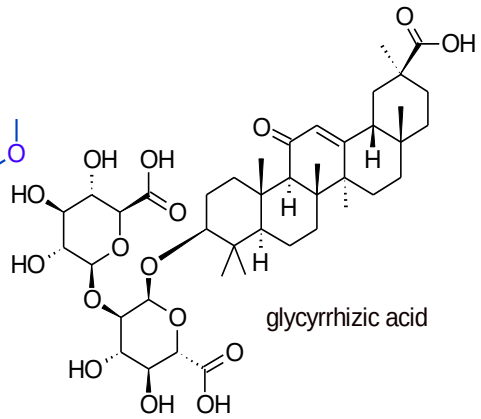
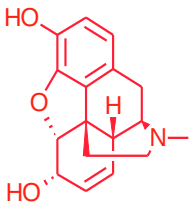


glycyrrhizic acid



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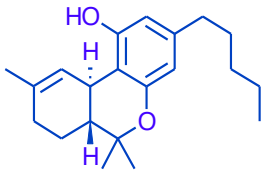

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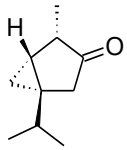
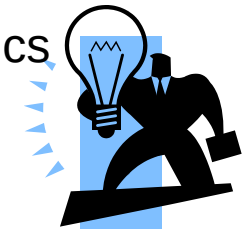
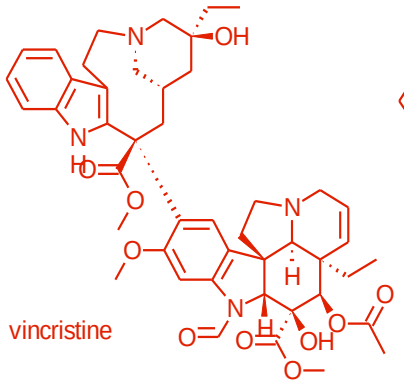


β-thujone

alkaloids phenolics terpenes





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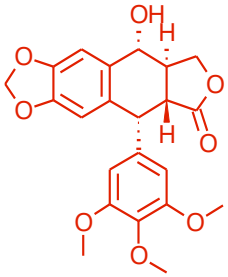

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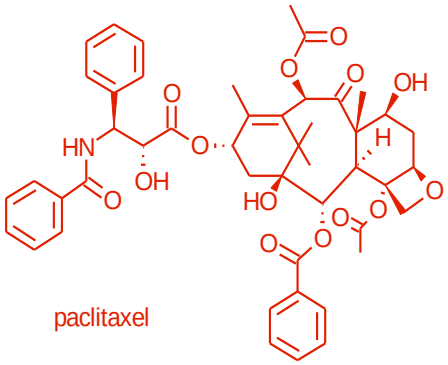

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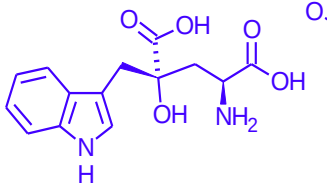
cytostatics



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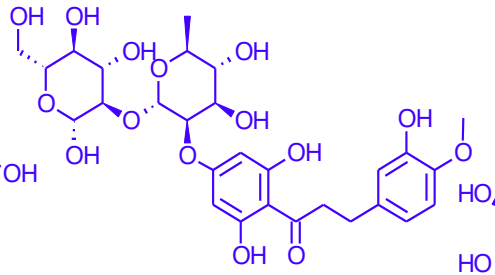

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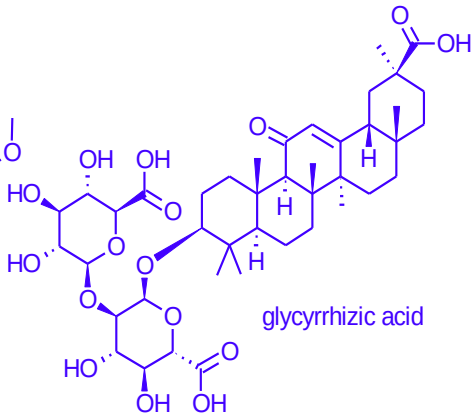
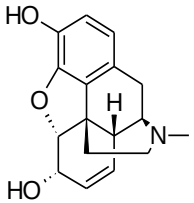


glycyrrhizic acid

sweeteners



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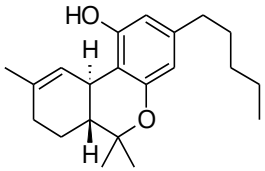
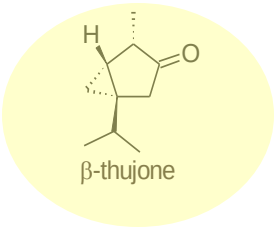


tetrahydrocannabinol



illicit drugs

Bioactive Natural Compounds

Basic terms

- PHARMACOGNOSY

  (from Greek *pharmacon* –poison, *gnosis* – knowledge)

  study of the starting natural materials and substances intended for therapeutics. Nowadays, the field of pharmacognosy is limited to plants and a limited number of fungi.

- PHARMACOPOIEA – A law defining assortment of materials used for preparation of remedies.

- DRUG – dried part of a medicinal plant or dried amorphous product used for preparation of remedies.

Bioactive Natural Compounds

Basic terms

- SYNERGY – complementary action of two or more substances
  (the effect is higher than would correspond to a simple addition of individual effects)

- ANTAGONISM – opposite action
  - Antagonists are used in order to suppress action of particular compounds,
  - Example: Use of antagonistically acting compounds to treat poisonning

Bioactive Natural Compounds

Basic terms

- HOMEOPATHY – one branch of “alternative medicine“. Homeopathy is based on idea, that a complaint can be cured with small doses of drugs, which cause simmilar symptoms when administerd in higher doses (“similia simile curantur“). Homeopathic remedies are highly diluted solutions of substances that in higher concentrations cause symptoms similar to the respective illness. (Samuel Hahnemann, 1755-1853).

- PLACEBO – psychological effect of a treatment.

Bioactive Natural Compounds

Basic terms

- ANATOMICAL PARTS OF PLANTS:
- AERIAL-
- Tops – *herba*, stalk – *caulis*, bud – *gemma*, leaf – *folium*, wood – *lignum*, bark, peel – *cortex*, flower – *flos*, fruit – *fructus*, seed – *semen*, spore – *sporae*.
- UNDERGROUND-
- root – *radix*, rhizome – *rhizoma*, tuber – *tuber*, bulb (onion) – *bulbus*.

Bioactive Natural Compounds

Basic terms

- DRUG EFFECTS:

- DIAPHORETICS – drugs that cause sweating
- (*Salix alba* – willow, *Tilia cordata* – linden, *Sambucus nigra* – European elder)

- ANTIHYDROTICS – drugs that decrease sweating
- (For local applications e.g.: *Quercus robur* – oak tree (bark), *Juglans regia* – wallnut tree (leaves), *Agrimonia eupatoria* – agrimony)

- CYTOSTATICS – substances that block the cell cycle, they are used against cancers. *Vinca rosea* – Madagascan periwinkle, *Taxus* sp. – yew..

Bioactive Natural Compounds

Basic terms

- DRUG EFFECTS:

- CARDIOTONICS – drugs influencing cardiac action. (Usually poisonous).
- *(Digitalis lanata* – Grecian foxglove)

- HYPOTENSIVES (ANTIHYPERTENSIVES) – blood pressure decreasing drugs
- (*Valeriana officinalis* – valerian, *Humulus lupulus* – hops)

- DIURETICS – kidney function supporting drugs

Bioactive Natural Compounds

Basic terms

- DRUG EFFECTS:

- ANTITUSSIVES and EXPECTORANTS – drugs for treating cough
- Mucus dissolving drugs
- *Glycyrrhiza glabra* – licorice, *Primula veris* – primrose, *Saponaria officinalis* – soapwort
- Irritation decreasing drugs:
- *Bellis perennis* – daisy, *Plantago lanceolata* – ribwort
- Mucous:
- *Verbascum thapsiforme* – mullein, *Tussilago farfara* – coltsfoot
- Disinfectively acting:
- *Thymus serpyllum* – thyme, *Pinus silvestris* – pine

Bioactive Natural Compounds

Basic terms

- STOMACHICS – drugs influencing stomach function. (Often drugs containing biter substances).

- CARMINATIVES – drugs against flatulence.
- (*Carum carvi* – caraway (caraway seed), *Coriandrum sativum* – coriander, *Majorana hortensis* – majoram, *Pimpinella anisum* – anise (aniseed)

- OBSTIPANTS – anti-diarrheal drugs.
- (*Daucus carota* – carrot, *Salvia officinalis* – sage, *Vaccinum myrtilus* – cranberry)

Bioactive Natural Compounds

Basic terms

- LAXATIVES (purgatives, catharacts)

- 1. Bulk laxatives: materials that bind water and thus influence the volume and consistency of intestinal content. E.g. pectins, mucins, agar-agar.
-
- 2. Substances influencing the motility of smooth muscles. Anthrachinon laxatives, e.g. *Frangula alnus* – buckthorn, *Cassia senna*.

- 3. Drastic purgatives. *Ricinus communis* – castor (castor oil).

Bioactive Natural Compounds

Basic terms

- EXTERNA – drugs for external use (used for skin care or for mouthwashes). Antibacterial and balmy drugs.
- *Matricaria chamomilla* – camomile, *Symphytum officinale* – coltsfoot, *Agrimonia eupatoria* – agrimony.

- PANACEA – miraculous universal remedy.
- Panacea (*Panakeia*) and *Hygieia* were daughters of Greek god *Asclepios* (Aesculapos). Panacea knew remedies for every disease. Her name sounds in the Latin *Panax ginseng*.

Bioactive Natural Compounds

Modes of action

- Biologically active substances act by interaction with different signaling systems, modulation of enzymes, stimulation or inhibition of the cell growth, antibiotic effects etc.

- Numerous compounds are able to influence several systems at the same time. Resulting effect depends on dosage, on the way of application and on sensitivity of the recipient.

Bioactive Natural Compounds

Modes of action

- 1. Substances influencing MEMBRANE BOUND RECEPTORS or ION CHANNELS act usually very rapidly. Many of them are severe poisons (e.g. cardiac glycosides). Also psychotropic substances act via the membrane-bound receptors.
  G-protein associated membrane bound receptors are responsible for perception of tastes and smells.

- 2. NUCLEAR RECEPTORS control gene expression.
  Compounds that interfere with this system mediate relatively slow effects that occur over a longer period of time (e.g phytoestrogens).

Bioactive Natural Compounds

Modes of action

- 3. ENZYMES may be either inhibited or activated.
  Especially the inhibition of enzymes synthesizing hormones and mediators may lead to dramatic consequences
  (see e.g. inhibition of acetylcholine esterase, thyroidal peroxidase, cyclooxygenase)

- 4. INTERACTION WITH CYTOSKELETON components
  is the principle of action of such cytostatics as are
  e.g. podophylotoxin, vincristin and paclitaxel.

- 5. INTERACTION WITH DNA may lead to carcinogenesis
  (e.g. aristolochoic acids).

Bioactive Natural Compounds

Modes of action

- 6. ANTIOXIDANTS protect cell structures from damage caused by reactive oxygen species. (e.g. vitamins C, E, flavonoids etc.)
- 7. CHELATION OF IONS OF TRANSIENT METALS protects from their toxicity and from their oxidative effects (pectins, tannins)
- 8. ANTIMICROBIAL and ANTIPARASITARY effects.
- 9. BINDING OF WATER, modification of MECHANICAL PROPERTIES of mucous secrets, protecting the surfaces of mucosas and skin. etc. (mucins, pectins, fibers etc.)

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

2. Primary metabolites - Proteins

Lecturer: Oldřich Lapčík

METABOLISM

Primary

Secondary

Primary metabolites are involved in

- building of body structures
- energetic metabolism

Biochemical pathways of primary metabolism are broadly common in living organisms

METABOLISM

Primary

Primary metabolites are involved in

- building of body structures
- energetic metabolism

Biochemical pathways of primary metabolism are broadly common in living organisms

Secondary

Secondary metabolites are not involved in energetic metabolism.

Possible physiological roles:

- Phytoalexins (protection from pathogens and predators)
- Allelochemicals (interspecies signalling)
- Molecular waste
- Unclear role

Biochemical pathways may be specific for a particular taxon

METABOLISM

Primary

Interconversion of compounds in frame of primary metabolism

Secondary

Secondary metabolites are derived from primary metabolites

METABOLISM

Primary

Interconversion of compounds in frame of primary metabolism

Carbohydrates (Saccharides)

Lipids

Amino acids

Proteins

Nucleotides

Secondary

Secondary metabolites are derived from primary metabolites

METABOLISM

Primary	Secondary
Interconversion of compounds in frame of primary metabolism	Secondary metabolites are derived from primary metabolites
Carbohydrates (Saccharides)	Phenolics
Lipids	
Amino acids	Terpenoids including Steroids
Proteins	
Nucleotides	Alkaloids

METABOLISM

Primary

Interconversion of compounds in frame of primary metabolism

Carbohydrates (Saccharides)

Lipids

Amino acids

Proteins

Nucleotides

Secondary

Secondary metabolites are derived from primary metabolites

Phenolics:

- Phenylpropanoids
- Polyketides

Terpenoids including Steroids

Alkaloids

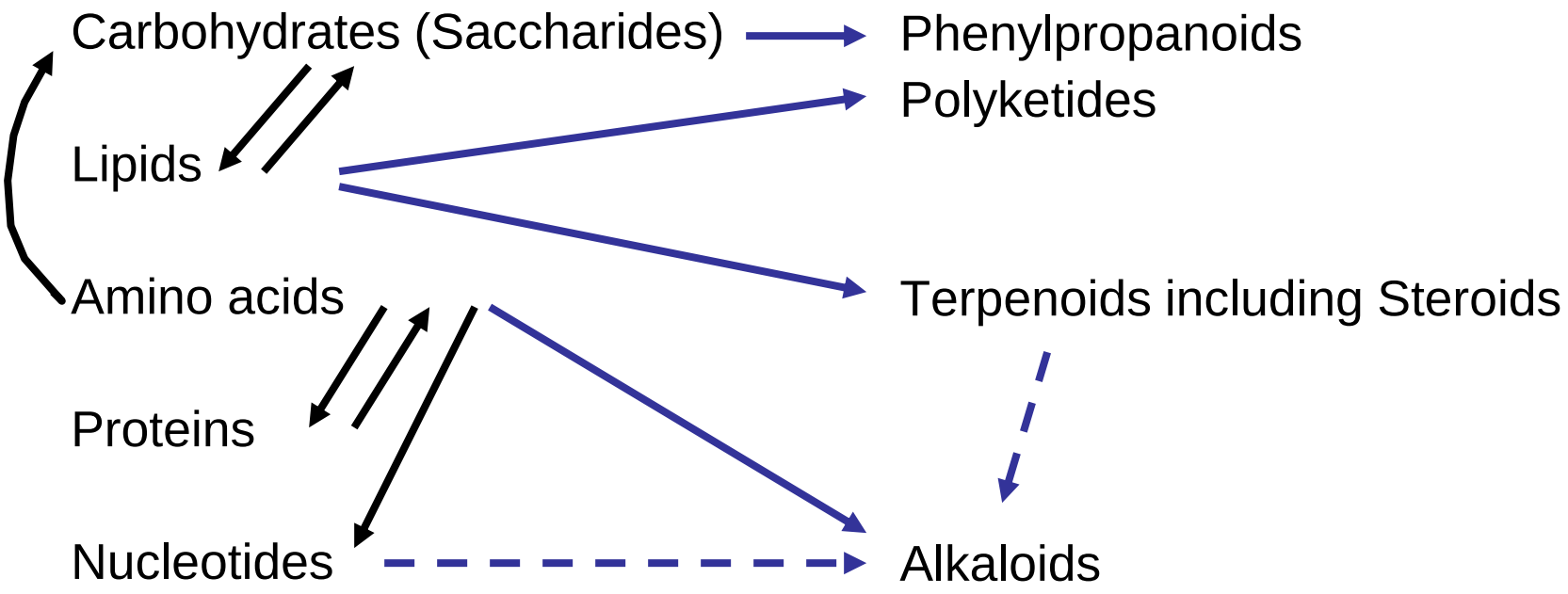
METABOLISM

Primary

Interconversion of compounds in frame of primary metabolism

Secondary

Secondary metabolites are derived from primary metabolites



Bioactive proteins

Enzymes

- Papain, chymopapain: endopeptidases, -SH active groups.
  Papain: 212 aminoacids, 23 kDa, chymopapain: 218 mino acids, .

- Plant: *Carica papaya* (Caricaceae), Carica pentagona etc.
  Perrenial herb, up to 6 m height. Latex from unrippen fruits is the source of papain and chymopapain.

- Producers: intertropical Africa, Asia, America

- Use: Chymopapain – injection formulation for improving absorption of disc prolapses, etc.
- Food technology: papaya has been used as meat tenderizer.

Bioactive proteins

Enzymes

- Bromelain: endopeptidases, -SH active groups.

  A mixture of basic glycoproteins, 18-28 kDa

- Plant: *Ananas comosus* (Bromeliaceae).

  Herbaceouas plant, native to Central America, cultivated in all tropical regions of the world

- Producers:
- Use:

Bioactive proteins

Lectins

- From the latin *lego, legere* (*lectum*) = to read, to choose, to select
-
- LECTINS are specific saccharide-binding proteins without enzymic activity.
- TOXIC LECTINS – parenteral application: hemagglutination, hemolysis.
  Proteolytically stable lectins – toxicity *per os*
- APPLICATIONS OF LECTINS – biology, immunology
  - Blood cell typing (lectins from *Dolicho bifloris, Ulex europeus*)
  - Specific mitogens: PHA – phytohemmagglutinin from *Phaseolus vulgaris*,
    ConA – concanavalin A from *Canavalia ensiformis*
    PWM – pokeweed mitogen from *Phytolacca americana*

Bioactive proteins

Toxins with a lectin subunit

- Ricin from *Ricinus communis* (Euphorbiaceae)
- Abrin A-D from *Abrus precatorius* (Fabaceae)

- Lectin subunit A, galactose specific:
  – binding to the cell membrane, internalization
- RNAase subunit B:
  – destruction of ribosomes (28S subunit rRNA)binding

Lethal doses (rat): ricin - 0.4 μg/kg bw
abrins – 0.1 – 0.3 μg/kg bw

Abrus precatorius

Bioactive proteins

Protein sweeteners

Thaumatin – found in fruits of *Thaumatococcus danielli*, Marantaceae.
(Indigenous in Ghana, Togo, Sierra Leone)

Preparation: Aqueous extraction of frozen fruits.

Soluble in water and in diluted alcohols, thermostable.

Sweet sensation is delayed, with licorice aftertaste.

Used in chewing gums, breath fresheners, etc.
Approved by FDA (USA) and by EU (E_{957}).

Bioactive proteins

Protein sweeteners

Monellin – found in fruits of *Dioscoreophyllum ccuminsii*, Menispermaceae.
English names: wild red berry, guinea potato or serendipity berry.
(Indigenous in western Africa)

Preparation: Aqueous extraction of frozen fruits, membrane filtration of the extract.

Soluble in water and in diluted alcohols, unstable at low pH, thermally unstable. Lysine residues play role in the sweet taste.

Limited applications

Bioactive proteins

Protein sweeteners

Miraculin – found in fruits of *Synsepalum dulcificum*, Sapotaceae.
(A shrub indigenous in western Africa,)

Preparation: Aqueous extraction of frozen fruits, membrane filtration of the extract.

473 AA glycoprotein, virtually tasteless on its own, it transforms acidic taste into a sweet taste and modifies perception of of numerous flavors. The ability to modify taste persists for up to two hours.

Miraculin gene bearing transgenic tomatos have been prepared

Bioactive proteins

Protein sweeteners

Thaumatin – found in fruits of *Thaumatococcus danielli*, Marantaceae.
(Indigenous in Ghana, Togo, Sierra Leone)

Preparation: Aqueous extraction of frozen fruits.

Soluble in water and in diluted alcohols, thermostable.

Sweet sensation is delayed, with licorice aftertaste.

Used in chewing gums, breath fresheners etc
Approved by FDA (USA) and by EU (E_{957}).

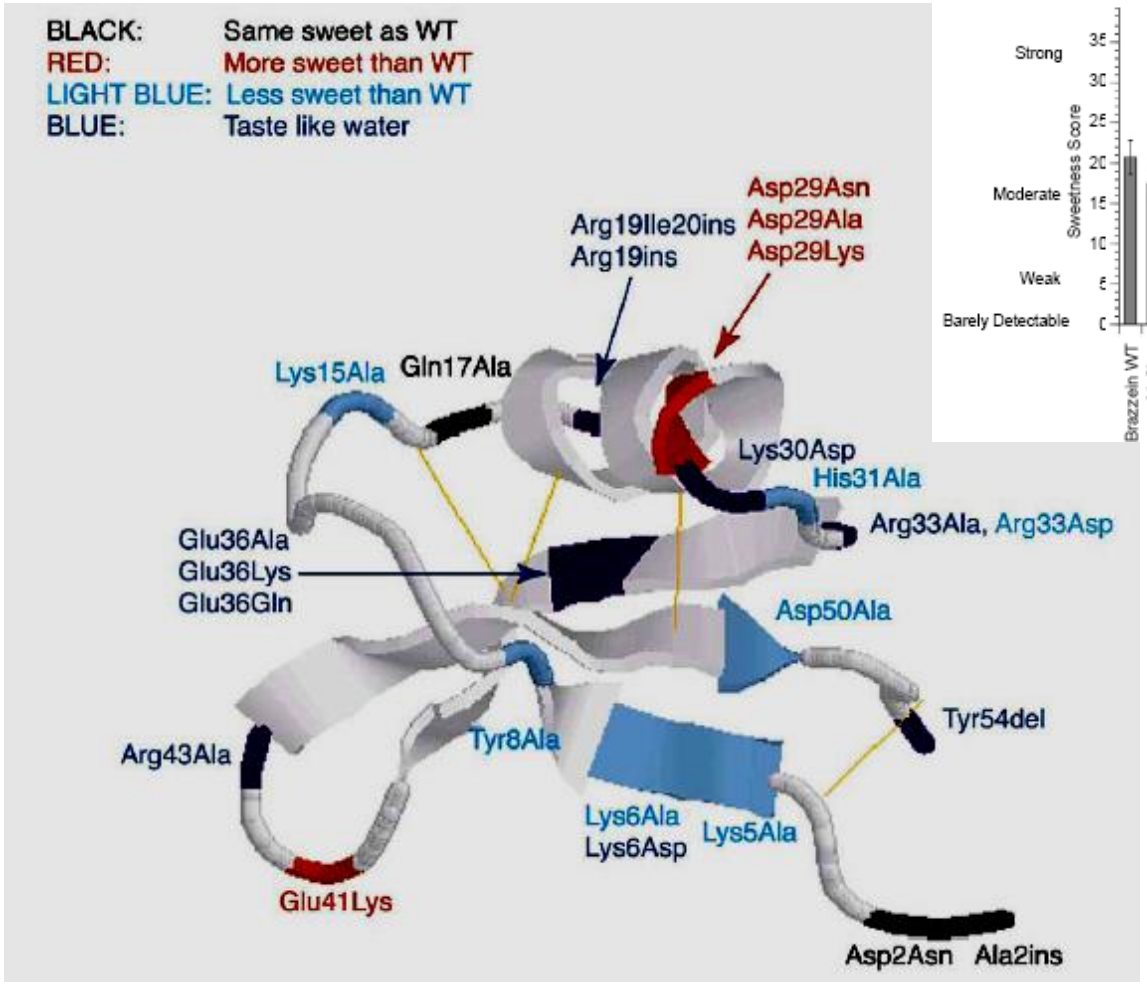
Sweet Proteins

Thaumatococcus daniellii Benth (Marantaceae)
thaumatin, 3000 – 15000 times sweeter than sucrose
E_{957} (EU), GRAS (USA)

Dioscoreophyllum cumminsii (Menispermaceae)
monellin, 3000 times sweeter than sucrose

Pentadiplandra brazzeana (Capparaceae)
brazzein, 1200 times sweeter than sucrose

Mutants of brazzein



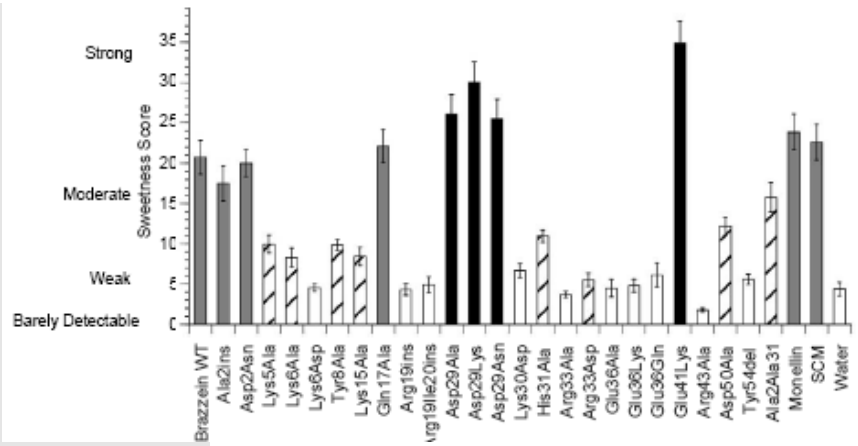


Fig. 3. Diagram showing the three-dimensional backbone of brazzein [10] with the position of mutations studied. The residues are color-coded to indicate the taste properties of mutants at these positions relative to those of WT brazzein: red, increased sweetness; black, the same sweetness; light blue, decreased sweetness; dark blue, taste equivalent to water. Intramolecular disulfide bonds are indicated as yellow lines.

Z. Jin et al./FEBS Letters 544 (2003) 33–37

Sweet Proteins

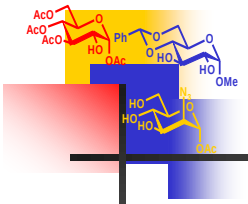
Capparis masaikai (Capparaceae)
mabinlin I-IV, 1000x sweeter than sucrose

Curculigo latifolia, (Liliaceae)
curculin a neoculin, 500 times sweeter than sucrose

Synsepalum dulcificum syn. *Richardella dulcifica*, Sapotaceae
miraculin

Sweet proteins: do they have common structural features?

Protein	SubU.	AA	m.w. (kDa)	SS	Sweetness	note.
Thaumatin I	1	207	222	8	3000–15 000	E_{957}
Thaumatin II	1	207	22.2	8		
Monellin	2	44 + 50	10.7	0	3000	
Brazzein	1	54	6.5	4	1200	
Mabinlin I	2	32 + 72	12.3	4		
Mabinlin II	2	33 + 72	12.4	4	1000	
Mabinlin III	2	32 + 72	12.3	4		
Mabinlin IV	2	28 + 72	12.1	4		
Curculin	2	114 + 114	23	4	500	
Neoculin	2	114 + 113	23-24	4		
Miraculin	4	191	24,6		tasteless	
Lysozym (hen)	1	211	14.4	4		



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MINISTRY OF EDUCATION, YOUTH AND SPORTS

Gums, mucins (polysaccharides)

Jitka Moravcová, Oldřich Lapčík
Department of Chemistry of Natural Compounds
UCT Prague

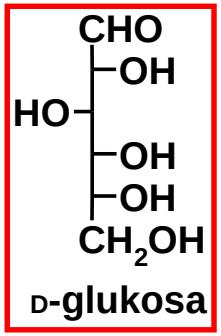
Generalities

Sacharides – natural compounds

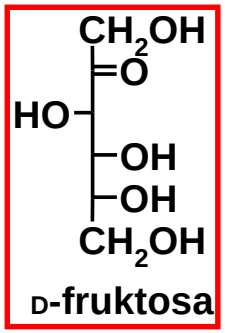
monosaccharides

polyhydroxyaldehydes (aldoses)

polyhydroxyketones (ketoses)

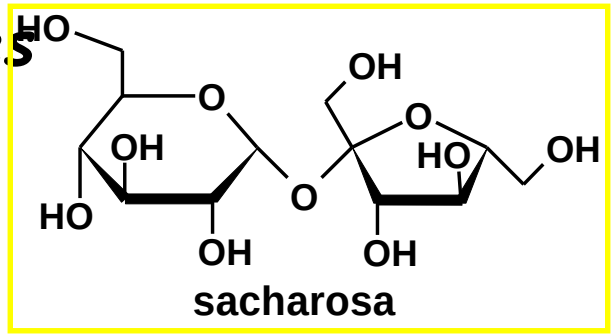


D-glukosa

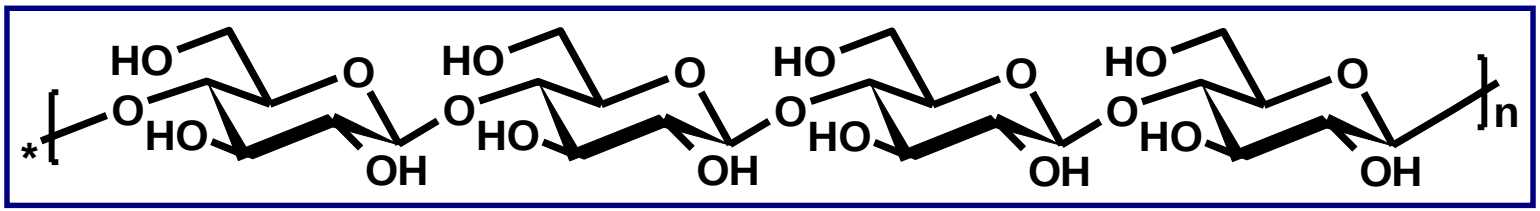


D-fruktosa

oligo- and polysaccharides



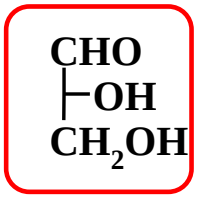
sacharosa

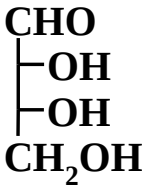


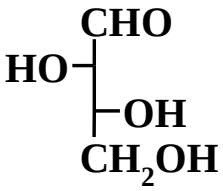
cellulose

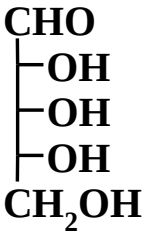
Generalities

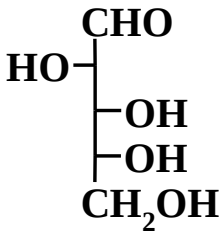
Saccharides - configuration

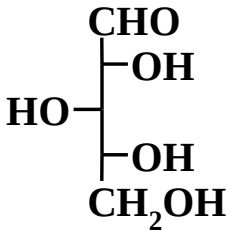


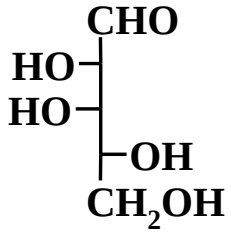


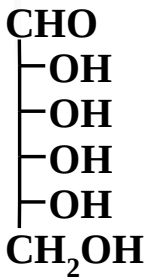


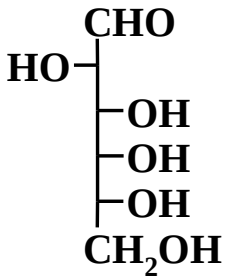


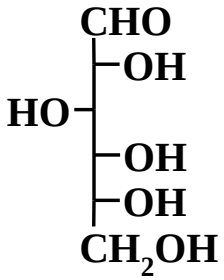


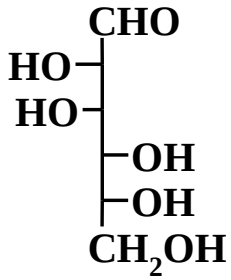


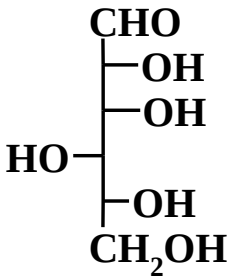


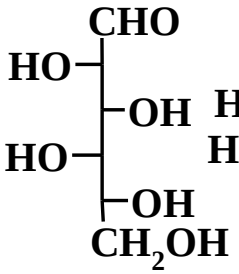


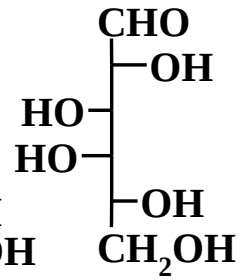


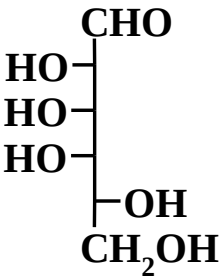










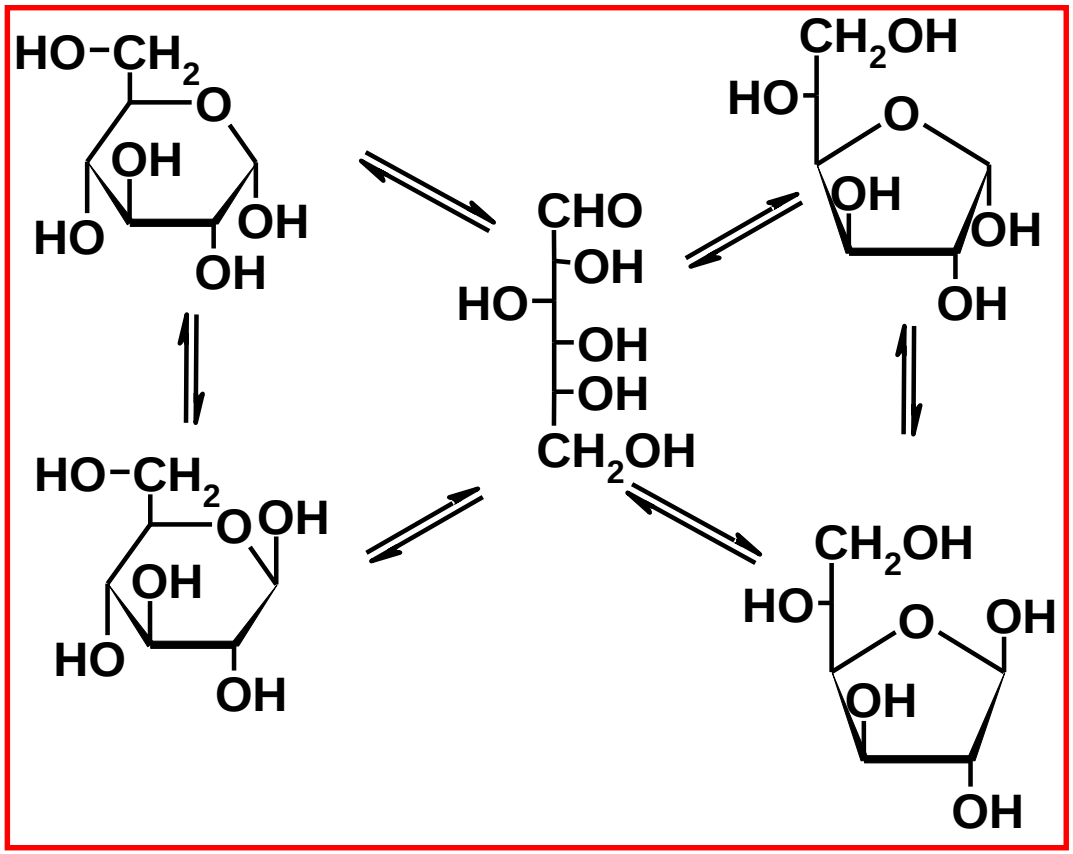


Generalities

Sacharides - mutarotation

α-pyranose

β-pyranose



α-furanose

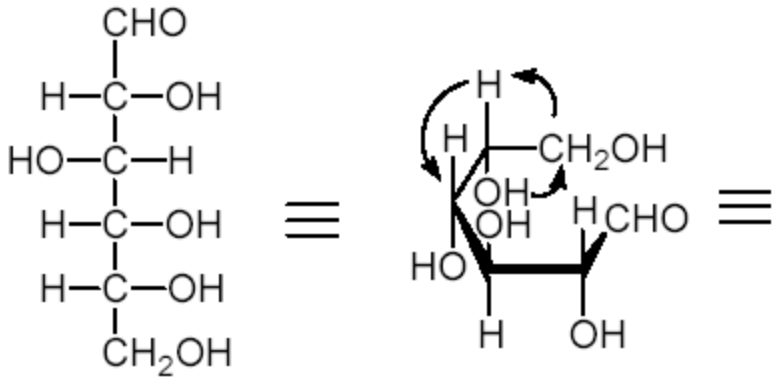
β-furanose

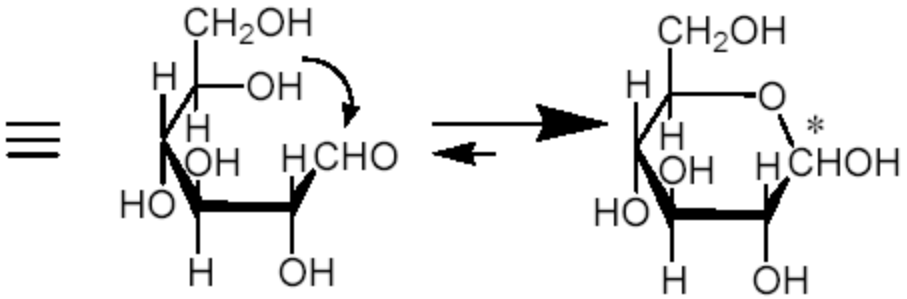
Generalities

Sacharides - mutarotation

Fischer projection

D-glucose

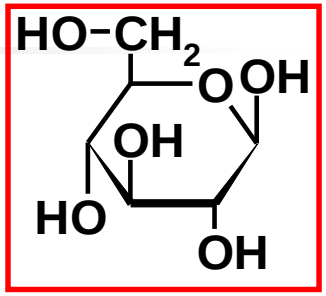




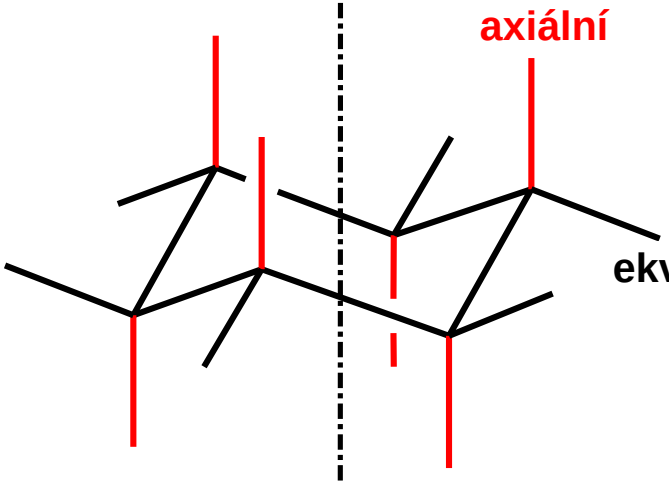
Formation of hemiacetal binding pyranose

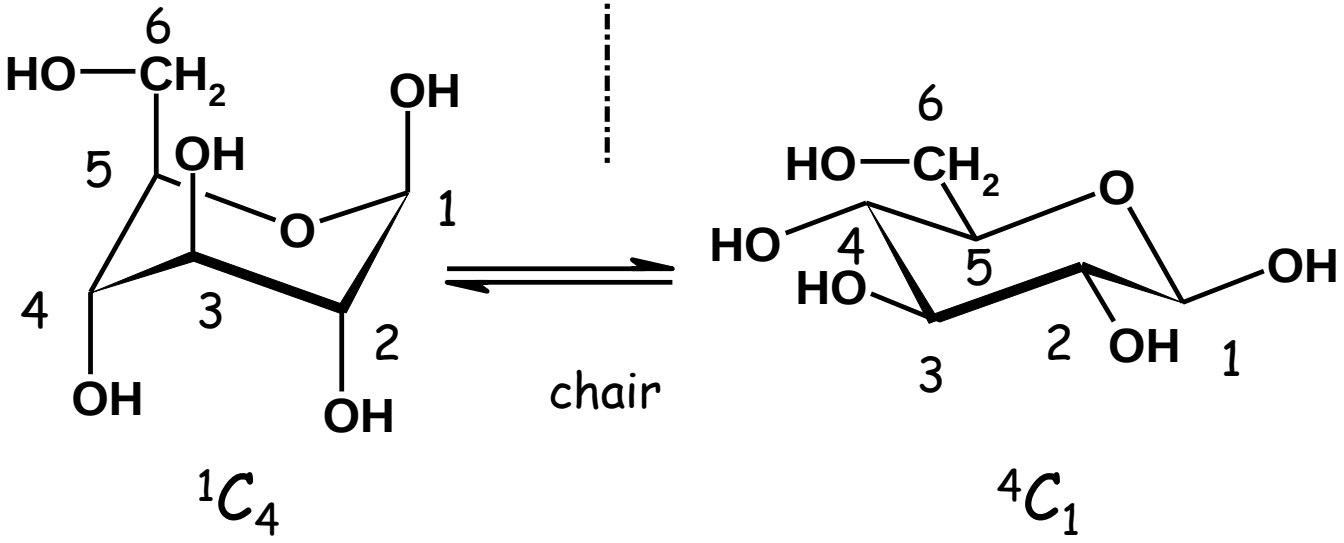
Generalities

Saccharides - conformation



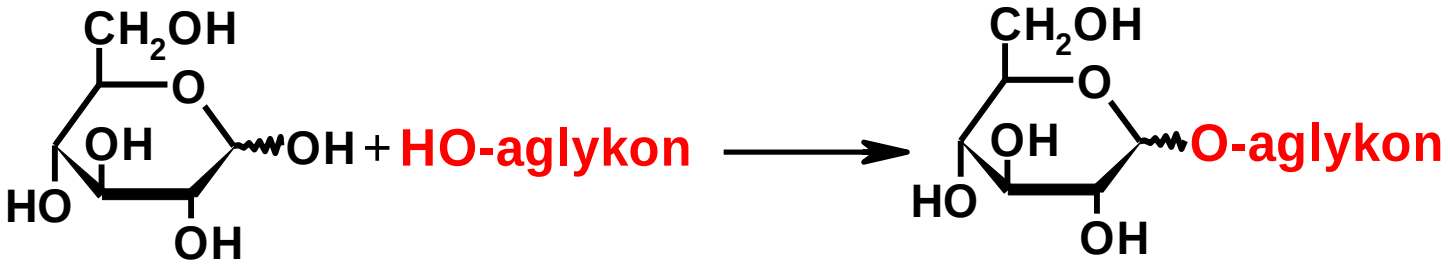
β-D-glucopyranose



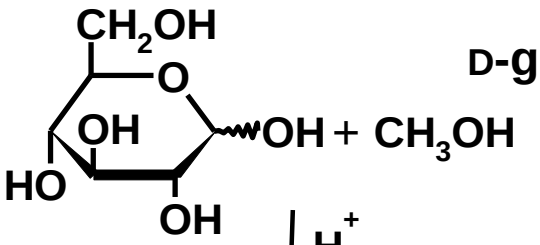


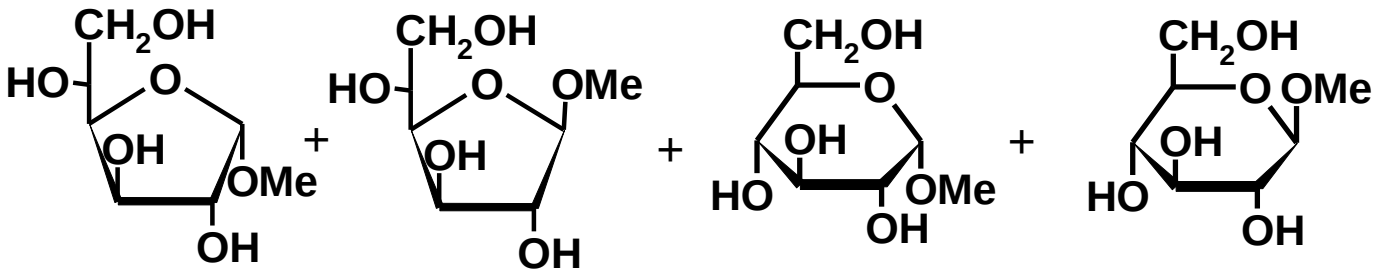
Generalities

Saccharides – glycoside formation



D-glukopyranosid



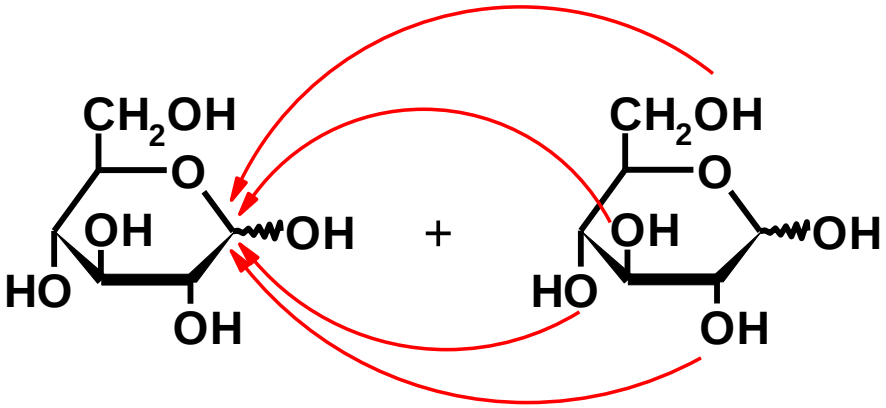


kinetically driven product

thermodynamically driven product

Generalities

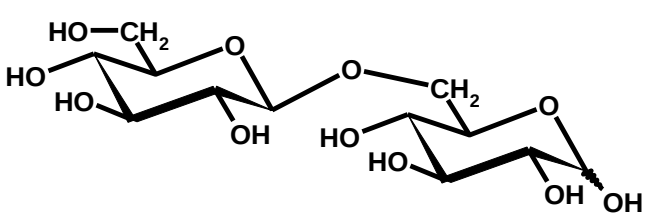
Saccharides – glycosylation of another sacchcharide

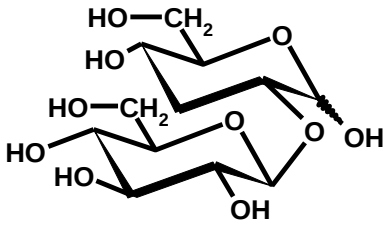


oligosaccharides – up to 10 units
polysaccharides – more than 10 units

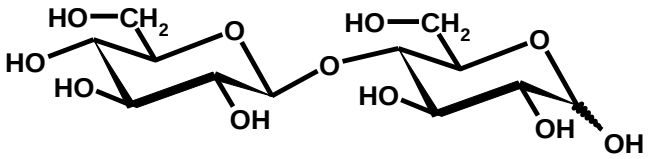
Generalities

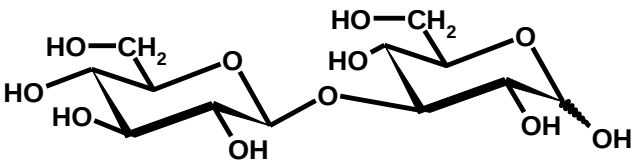
Sacharides – glycosides formation

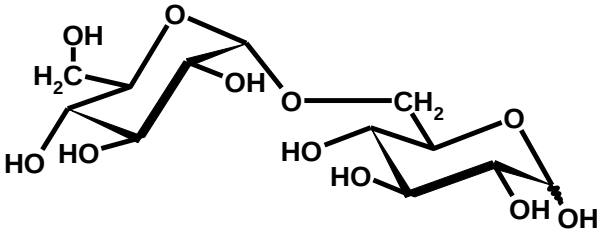




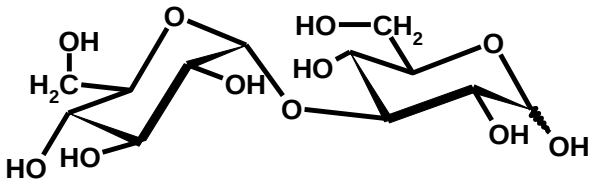
β

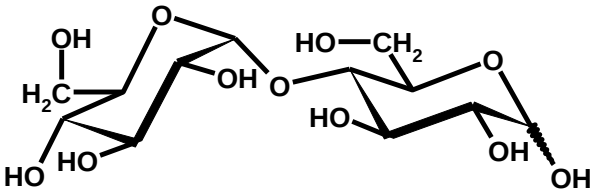


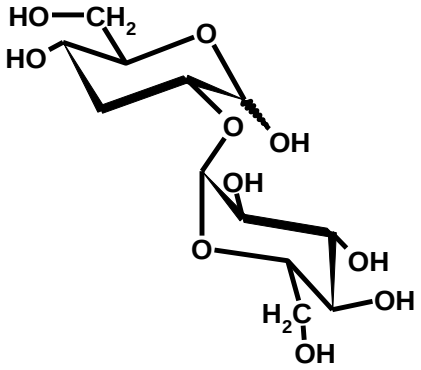




α

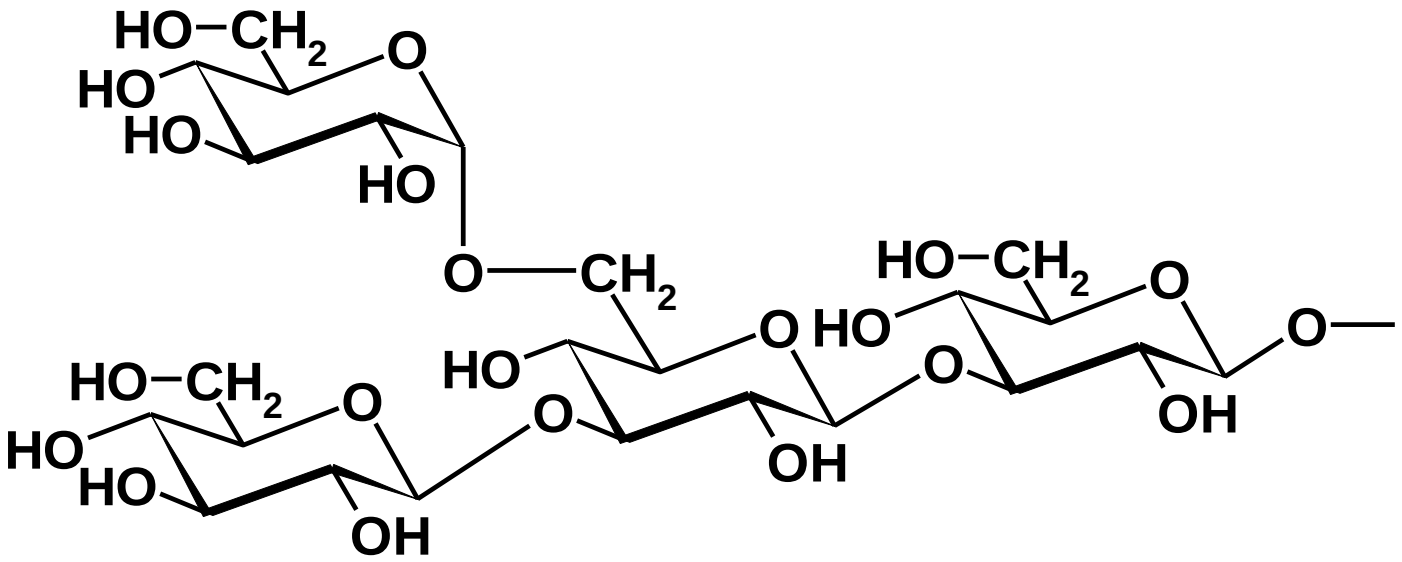






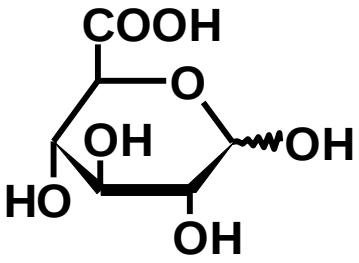
Generalities

Sacharides – glycosides formation

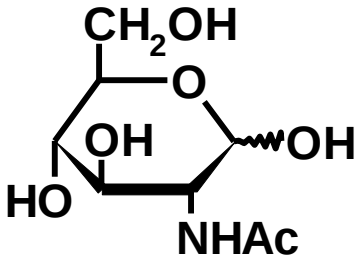


Generalities

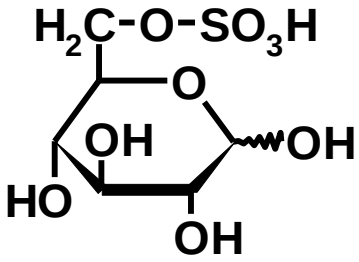
Saccharides – derivatives



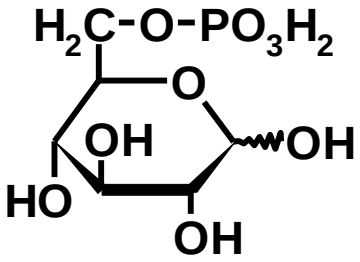
D-glukuronová kyselina



N-acetyl-D-glukosamin



ester s kyselinou sírovou



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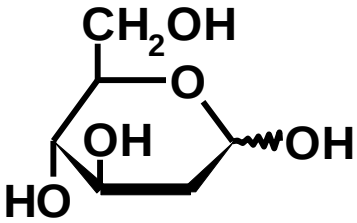
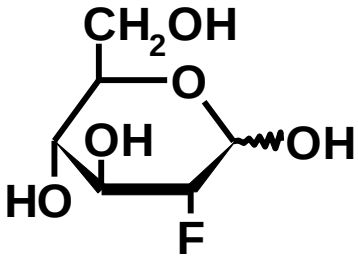


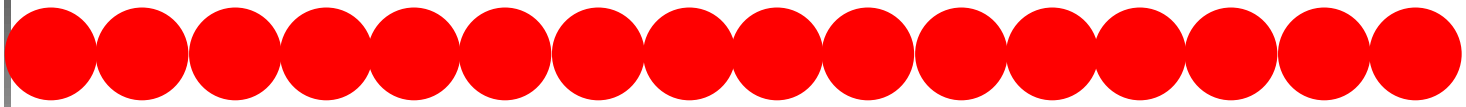
2-deoxy-D-glukosa



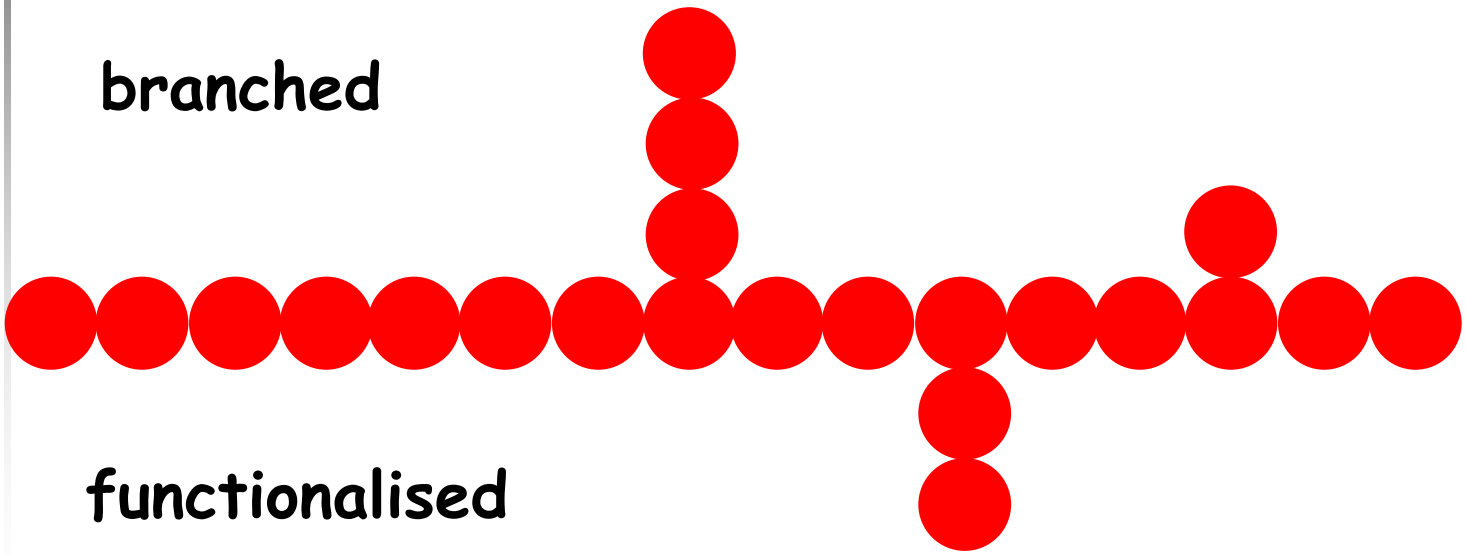
2-deoxy-2-fluor-D-glukosa

Polysaccharides

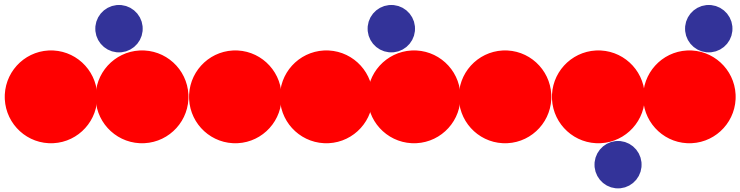
linear



branched



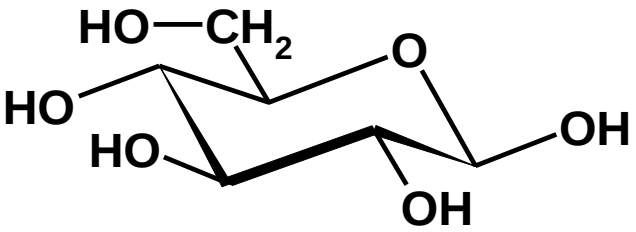
functionalised

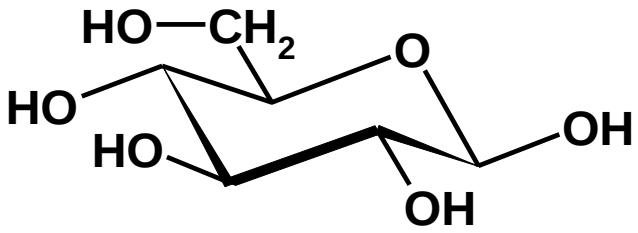


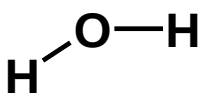
COOH, COOMe, Ac, SO_3H

(Poly)saccharides – common properties

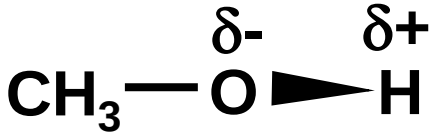
Hydrogen bonds



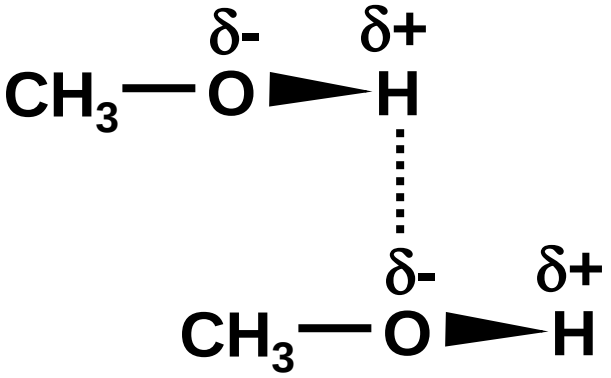




Hydrogen bonds network



Polarized bond
Different electronegativity
Shifting of σ electron pair

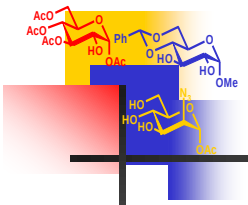


Hydrogen bond
- Intermolecular
- Intramolecular

Renewable sources

10^{14} of metric tons
Of renewable sources/year

sucrose
D-glucose
D-fructose
starch
cellulose



Polysaccharides in Nature

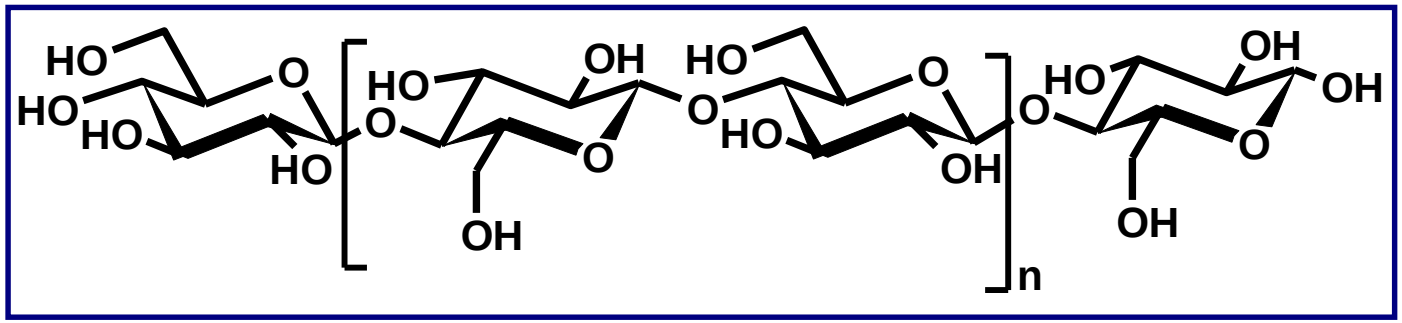
building (cellulose, hemicelluloses, chitin),

stock (starch, inulin),

gums (exudates of damaged plant tissues, protecting role),

mucins (keeping of water, found particularly in perisperms)

Cellulose



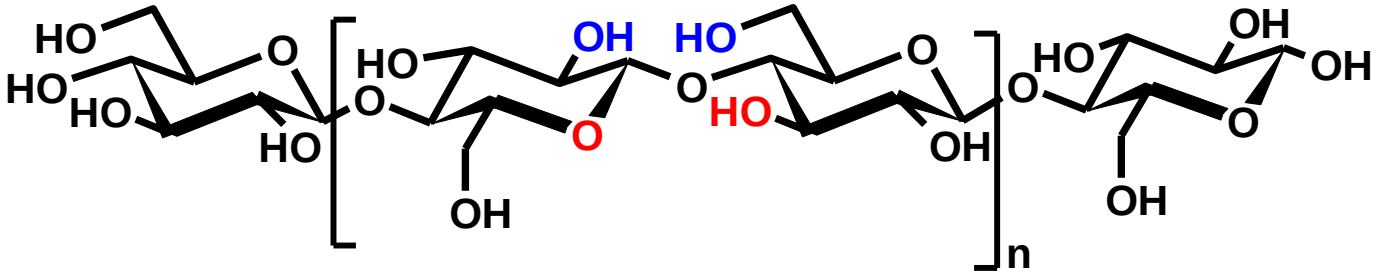
linear polymer, β-(1⟶4)-D-Glcp, $^4C_1$ conformation

2 000 – 14 000 units, water insoluble

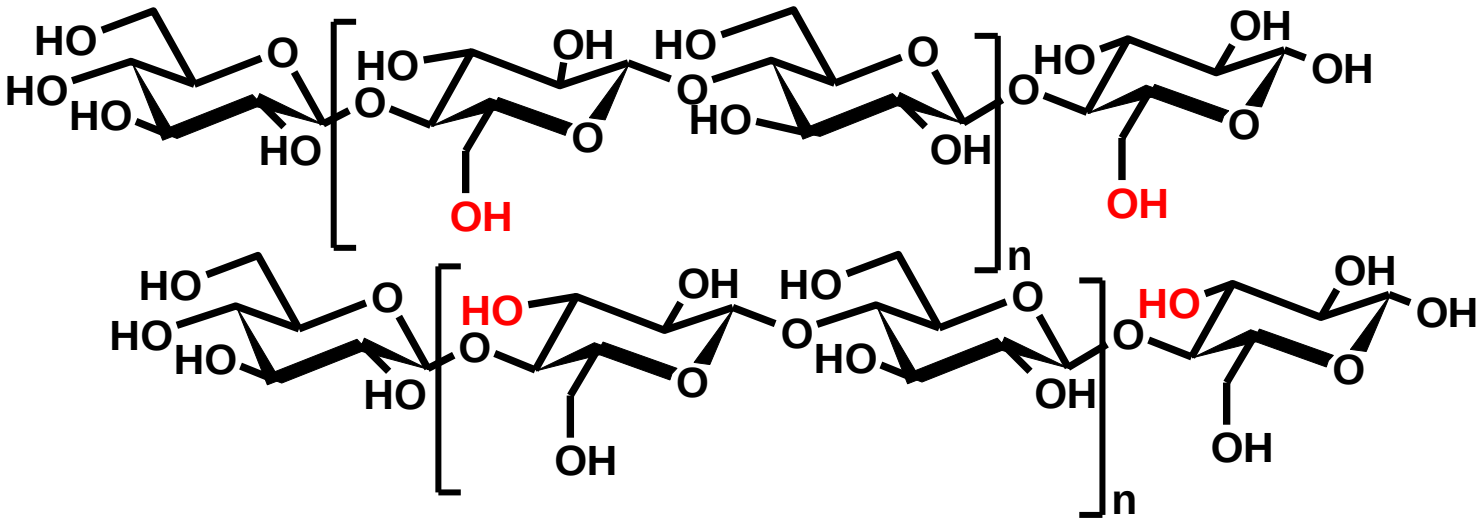
Raw material:
wood
cotton

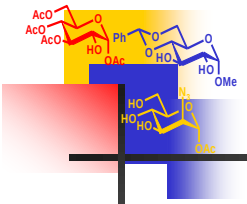
Cellulose – hydrogen bonds

intramolecular



intermolecular, between two paralell chains





Cellulose – use

Food Industry:
emulgator
stabilizer
filling
gelating agent

Other:
paper
bandage material
filters, carriers

cellulose based plastics

Chitin, chitosan

The second most abundant polysaccharide

Invertebrate exoskeleton main component (crustaceans, insects), cell walls of fungi

chitin – polysaccharide composed of *N*-acetyl-glucosamine

chitosan – deacetylated chitin

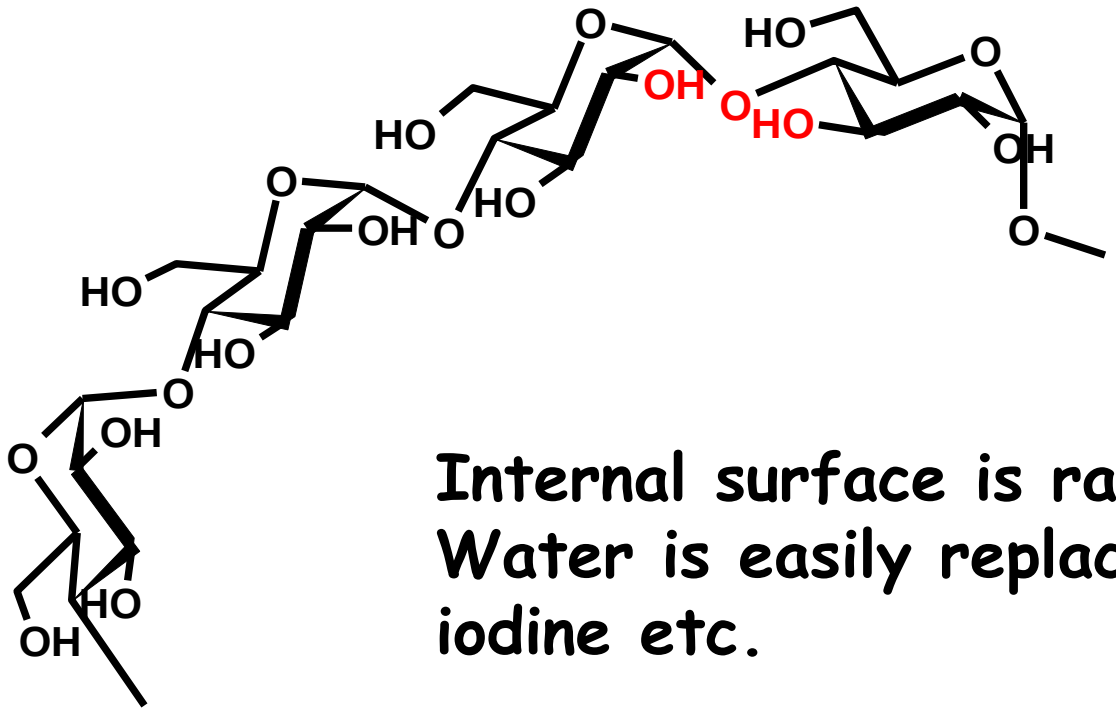
Food complements,
Reduction diets,
Arthrose treatement

Starch

amylose

linear polymer, α-(1⟶4)-D-Glcp, $^4C_1$ conformation

500 – 2 000 units, helical molecule



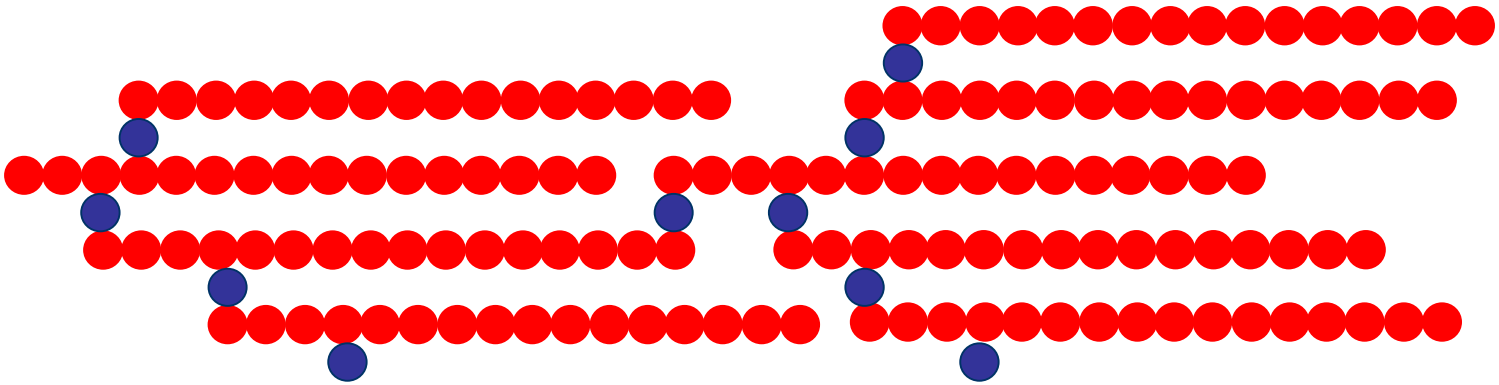
Internal surface is rather hydrophobic,
Water is easily replaced with lipids,
iodine etc.

Starch

amylopectin

linear polymer, α-(1⟶4)-D-Glcp chains regularly branched by α-(1⟶6)-glycosides

Up to 2 000 000 units, some groups may be derivatised



Raw material:

Potatoes, maize, etc.

Starch – use

Both amylose and amylopectin are polydispersal

Properties depennd on amylose/amylopectin ratio (30 - 70 %)

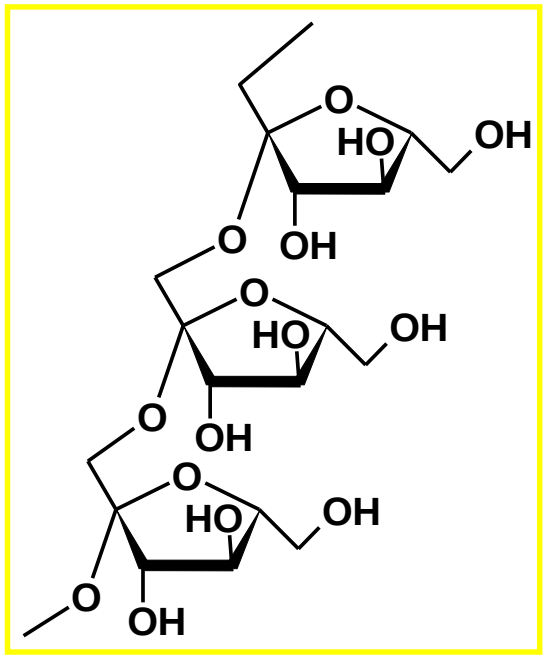
<u>Food industry</u>:
pastry
pudings
filler
meat products additive

glucose sirups
fructose sirups

<u>Other</u>:
D-glucose
modified starch
textile apertures

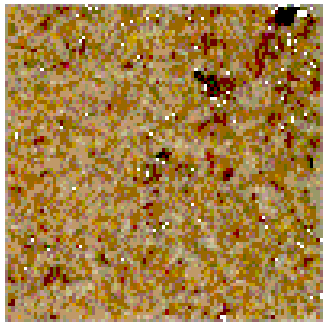
Inulin

fructane, linear polymer, β-(2 → 1)-D-Fruf often terminated with D-Glcp bound α-(1 → 2)
Obtained from cichory (*Cichorium intybus*), roots, 12 – 20 % of inulin, 5 – 10 % oligofructose
Non digestible,
Fiber, used in low-calorie foods, enhances perception of a fruit taste



Gum Arabic

Dried exudate from incisions in stem bark of *Accacia. senegal, A. seyal, A. nilotica*



Highly branched, acid polysaccharide:
β-D-galactose (main chain),
L-arabinose, L-rhamnose, D-glucuronic acid

E414, additive for puddings, pastry,
emulsion stabilizer

glue,
pharmaceutical industry

Tragacanth

Exudate from incissions in bark of *Astragalus gummifer*

tragacanthin (neutral polysaccharide, D-galactose, L-arabinose)
bassorin (poly galacturonic acid Partly methylated)

E413, emulsion stabilizer (pharmaceutical industry, food industry)

Konjac

Amorphophallus conjac (Araceae)

- originally from Asia
- solitary foul-smelling flower
- large leaf

- voluminous tuber,
  rich in 1→4 glucomannan

- konjac flour in food preparation:

  filling in low caloric diets (bakery)
  jellies, capsules.

Guar

Cyamopsis tetragonolobus (Fabaceae)
annual herb cultivated in India, Pakistan, USA, Central America.

The endosperm of the seeds is a D-galacto-D-mannan.

Creamy white powder, insoluble in organic solvents gives viscous water solutions.

Use: Reduction diets, appetite suppressant. Emulsifier and gelifier (E_{412}).

Carob gum (Locust gum)

dried endosperm of seeds of *Ceratonia siliqua*

carob (neutral polysaccharide, D-galactose, D-mannose)

Easily soluble in hot water

E410, ice creams, creamers, reduction diets

Isabgol, Ispaghul

Psylium ovata (chmelík vejčitý)
Endosperm of a perenial herb

Mucilaginous materials isabgol, ispaghul (neutral polysaccharides,
D-xylose, L-arabinose)

Highly swells in water

Adjuvant treatment of intestinal catarh,
Mild laxative, reduction diets

Pectins

Source: fruits, vegetables

Acidic polysaccharides,
Poly D-galacturonic acid, partly methylated,
ocassionaly L-rhamnose, D-galactose,
L-arabinose,

E440a – pectine, E440b – amide

Gelators, stabilizers
Ice creams, marmelades, jellies,
candies.
Chelation of ions of heavy metals

Polysaccharides from algae

Alginates

Products of brown algae (*Fucaceae* family)

acidic polysaccharides, D-mannuronic acid and L-guluronic acid

Block copolymers

polyanionts (as salts)

E400, E401 – Na^+ alginate, E402 – K^+ alginate, E403 – NH_4^+ alginate

Emulsion and suspension stabilizers, antacides, hemostatic powders

Polysaccharides from algae

Carragenans

Products of red algae, the *Rhodophyceae* family

polygalactans,

Polymers of D-galactose,

Partly esterified with sulphuric acid

Several types, (κ, τ, λ)

Viscous solutions, not gels

E407

Inhibitors of ice crystals, mild laxatives,

carriers – pharmaceutics and hygienical preparations

Polysaccharides from algae

Agar

Product of red algae, *Gelidium* sp.and *Gracillaria* sp.

agarose – linear polygalactan, low sulfation,
agaropectin – heterogenous structures

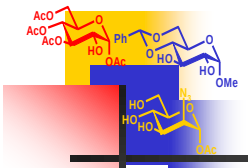
E406
king of gelating polysaccharides,
carrier of solid bacteriological media
electrophoresis carrier

Polysaccharides from microbes and fungi

β-glucans (D-glucose, β(1-3) glycosidic bond, More or less branched

Yeast cell walls

Immune system modulation
Decrease of blood cholesterol,
cosmetics,



Polysaccharides from microbes and fungi

Dextranes

α(1-6) glycosidic bond, linear polymer of D-glucose partly branched by α(1-4), α(1-2) and α(1-3)

Leuconostoc mesenteroides, Streptococcus mutans

Native dextranes – m.w. more than 5×10^6 Da

Partly hydrolysed – fractions around 60 kDa:
blood plasma substitution – emergency
artificial tears - ophtalmology

Crosslinked dextranes: chromatographic carriers

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Bioactive Natural Compounds

4. Alkaloids – introduction
– protoalkaloids

Lecturer: Oldřich Lapčík

Alkaloids - generalities

- The term introduced at the beginning of 19-th century (W. Meissner)
- *Al kaly* = soda (Arabic); eidos = appearance (Greek)

- "**True" alkaloids**:
  - **Nitrogen** as a part of a heterocyclic system
  - Biosynthetically derived from amino acids
  - Pharmacologically active

Alkaloids - generalities

- **Pseudoalkaloids** – the skeleton not derived from amino acids – mostly isoprenoids

- **Protoalkaloids** – simple amines, the nitrogen atom is not part of a heterocyclic ring. Basic, derived from amino acids.

- **Purine alkaloids** – derived from purine bases (e.g. caffein, theobromin)

Alkaloids – occurence

- **rarely produced by**

  bacteria

  (e.g. pyocyanin from *Pseudomonas aeruginosa*)

  fungi

  (e.g. ergoline alkaloids from the genus *Claviceps*, tryptophan derived alkaloids from *Psilocybe* spp.)

  animals

  (e.g. histrionicotoxin from the frog *Dendrobates histrionicus*, solenopsin A from ants)

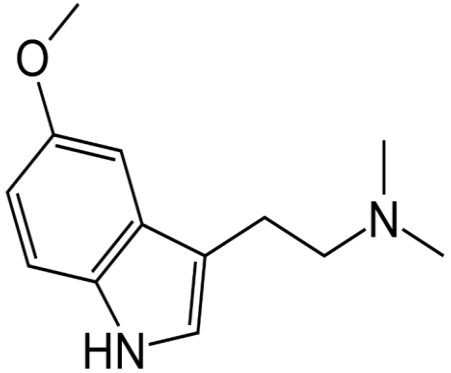
  certain animals accumulate alkaloids from nutritional sources – e.g. certain butterflies, beavers, birds

Dendrobatidae – toxic frogs

Dendrobates azuleus

Dendrobates auratus

Significant sources of 5-methoxy N, N-dimethyltryptamine



- seeds and bark of *Anadenanthera peregrina* tree
- resin and bark of the genus Virola
- skin secret of colorado toads


Bufo alvarius


Virola sebifera


Anadenanthera peregrina

Tryptamines containing fungi

- Genera

Psilocybe, *Pluteus*, *Panaeolus*, *Inocybe*, *Gymnopilus*, *Panaeolina*, *Pholiotona*

Panaeolus subalteus

Gymnopilus dilepsis

Psilocybe baeocystis

Panaeolina foenisecii

Inocybe aeruginascens

Alkaloids – occurence

- **limited repertoir of alkaloids produced by**

  Pteridophytes and **Gymnosperms**

  (lysine – derived alkaloids in certain Lycopodiae)

Alkaloids – occurence, distribution

- **Angiosperms:**
  - **10-15% of angiosperms synthesize alkaloids**
  - **Certain families have a marked tendency to elaborate alkaloids**

  - **Monocots: Amarylidaceae, Liliaceae**
  - **Dicots: Annonaceae, Apocynaceae, Fumaricaceae, Lauraceae, Magnoliaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae, Solanaceae**

Alkaloids – occurence, distribution

Chemotaxonomy

The occurence of certain alkaloids is limited to a specific taxon (e.g. morphinans - the *Papaveraceae*, morphine in *P. somniferum* exclusively)

while some others are found in several distant taxa (e.g. caffeine)

Anatomy

The alkaloid content may vary by several orders of magnitude in different anatomical parts

Different alkaloids may be present in one plant, usually of the same metabolic origin

Alkaloids – Physico-chemical properties

- **m.w. – 100-1000 Da**
- **Relatively stable**

- **Oxygen-containing alkaloids:**
  - usually colorless, optically active
  - crystalizable solids
  - sharp melting points, without decomposition

- **Oxygen-free alkaloids:**
  - liquids, some of them relatively volatile

Alkaloids – Physico-chemical properties

- **Soluble in**
  - slightly polar organic solvents (CH_2Cl_2)
  - alcohols
  - acified water,

- **Rather limited solubility in**
  - water, alkaline solutions

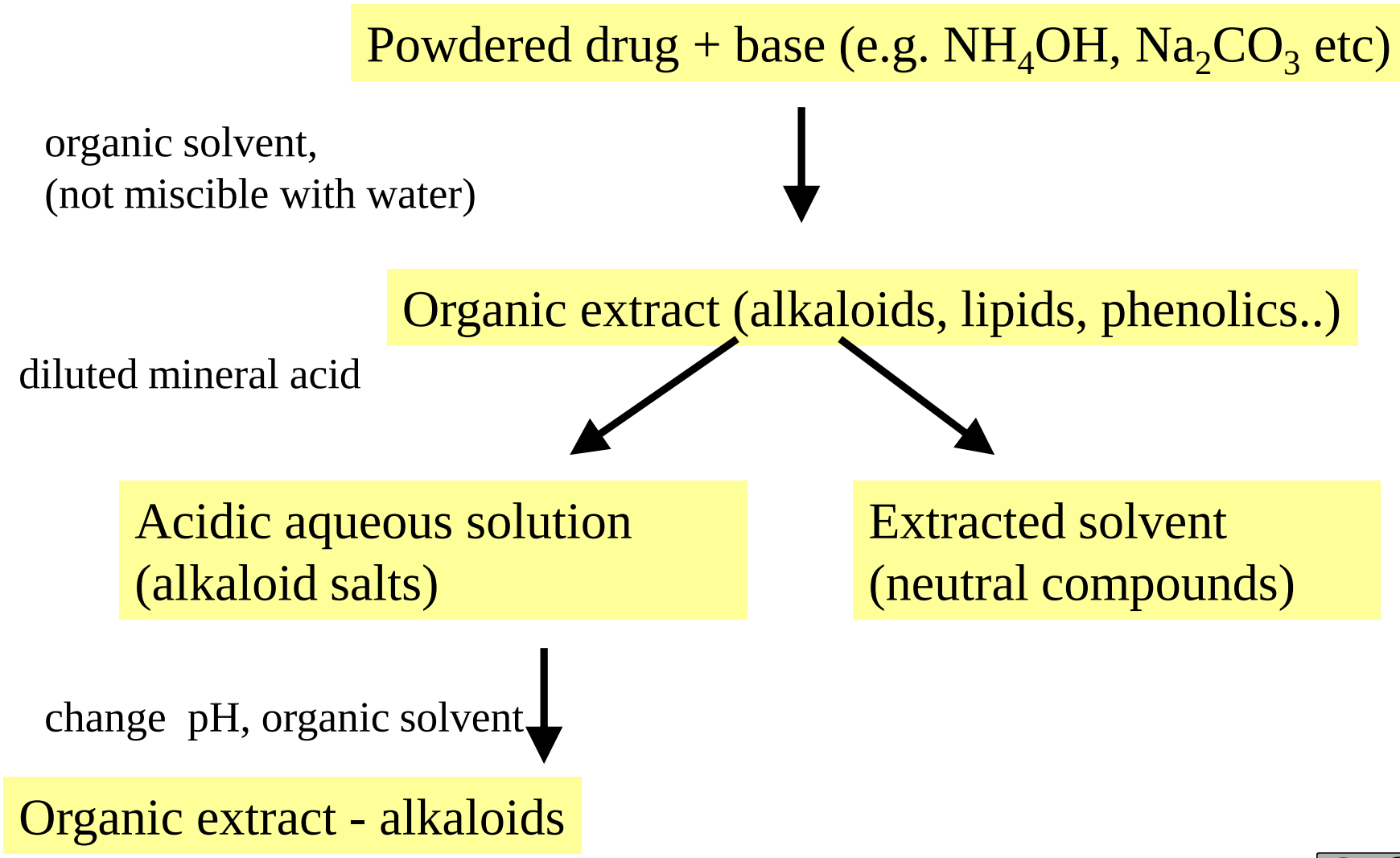
Alkaloids – Detection and Characterization

TLC

- **Mayer´s reagent** - ($KI + Hg_2Cl_2$)
- **Dragendorf´s reagent** – ($KI + BiNO_3$)

- *p*-dimethylaminobenzaldehyde (ergot alkaloids)
- ninhydrin (amines)
- $Ce(SO_4)_2 + (NH_4)_2SO4$ (indoles,dihydroindoles)

Alkaloids – Extraction in Alkaline Medium



Alkaloids – Extraction in Acidic Medium

Powdered drug
+ mineral acid (e.g. HCl, H_2SO_4 etc.) (+ alcohol)

change pH (alkaline)
organic solvent,
(not miscible with water)

Organic extract (free alkaloid bases)

diluted mineral acid

Acidic aqueous solution
(alkaloid salts)

change pH, extraction with
organic solvents or ion-exchange resins

Organic extract - alkaloids

Alkaloids – Special Extraction Techniques

Optimized for individual alkaloids and alkaloids sources

- Steam distillation (e.g. spartein)
- Adsorption on – styrene-divinylbenzene resins
  - diatomaceous earths
-

Alkaloids – Pharmacological activity, uses and abuses

CNS depressants (e.g. morphine, scopolamine)
CNS stimulants (e.g. strychnine, caffeine)

Sympathomimetics (e.g. Ephedrine)
Sympatholytics (e.g. Yohimbine)

Parasympathomimetics (e.g. Pilocarpine)

Anticholinergics (e.g. Atropine)

Local anesthetics (e.g. Cocaine)

Alkaloids – Pharmacological activity, uses and abuses

Antibacterials (e.g. Sanguinarine)

Antimalarials (e.g. Quinine)

Amebicides (e.g. Emetine)

Cytostatics (e.g. Vinblastine, Colchicine)

Alkaloids – Pharmacological activity, uses and abuses

Therapeutics

Psychotropic illicit drugs,

Poisons – criminal, hunting, war poisons
- ordeal poisons
- criminal abortives

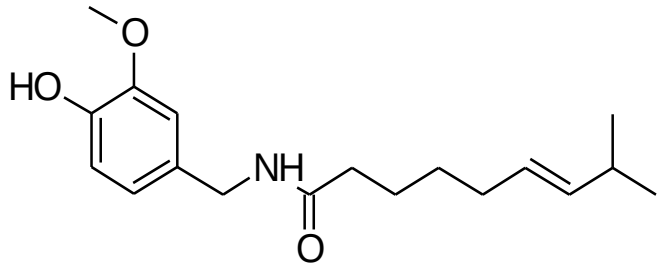
Pesticides

Food contaminants (solanine, ergot alkaloids)

Culturally accepted addictions (caffeine, nicotine, arecoline)

Protoalkaloids

- Capsaicin from *Capsicum sp.*, Solanaceae

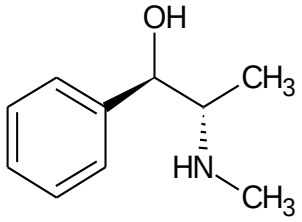


Capsaicin – the base of pungent taste in hot peppers: *Capsicum annuum*, *Capsicum frutescens* etc.

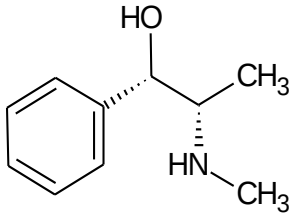
Target structure:	Vanilloid receptor TRPV1
Vay of action:	ligand opened Ca^{2+} channel.

Protoalkaloids

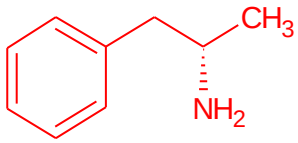
- Ephedrine from *Ephedra sp.*, Ephedraceae



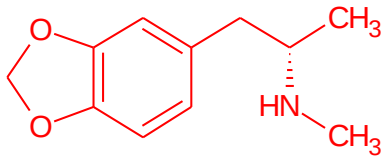
Ephedrine



Pseudoephedrine



Amphetamine



Ecstasy

3,4 (Methylenedioxy)methamphetamine

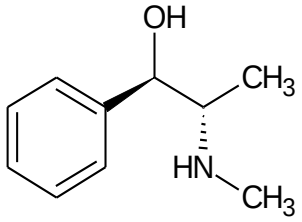
Synthetic analogues

Ephedrine, Pseudoephedrine –
sympathomimetics
originaly obtained from *Ephedra sinensis* and other *Ephedra sp.*
recently synthesized by fermentation

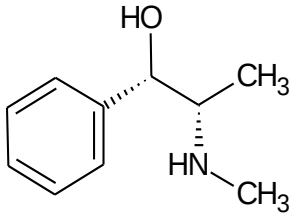
Amphetamines – synthetic analogues of ephedrine, illicit drugs

Protoalkaloids

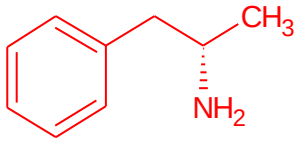
- Ephedrine from *Ephedra sp.*, Ephedraceae



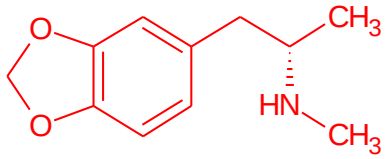
Ephedrine



Pseudoephedrine



Amphetamine



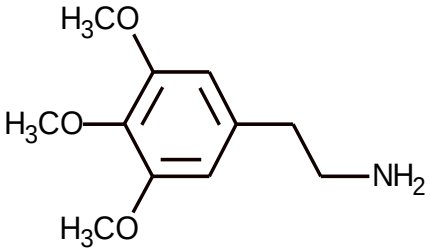
Ecstasy

3,4 (Methylenedioxy)methamphetamine

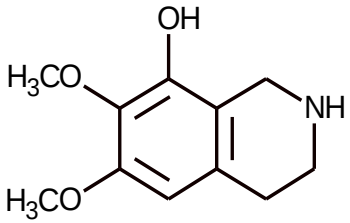
Ephedrine, Pseudoephedrine –
sympathomimetics
originally obtained from *Ephedra sinensis* and other *Ephedra sp.*
recently synthesized by fermentation

Amphetamines – synthetic analogues of ephedrine, illicit drugs

Protoalkaloids



Mezcaline



Anhalmine

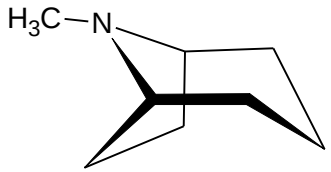
- Peyote – *Lophophora williamsi*, Cactaceae
- Considered a divine plant by the Aztecs
- A potent hallucinogen
- Globular cactus, up to 20 cm in height, 5-10 cm in diameter
- Peyote grows in North Mexico and in Texas

- About 50 alkaloids, phenetylamines and tetrahydroisoquinolines (up to 6% dry weight)
- Causes psychic effects (similar to those of LSD) –

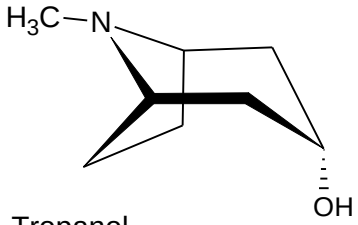
Peyote – *Lophphora williamsi*

- distortion of the perception of shapes, intensification of colors, auditory hallucinations
- Tachycardia, bradypnea, nausea, anxiety
- High doses – memory loss, hypertensive encephalophathy, intracranial hemorrhage.

Tropane Alkaloids

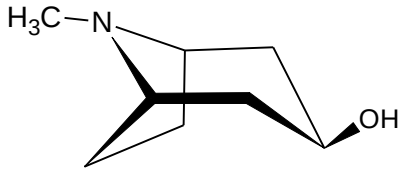


Tropane



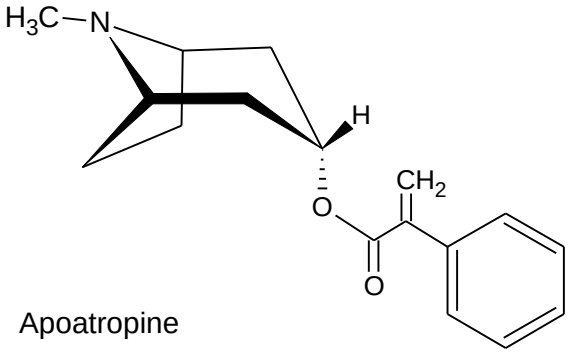
Tropanol

3α-hydroxytropane

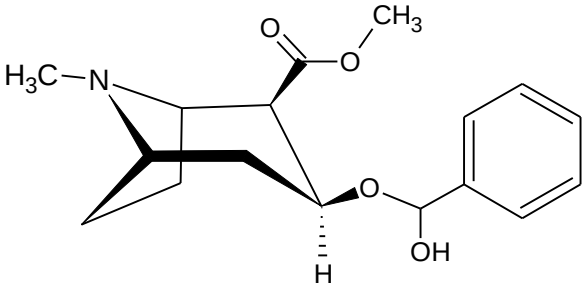


Pseudo-tropanol

3β-hydroxytropane



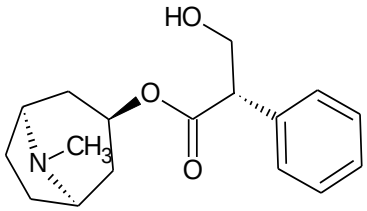
Apoatropine



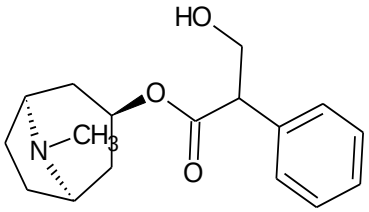
Cocaine

Tropane Alkaloids

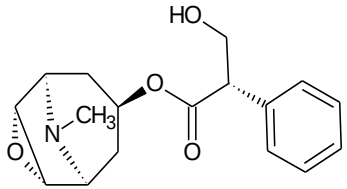
Deadly Nightshade
– *Atropa belladona*



Hyosciamine



Atropine

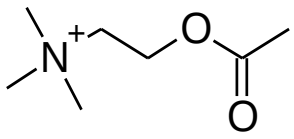


Scopolamine

Indigenous to western Europe
Drug: folium, radix

0.3-0.6% alkaloids, hyoscyamine (90%)
scopolamine (2%)
Racemization of hyoscyamine - Atropine

Reversible inhibition of acetylcholine binding to the receptors
Inhibition of muscarinic receptors



acetylcholine

Tropane Alkaloids

Deadly Nightshade – *Atropa belladona*

Atropine

- **Heart:** temporary bradycardia, followed by tachycardia
- **Smooth muscles** – relaxation, motor inhibition
  (decrease of the intestinal tone, amplitude and frequency)
  (blocking of acetylcholine effect on bronchoconstriction)
- **Sweat and saliva** secretion – decreased
- **Eye** – passive mydriasis
- **CNS** – high doses - excitation, disorientation, exaggerated reflexes, hallucinations, mental confusion, insomnia.

Tropane Alkaloids

Black henbane

– *Hyoscyamus niger*

Tropane Alkaloids

Thorn apple, Jimsonweed (US)
– *Datura stramonium*

Tropane Alkaloids

Henbane

– *Hyoscyamus niger*

0.04-0.15% total alkaloids

Hyoscyamine, scopolamine

Thorn apple

– *Datura stramonium*

0.2-0.5% total alkaloids

Hyoscyamine, scopolamine

Tropane Alkaloids

Atropine – indications

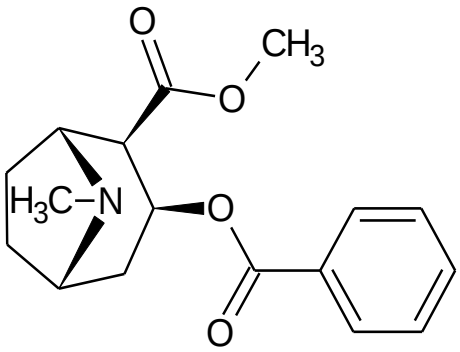
- A-V block, myocardial infarction,
- Anesthesiology (preanesthesia)
- Symptomatic treatment of GIT, biliary tract
- Antispasmodic for ureteral colic
- **Specific antidote to treat acetylcholinesterase poisoning** (e.g. organophosphates)
- Eye drops
- Parkinson disease

Tropane Alkaloids

Cocca

– *Erythroxylon cocca*

Tropane Alkaloids



Cocaine

- Local anesthetic
- Parasympathomimetic

- Causes:

  hyperthermia, mydriasis, vasoconstriction,

  high blood pressure, tachycardia

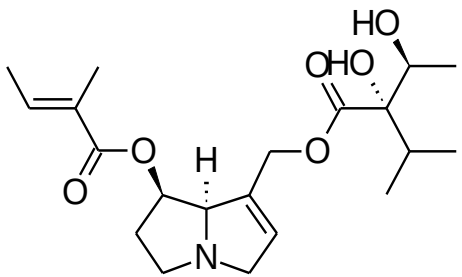
  CNS – euphoria, hyperactivity, sensation of intelectual stimulation,

  - psychic dependence develops rapidly

    (especially in IV users and smokers)

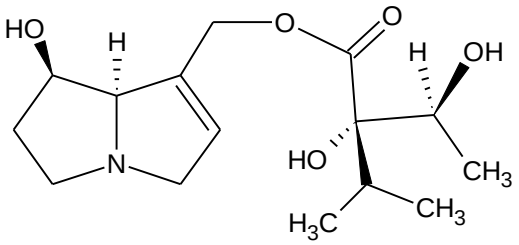
Pyrrolizidine Alkaloids

- Common Comfrey
  - *Symphytium officinale*
  - Healing properties, topical use.



Symphytine

Pyrrolizidine Alkaloids



Lycopsamine

Borago officinalis

- Borage
  - *Borago officinalis*
  - Borage oil – unsaturated fatty acids
  - Leaves

    traditionally used as diuretics

    and expectorants

    Not reccomended or even forbidden in some EU countries

Pyrrolizidine Alkaloids

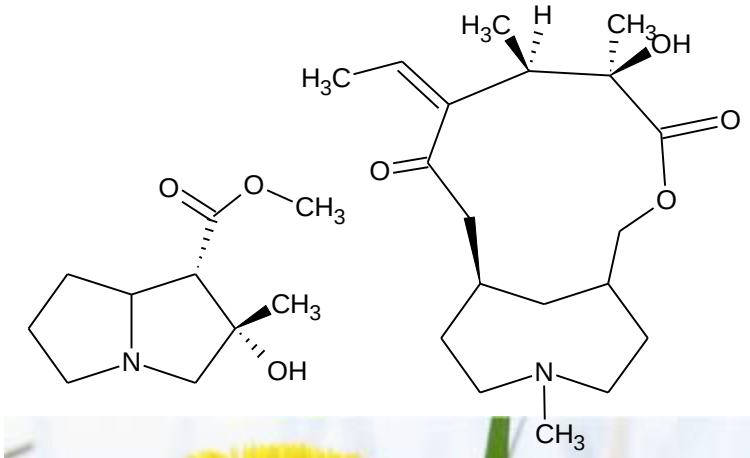
- Coltsfoot
  - *Tussilago farfara*
  - Capitulum contains:

    acidic mucilage, flavonoids, carotenoids, triterpenes and pyrrolizidine alkaloids

    Traditionally used as

    antitussive, expectorant.

    Not reccomended or even forbidden in some EU countries



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

5. Alkaloids – "True alkaloids" (continuation)

Lecturer: Oldřich Lapčík

Isoquinoline alkaloids

- Simple Tetrahydroisoquinolines
- Benzyltetrahydroisoquinolines
- Morphinanes
- Protoberberines
- Phenetylisoquinolines

Tetrahydroisoquinolines

- Peyote – *Lophophora williamsi*, Cactaceae
- Considered a divine plant by the Aztecs
- A potent hallucinogen
- Globular cactus, up to 20 cm in height, 5-10 cm in diameter
- Peyote grows in North Mexico and in Texas

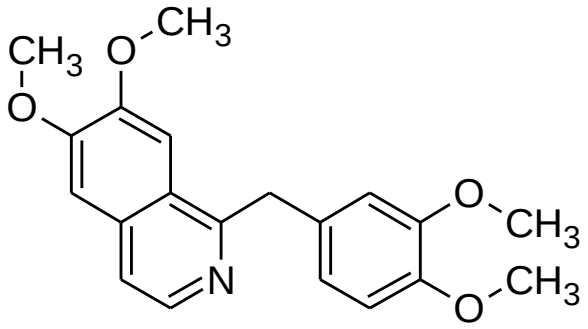
- About 50 alkaloids, phenetylamines and tetrahydroisoquinolines (up to 6% dry weight)
- Causes psychic effects (similar to those of LSD) –

Peyote – *Lophphora williamsi*

- distortion of the perception of shapes, intensification of colors, auditory hallucinations
- Tachycardia, bradypnea, nausea, anxiety
- High doses – memory loss, hypertensive encephalophathy, intracranial hemorrhage.

Benzyltetrahydroisoquinolines

Papaverine



- Practically no effects on CNS
- Potent spasmolytic, relaxes smooth muscle fibers in cerebral, pulmonary and systemic perpheral blood vessels.
- Used as a smooth muscle relaxant
- Organic synthesis available

Bisbenzyltetrahydroisoquinolines

- Over 400 compounds occuring in about ten families (*Menispermaceae*, *Ranunculaceae*, *Berberidaceae*, *Monimiaceae*, *Annonaceae*, *Lauraceae* etc.)

- CURARE

  - complex products, of variable botanic origin

  - almost identical pharmacological properties.

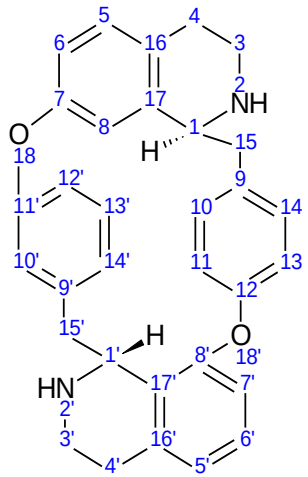
Used for coating the tips of the blow darts or the arrows by the natives of South America

• CURARE

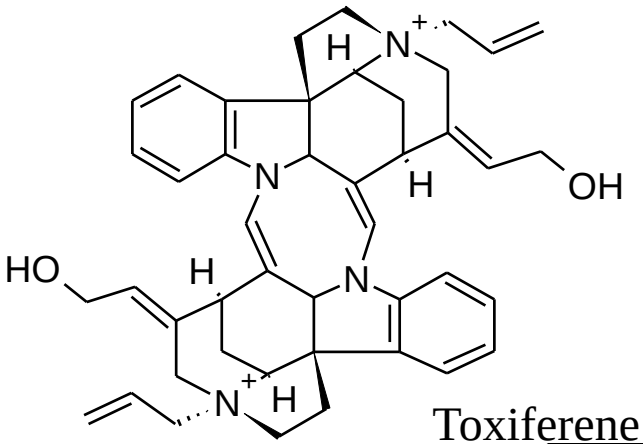
- Immediate muscle relaxation – the animal cannot flee
- Animals fall to the ground
- Curare is toxic only by the parenteral route, so that the game caught with it can be consumed safely

- Used as hunting poison, not as war poison

- Tubocurarine
- Toxiferine



Tubocurarane



Toxiferene

- CURARE

- Active only in parenteral route
- Competes with acetylcholine for the cholinergic receptors

- Decrease in muscular tone – atony – progressive paralysis

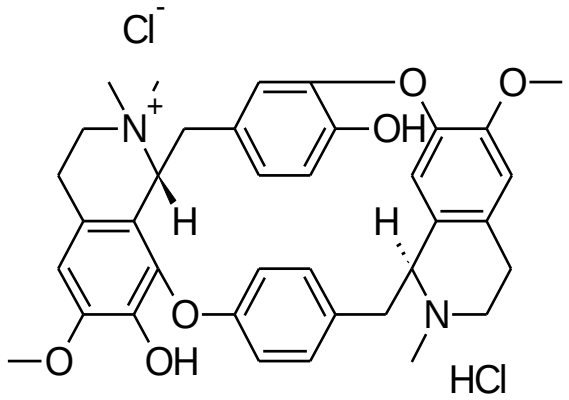
- Peripheries, eyelids and face, then abdominal and respiratory muscle, diaphragm.

- CURARE

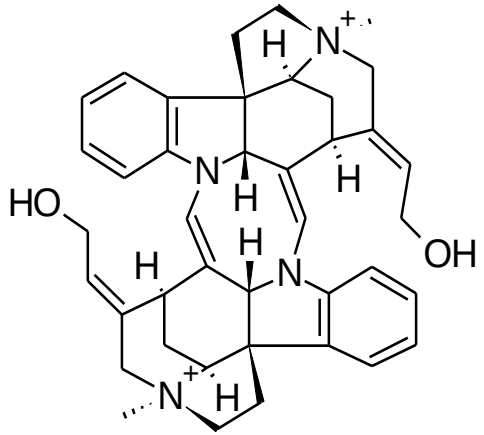
- 1820 – Charles Walerton – the effect of Curare is temporary

- 1935 – Frederick Prescot (*Borroughs Wellcome*) – use of curare to achieve myorelaxation before surgery or intubation

- Currently – semisynthetic derivatives of C-Toxiferine are used.

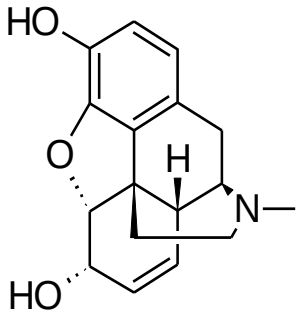


Tubocurarine - chloride



Toxiferine I

Morphinanes



- Morphinan alkaloids are typical for the *Papaver* genus (about 100 species)
- About 10 *Papaver* sp. synthesizes thebain
- Only *P. somniferum* and *P. setigerum* synthesize morphine

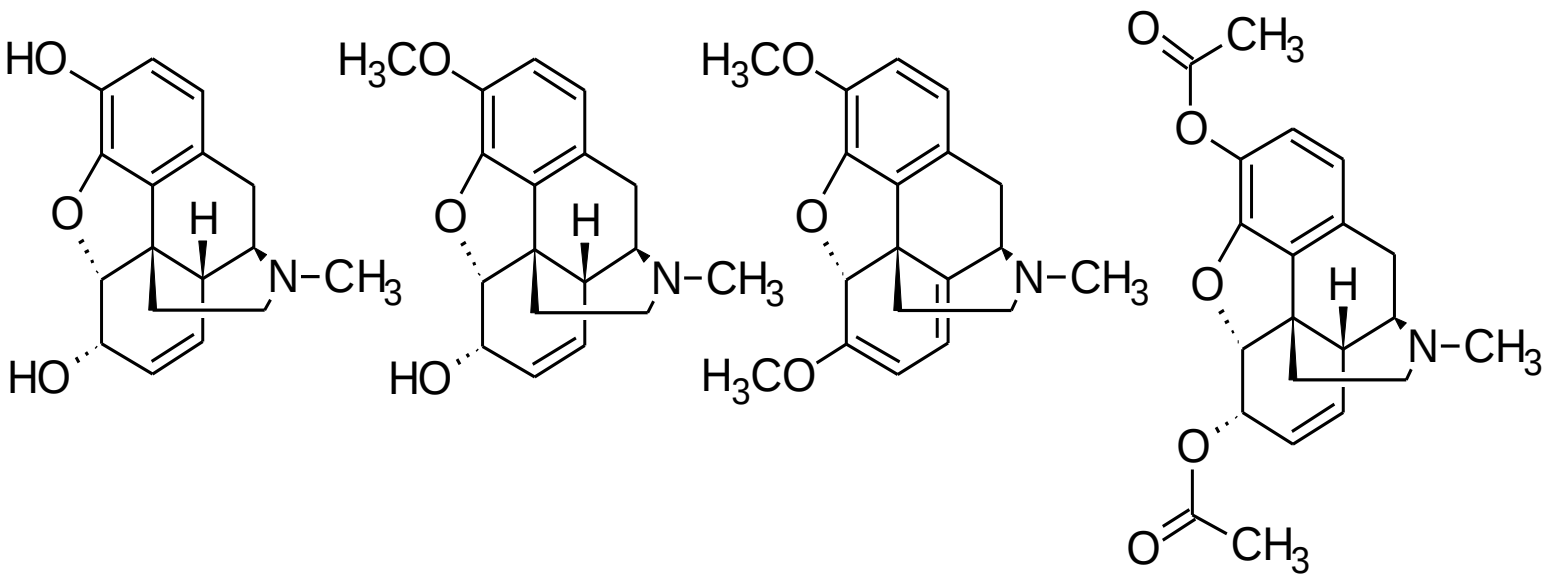

Papaver somniferum


Papaver rhoeas

Morphinanes

- *Papaver somniferum* – Opium poppy
- About 10 *Papaver* sp. synthesize thebain
- Only *P. somniferum* and *P. setigerum* synthesize morphine
- Raw opium: the air dried latex obtained by incision of unripe capsules of *P. somniferum*:
  about 10% of morphine, about 2% of codeine

Morphinanes



morphine codein thebain heroin

(diacetyl morphine, semisynthetic)

Morphinanes

- Opium production – 330 t of morphine per year (1997)
  (main exporters: India, France, Australia, Turkey)

- Opium: 10-15% water
  20% sugars
  organic acids (lactic, fumaric, oxaloacetic, meconic a.)
  up to 20% alkaloids: morphine, codeine, thebaine, codeinone, oripavine.

Morphine: pharmacological activity

- CNS
- Analgesia – morphine raises the threshold of pain perception

- Psychomotor activities depend on the animal species and on pre-existing pain.

  Subjects in pain: sedation, sometimes a state of euphoria

  Normal subjects: agitation, delirium, anxiety, nausea

Morphine: pharmacological activity

- Respiratory effects: Reduced sensitivity to CO_2

  Bradypnea, irregular rhythm

  Depression of the cough center

- Pituitary: decrease of secretion of FSH, LH, ACTH

- Dependence: Psychic dependence – the transient sensation of well being, euphoria, soon followed by tolerance (the requirement for increasing doses to obtain the same effect)

  Withdrawal: rhinorrhea, sweating, lacrimation, mydriasis, pain in the joints and muscles, anxiety, insomnia. Later: tachycardia, polypnea, nausea.

  Medical intervention needed.

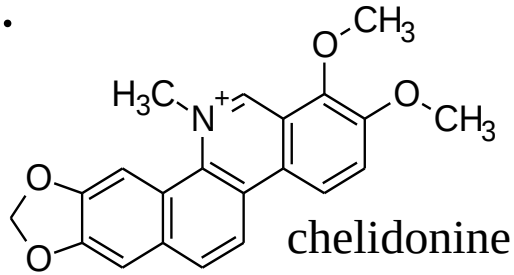
- CODEINE

- Antitussive and analgesic activity – used as codeine phosphate in combinations with other substances (e.g. Paracetamol)

- Codeine *per os* is used as a heroin substitute by some addicts.

Protoberberine alkaloids

- Found in the *Berberidaceae*, *Menispermaceae*, *Papaveraceae*, *Ranunculaceae*, *Annonaceae*.



chelidonine

- Greater celandine – *Chelidonium maius*

  A perrenial herb, flowers with yellow petals. When damaged, the plant produces an orange colored latex.

  2% of alkaloids: chelidonine, chelerythrine, sanguinarine.

  In folk medicine used as remedy for warts.

Chelidonium maius

Protoberberine alkaloids

- Bloodroot – *Sanguinaria canadensis*
  A perennial herb, producing a red latex, common in North America.

  Up to 7% of alkaloids in the rhizome: chelerythrine, sanguinarine.

  Sanguinarine has antimicrobial, antifungal and anti inflammatory properties.

  Sanguinarine HCl: used in mouthwashes and toothpastes.

Phenethylisoquinoline alkaloids

- Autumn crocus (meadow safron)– *Colchicum autumnale*

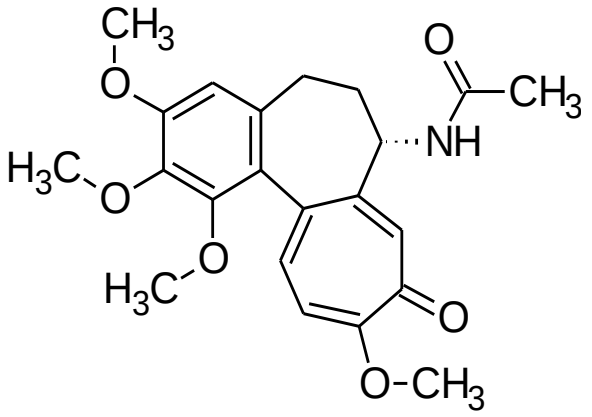
Known to Antic Greeks (Dioskorides).

Used in the Byzantine empire since the fifth century to treat gout.

Seeds: 0.3-1.2% of alkaloids, only weakly basic, some of them as glycosides.

Colchicine – about 0.6%.

Colchicine



- Toxic, the intoxication causes:

  swallowing difficulties, abdominal pains with diarrhea, hypotension, respiratory difficulties.

  Serious intoxications: the death several days after the intoxication – a respiratory or cardiovascular collapse.

- Antimitotic properties: Preventing the mitotic spindle formation, blocking the mitosis at metaphase.

  (Interaction with tubulin)

Galanthamin

- *Galanthus nivalis* - Snowdrop (Schneeglokchen)
- *Narcissus* sp. - Daphodile

- Galanthamin – acetylcholinesterase inhibitor
- Acts against curare
- Improves results of memory tests
- Treatment of Alzheimer disease – modulation of cholinergic nerves, improvement of cognitive performance.

Alkaloids derived from tryptophan

- Simple amines
- Indolines
- Ergolines
- Monoterpenoid Indole alkaloids

Tryptamines

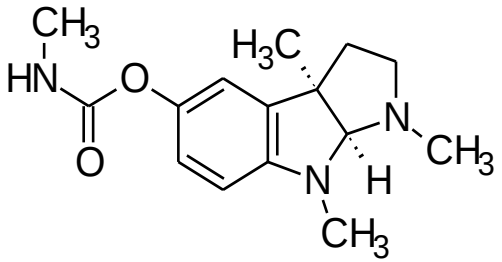
- The *Agaricaceae* family
- The genera: *Psilocybe*, *Conocybe*, *Panaeolus*, *Stropharia*.

- Hallucinogenic alkaloids – Psilocin, psilocybin

- Mydriasis, muscle relaxation
- Visual hallucinations with shape distortion, color intensification, distorted perception of time and space

- In some people – panic and psychosis may occur

Calabar bean -*Physostigma venenosum*



physostigmine
(eserine)

- Seeds: 0.2-0.3% of alkaloids
- Traditionally used as ordeal poison

- Physostigmine: reversible choliesterase inhibitor
- Parasympathomimetic

- Used as antidote against parasympatolytics (atropine, hyoscyamine)

- In therapeutics often replaced by synthetic analogues: Neostigmine, Pyridostigmine.

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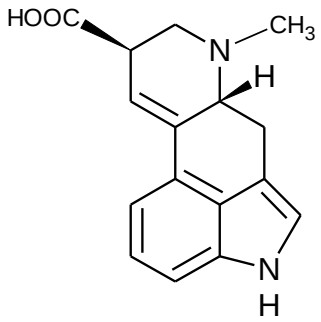
Bioactive Natural Compounds

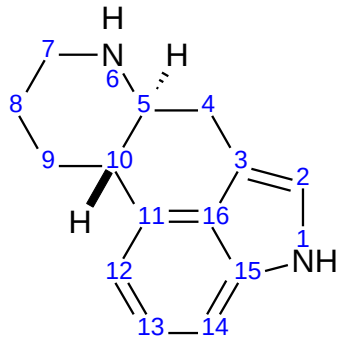
6. Alkaloids

- True alkaloids (continuation)
- Pseudoalkaloids

Lecturer: Oldřich Lapčík



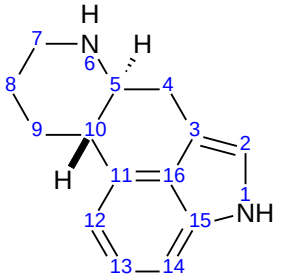
Ergoline alkaloids



- *Claviceps* sp. – about 50 species, several of them infest the *Poaceae*
- *C. purpurea* – Ergot of rye – grows on *Secale cereale*, *Triticale*; *C. paspali* – on *Paspalum* spp.; *C. fusiformis* – on *Pennisetum* spp.
- Other fungi: *Aspergillus*, *Balansia*, *Penicilium*, *Rhizopus*
- Plants: The *Convolucaceae* family – the genera *Argyreia*, *Ipomoea*, *Turbina*, *Strictocardia*

…ovice nachová

Ergoline alkaloids



- Around 1000 A.D.: St. Anthony´s fire epidemic in western Europe

- Ergotism:

  -Gangrenous form: inflammation of the extremities up to spontaneous loss of the joints (St. Anthony´s fire)

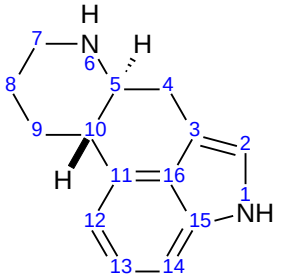
  -Convulsive form: mental agitation, delirium, sensory perturbations

- The prevalence of ergotism

  - In Europe the decrease connected with the agricultural and social improvement (19-th Century)

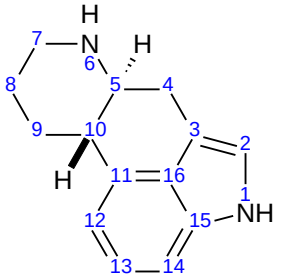
  - Soviet Union 1926, Ethiopia 1977-78 (47 deaths)

Ergoline alkaloids



- 1582 – LONICER – *Pulvis parturiens*
- Seventeenth century: infested rye was suspected to cause ergotism.
- 1711 – the fungal nature of ergot recognized
- 1853 – the development cycle of ergot elucidated
- 1918 – STOLL – isolation of ergotamine

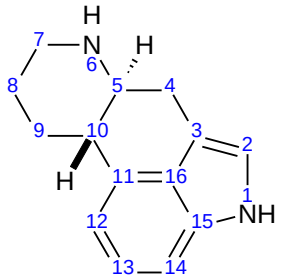
Ergoline alkaloids - production



- Field cultivation
  – *Triticale* (x *Triticosecale* sp.) used as the feeding plant.
  -Yield up to 1000 kg/hectare

- Industrial fermentation
  – Synthetic media, control of pH, oxygenation an nutrients
  – the possibility to influence the alkaloid production.

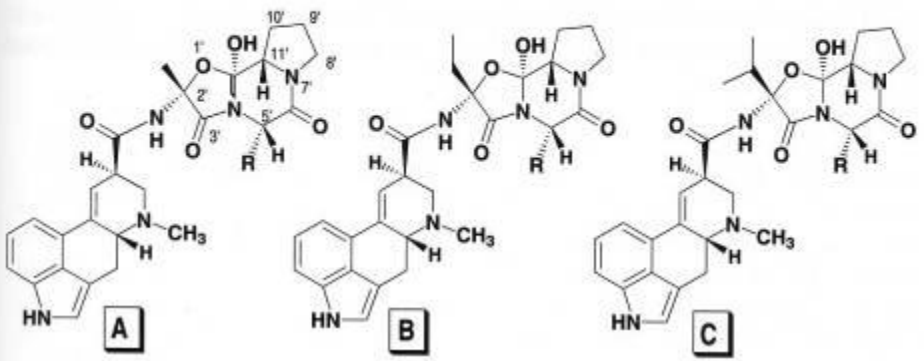
Alkaloids of *Claviceps purpurea*



- Simple amides of lysergic acid (cca. 20% of total alkaloids)

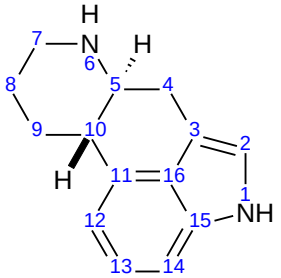
- Ergopeptines
  (80% of total alkaloids):
  Ergotamines
  Ergoxines
  Ergotoxines

Principal alkaloids of the ergot of rye, Claviceps purpurea



	A : Ergotamines	B : Ergoxines	C : Ergotoxines
R = CH_2 Ph	*Ergotamine*	*Ergostine*	*Ergocristine*
R = CH_2 $CH(CH_3)_2$	α-*Ergosine*	α-*Ergoptine*	α-*Ergocryptine*
R = $CH(CH_3)CH_2CH_3$	β-*Ergosine*	*	β-*Ergocryptine*
R = $CH(CH_3)_2$	*Ergovaline*	*Ergonine*	*Ergocornine*
R = CH_2CH_3	*Ergobine*	*Ergobutine*	*Ergobutyrine*

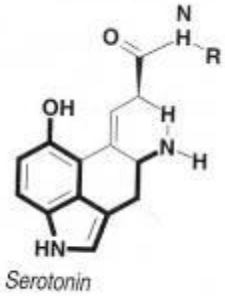
Pharmacological activity



- Complex, caused by interaction with several classes of receptors: Nor-adrenaline, dopamine, serotonin

Nor-adrenaline

Dopamine

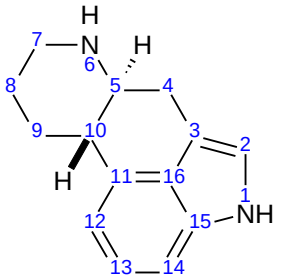


Serotonin

- Modulation of specific sub-types of receptors by individual alkaloids – tissue specific mode of action

- Different ability of individual compounds to cross the blood-brain barrier

Pharmacological activity



- Methylergometrine maleate

  – used in obstetric emergencies: post-partum hemorrhages, after cesarean section, after abortion, for uterine atony etc.

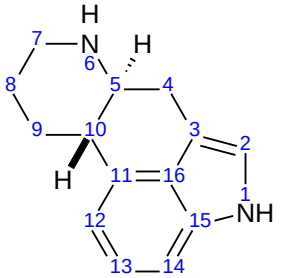
- Ergotamine tartarate

  - treatment of migrains and related vascular headaches

- Dihydroergotamine

  - migrains, vascular headaches, venous and lymphatic vessels insufficiency, orthostatic hypotension.

Pharmacological activity



- Dihydroergotoxine, dihydroergocristine

  – proposed for oral administration as a treatment of senile cerebral insufficiency.

- Nicergoline

  - treatment of the chronic arterial dissease of lower limbs

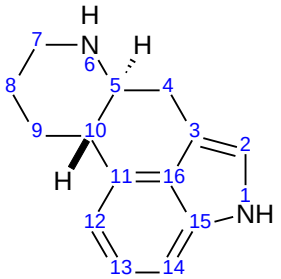
  - treatment of senile cerebral insufficiency

- Bromocriptine

  - basic treatment of prolactin secreting adenomas

  - treatment of clinical cosequences of hyperprolactinemia

Pharmacological activity



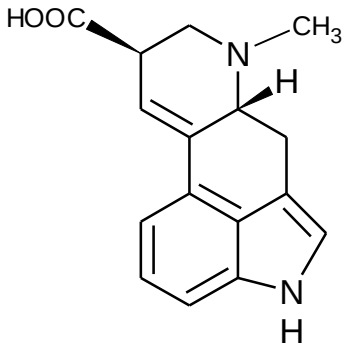
- LSD – diethylamide of lysergic acid

  – semisynthetic.

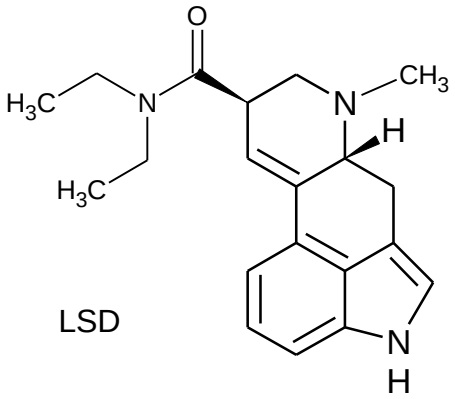
  - hallucinogenic

  - tested in psychiatric research in 70-ies

  - forbidden



Lysergic acid



LSD

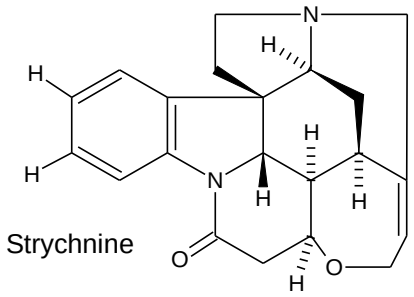
Monoterpenoid Indole Alkaloids

- Biosythetically derived from tryptamine
- 10 carbon building block originates in isoprenoid metabolic pathway

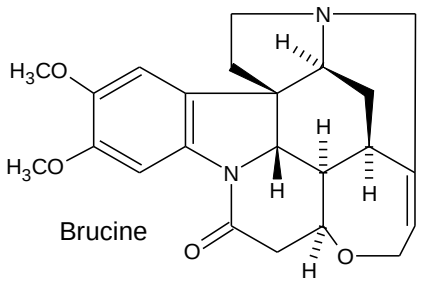
- Found in the families:
  - *Loganicaceae*: the genera *Strychnos*, *Gelsemium*
  - *Rubiaceae*: the subfamilies *Rubioideae* and *Cinchonoideae*
  - *Apocynaceae*: the subfamily *Plumeroideae*

Monoterpenoid Indole Alkaloids

- Nux Vomica - *Strychnos nux vomica, Loganiaceae*



Strychnine

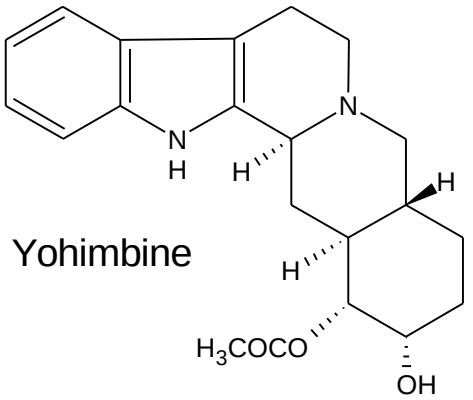


Brucine

- South Asian tree
- The seed = nux vomica: 1-3% alkaloids
- Introduced to Europe in 16-th century as a pesticide
- Strychnine intoxication resembles tetanus

Monoterpenoid Indole Alkaloids

- Yohimbe – *Pausinystalia yohimbe*, *Rubiaceae*



Yohimbine

- A tree widespread in Cameroon, Gabon and Congo.
- The bark of the trunk: 1-6% of alkaloids

- Yohimbine:
  - selective inhibitor of presynaptic α-2-adrenergic receptors
  - sympatholytic

- Biphasic effect on blood pressure
- Peripheral vasodilatation

Monoterpenoid Indole Alkaloids

- Cinchona – *Cinchona* spp. *Rubiaceae*
  (*C. pubescens*, *C. officinalis*, *C. calisaya*, ***C. ledgeriana***)

- The genus Cinchona – about 40 species, trees 15-20 m in height.
- Indigenous in South America – Amazonia, Columbia, Bolivia
- During the 19-th century cultivation set up in India and Java
- Nowadays planted in:
  Indonesia, Zaire, Burundi, Cameroon, Kenya, Peru, Bolivia, Ecuador

Monoterpenoid Indole Alkaloids

- Cinchona – the barks are obtained by beeting and peeling the trees

- Up to 6.5% alkaloids, 30-60% of them – quinine type alkaloids.

- Quinine

  - Antimalarial,

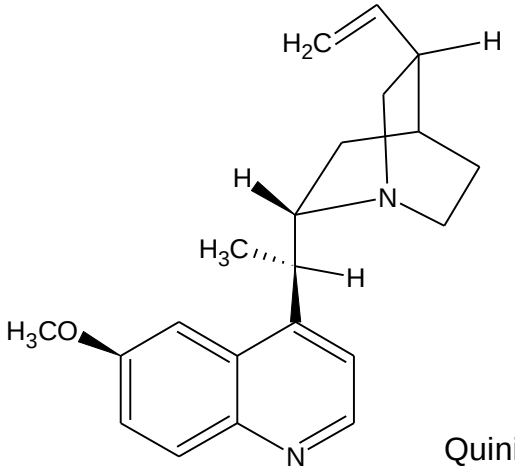
    active on the erythrocytic forms

    of *Plasmodium malariae*, *P. vivax*,

    P. falciparum, and *P. ovale*

  - modestly antypyretic and analgesic

  - a bitter taste standard



Quinine

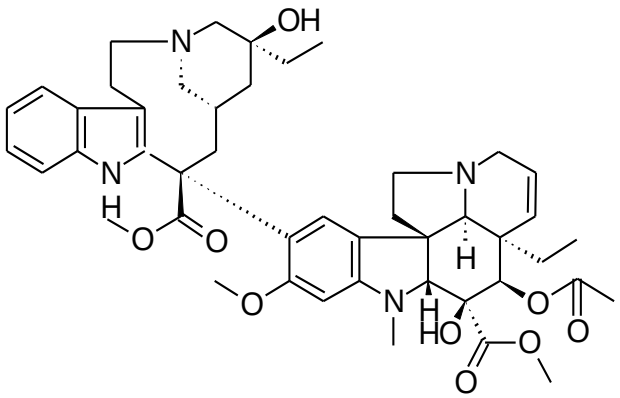
Monoterpenoid Indole Alkaloids

- Madagascan periwinkle – *Catharanthus roseus*, *Apocynaceae*

- A subshrub, originally from Madagascar, widespread in tropical regions.
- Planted as an ornamental species in Europe and North America.

- 0.2-1% alkaloids (in aerial parts)

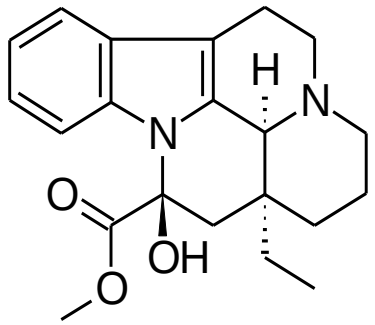
- Binary alkaloids with cytostatic activity:
  - Vincristine (about 3g/t)
  - Vinblastine



vinblastine

Monoterpenoid Indole Alkaloids

- Common periwinkle – *Vinca minor*, *Apocynaceae*
- A herbaceous plant, deep blue flowers with five lobes. The fruit consist of two follicles.
- 0.3-1% alkaloids. About 10% of them – Vincamine
- Indications: senility, disorders of vascular origin
  in ophtalmology and otorhinolaryngology.



Vinca minor

Monoterpenoid Indole Alkaloids

- Rauwolfia (Snakewood) – *Rauwolfia serpentina*, *Apocynaceae*

- Evergreen shrub, with a big root system.
- Pakistan, India, Thailand, Malaysia, Indonesia.

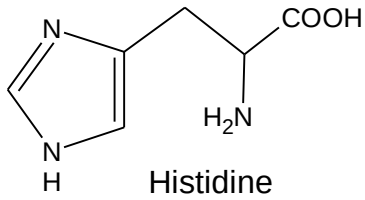
- Root: 0,5 – 2,5% alkaloids
  - Yohimbane alkaloids : **Reserpine**
  - Heteroyohimbanes : **Ajmalicine**
  - Dihydroindole alkaloids

- .

Nicotinic acid derived alkaloids

- 1492 Columbus - The discovery of America
- 1556 André Thévet - tobacco growing
- Jean Nicot – Propagation of tobacco to French queen as an anti-migraine remedy
- 1628 France – Ban on smoking in churches
- 1629 France - Tobacco Tax
- 1674 France – Tobaco state monopoly

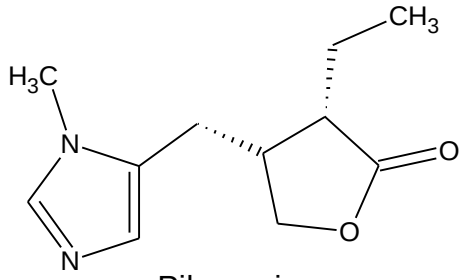
Imidazole Alkaloids



Histidine

- Jaborandi – *Pilocarpus microphyllus*, *Rutaceae*

- Shrubs, leaves are harvested during the dry season.
- Brazil

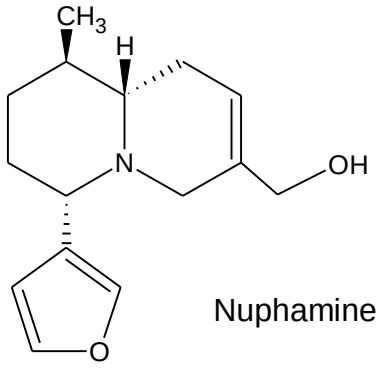


Pilocarpine

- Leaves: up to 0.8% alkaloids,
- Chief constituent: Pilocarpine
  - parasympathomimetic
  - used as a nitrate or a hydrochloride in eye drops
    - Causes myosis, decreases intraocular pressure
- .

Pseudoalkaloids

- Terpenoid alkaloids:
- Water lilies: *Nymphaea alba*
  Nuphar luteum
- Rhizoma: sesquiterpenoid alkaloids
- Clinically not important



Nuphamine

Pseudoalkaloids

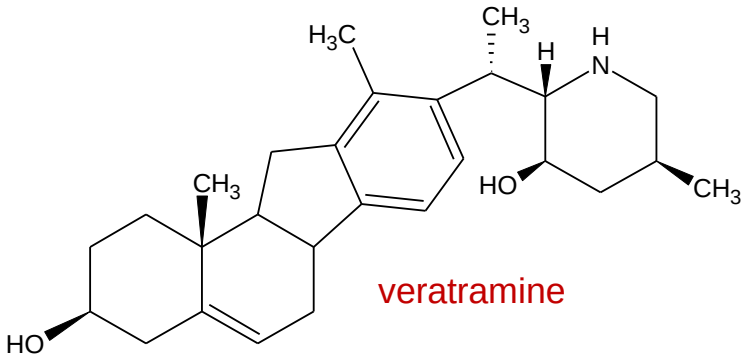
- Diterpenoid alkaloids:

- Aconite – *Aconitum napellus*, *Ranunculaceae*
- Perennial herb with a tuberized root.
- Root: 60% starch and other saccharides, 0.5-1.5% alkaloids

- Aconitine
  - Paralysis of peripheral nerves as well as the brain stem
  - Respiratory slowing, extinction of atrial impulses in AV node
  - Arrow poison, criminal poison, pesticide.
  - Lethal dose: about 25 μg/kg; no antidote known.

Pseudoalkaloids: Steroidal alkaloids

- White hellebore – *Veratrum album*, *Liliaceae*
- Perennial herbaceous plant, grows in mountains of Europe and Northern Asia.
- Rhizome: 1.5% steroidal alkaloids
- Pharmacological activity:
  - Toxic, increases permeability of sodium channels.
  - Causes bradycardia, hypotension, rhythm alterations, nausea, vomiting.
  - The powder irritates in eyes, nose and skin.



veratramine

Veratrum album

Pseudoalkaloids: Steroidal alkaloids

- Woody nightshade – *Solanum dulcamara*, *Solanaceae*

- Potato – *Solanum tuberosum*, *Solanaceae*

  - the alkaloids occur in the leaves (30-90 mg/100g)

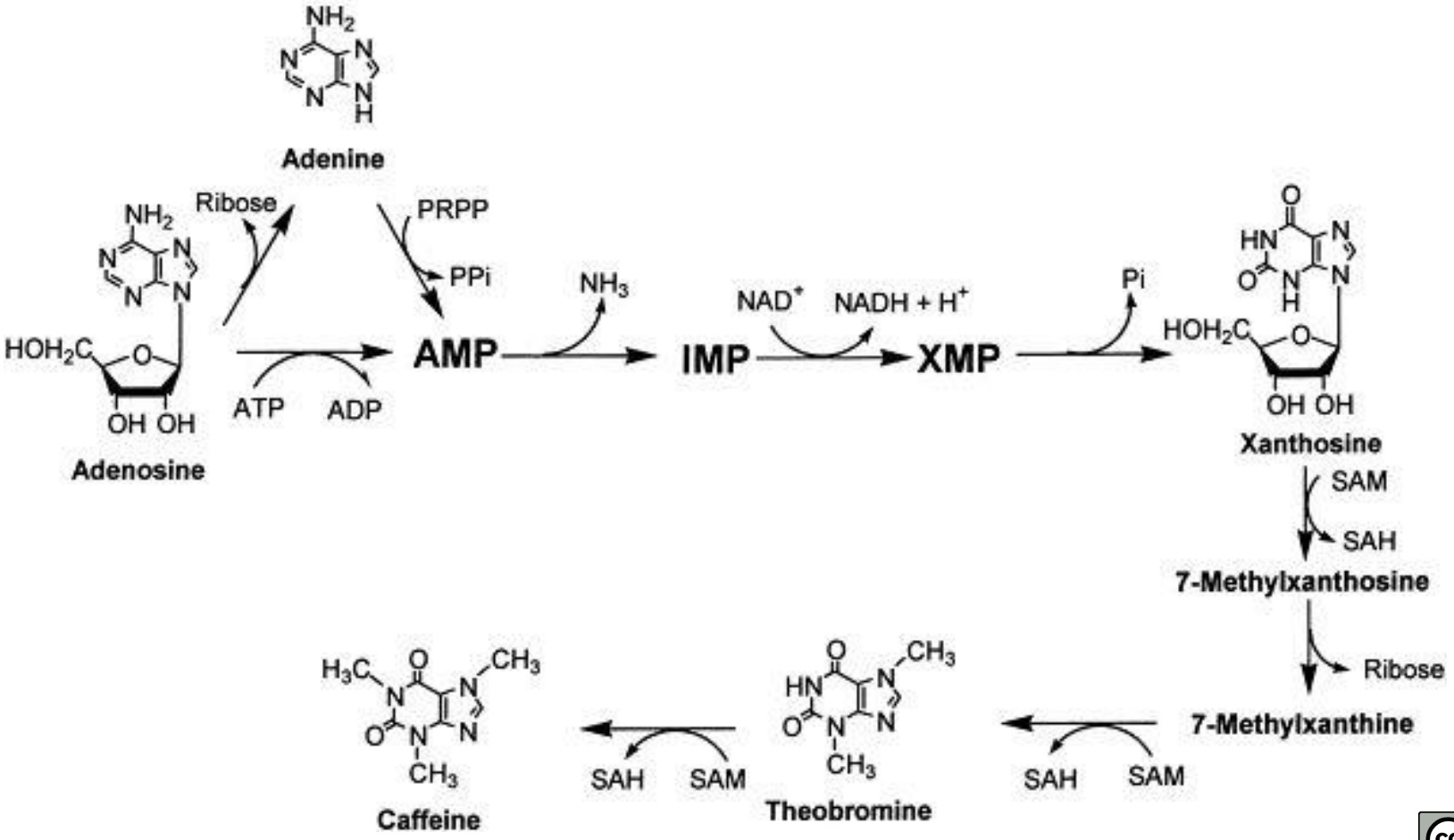
  - fruits (40-100 mg/100g), flowers (200-500 mg/100g), sprouts (500 mg/100g and more)

Low concentrations of alkaloids in the tubers under normal conditions.

Symptoms of the intoxication: gastrointestinal distress, fever, rapid breathing, a drop in blood pressure.

Teratogenic.

Purine alkaloids



Purine alkaloids - occurance

Camellia sinensis (tea) – Theaceae
Coffea arabica (coffee) – Rubiaceae
Cola acumitata (cola nuts) – Sterculiaceae
Paulinia cupana (guarana) – Sapindaceae
Illex paraguariensis (yerba maté)- Aquifoliaceae

Theobroma cacao (cocoa) – Sterculiaceae

Camelia sinensis

Coffea arabica

Theobroma cacao

Purine alkaloids

Caffeine:
CNS – stimulation, enhances allertness, decreases sensation of fatique
Respiratory center – stimulation (increased sensitivity to CO_2):
Cardiovascular system – stimulation of heart output,
Other effects - mild diuretic

Usually used as caffeine benzoate, often used in combination with analgetics (paracetamol, acetylsalicylic acid)

Theophylline:
Bronchial smooth muscle relaxant, respiratory stimulator,
diuretic activity (due to increased glomerular filtration)

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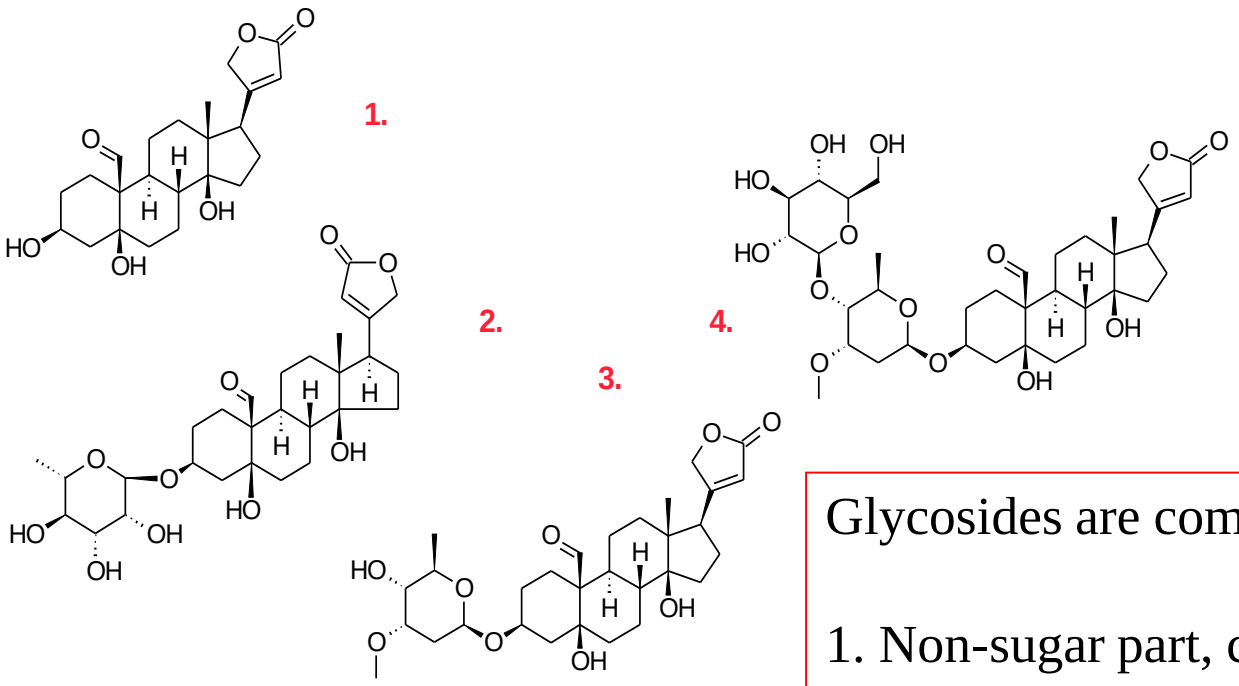
Bioactive Natural Compounds

7. Glycosides

– Generalities
– Specific bioactive glycosides

Lecturer: Oldřich Lapčík

Glycosides: aglycone (genin), glycone



1. Strophantidin
2. Strophantidin-α-L-Rhamnoside
3. Strorphantidin-D-cymaroside
4. Strophantidin-glucocymaroside

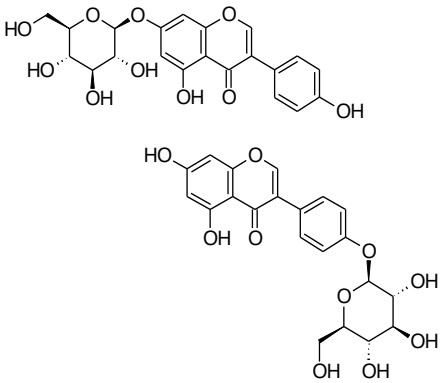
Glycosides are composed of two distinct parts

1. Non-sugar part, called aglycone or genin

2. Saccharide part, glycone

Different glycosides may be derived from the same aglycone (example: glycosides of strophantidin)

Glycosides: generalities



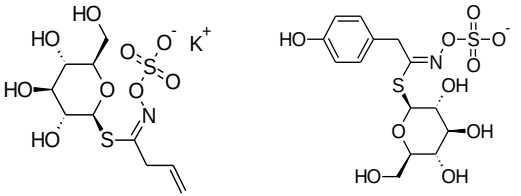
Genistin and genistein-4′-glucoside
O- glycosides of genistein

Glycosidic bond
– hydroxyl at C1 of the sachcharide is engaged (hydroxyls at C2-C6 may be esterified or etherified, but they do not form glycosidic bond)

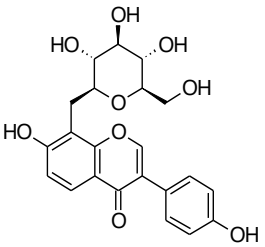
-S glycosides: -O- is replaced with -S-
-N glycosides: -O- is replaced with -NH-
-C glycosides -O- is replaced with $–CH_2$-

-O, S and N glycosides – easily hydrolysable (enzymic hydrolysis or acidic hydrolysis)

-C glycosides - stable



Sinigrin and sinalbin,
S-glycosides



Puerarin,
C-glycoside

Glycosides: generalities

Solubility
- in water : increased
- in organic solvents: decreased

Volatility: removed

Sensitivity to oxidation: decreased or removed

Toxicity: often decreased (masking of pharmacophores)

Specific groups of biologically active glycosides

Cardiac Glycosides

Found in a limited number of genera
belonging to about 15families:

Asclepiadeaceae, ***Apocynaceae***, *Brassicaceae*,
Celestraceae, *Crassulaceae*, *Fabaceae*, *Iridaceae*,
Liliaceae, *Moraceae*, ***Ranunculaceae***, ***Scrophulariaceae***, *Tiliaceae*

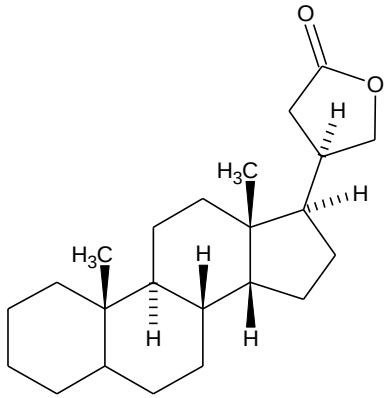
Aglycones found in amphibians (bufadienolides),

Certain insects are able to accumulate cardiac glycosides

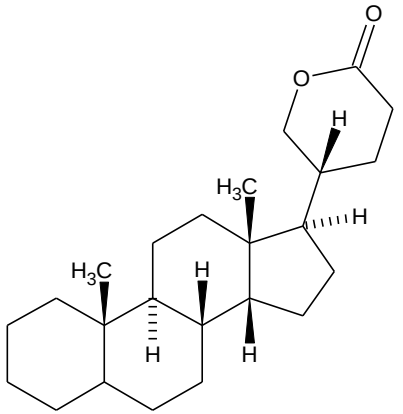
Cardiac Glycosides

Two main aglycone structures: CARDENOLIDES, BUFADIENOLIDES

Sugar structures - often unusual:
Diginose, Fucose, Digitalose,
Digitoxose, Oleandrose, Rhamnose,
Sarmentose, Boivinose, Thevetose etc.



Cardanolide



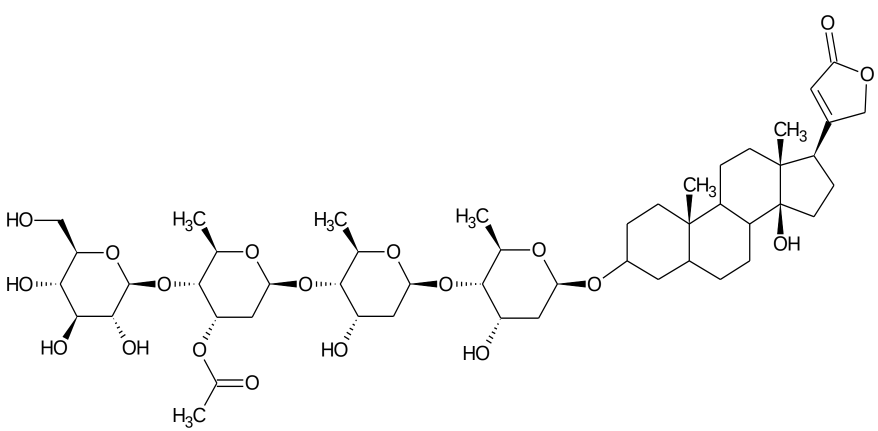
Bufanolide

Principal activity: Modulation of NaK ATPase

Cardiac Glycosides

Digitalis sp. Scrophulariaceae

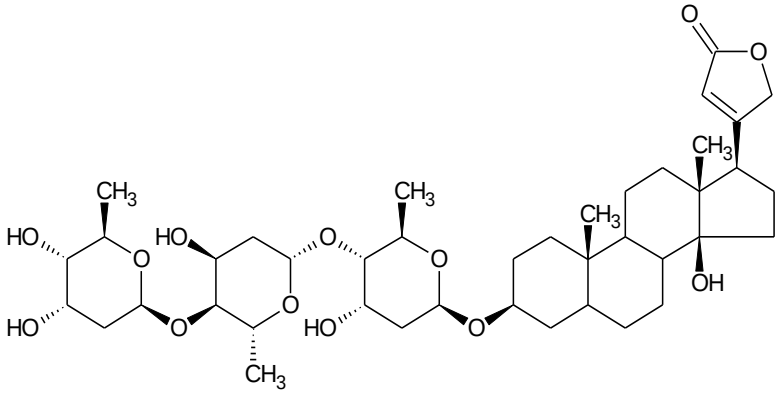
Digitalis lanata
Digitalis purpurea



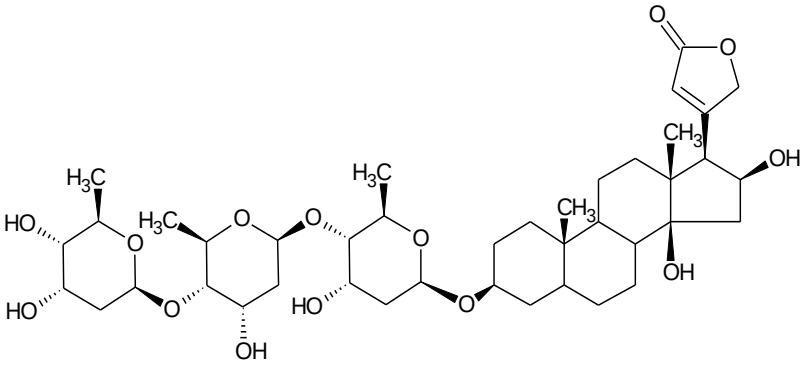
Lanatoside A

Cardiac Glycosides

Digitalis sp. Scrophulariaceae
Digitalis purpurea



Digitoxin



Gitoxin


Digitalis grandiflora

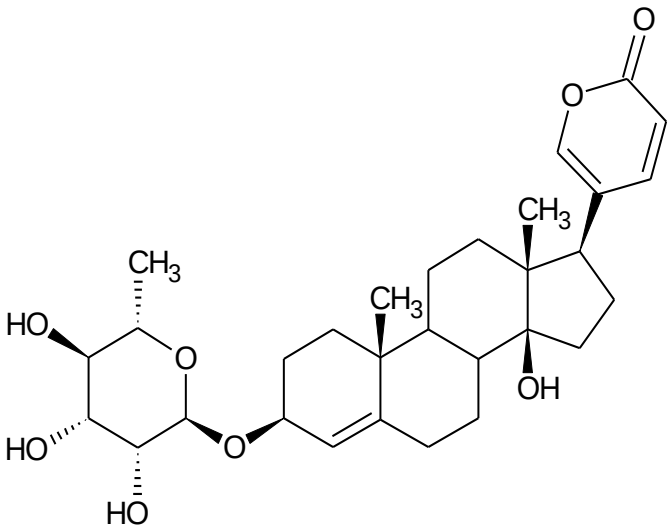
Cardiac Glycosides

Drimia maritima, Liliaceae, Squill

Voluminous bulbs up to 3 kg

Up to 4% bufadienolides

Heart tonic
Rat poison
Expectorant



Proscillaridin
Scillarenin-3-β rhamnopyranoside

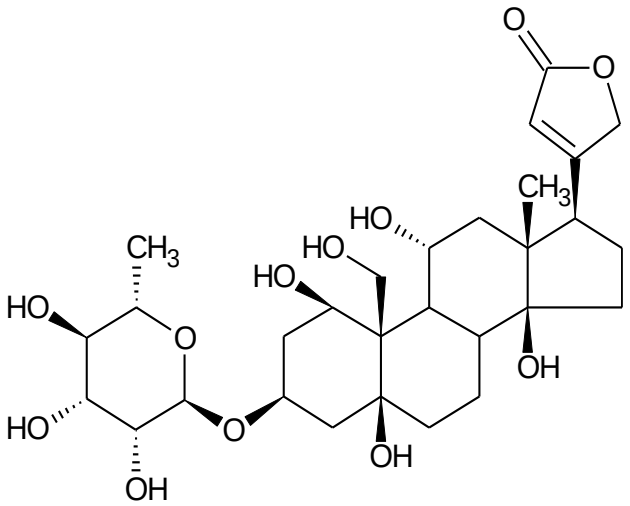
Cardiac Glycosides

Strophantus spp., Apocynaceae,

Bushes or vines, growing in Gulf of Guinea, Zaire, Tanzania, Kenya.

Formerly used for arrow poisons

Source of ouabain

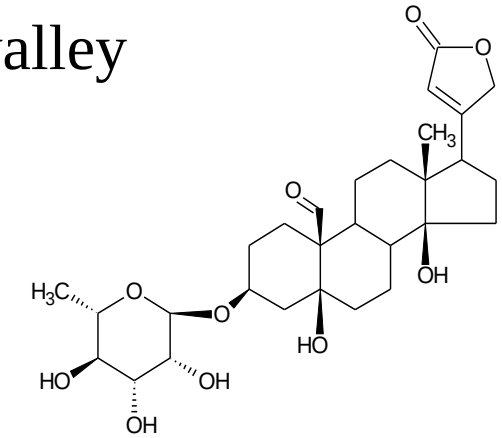


Ouabain

Cardiac Glycosides

Convallaria majalis, Liliaceae,

Lilly of the valley



Convallotoxin is poorly absorbed in the intestine
Intoxications usually are not serious – gastrointestinal symptoms.

Cardiac Glycosides

Helleborus sp., Ranunculaceae,

H. niger – Christmas rose

Bufadienolide glycosides

Intoxications are rare
Intoxications of domestic animals sometimes occur

Symptoms:
Tingling in the throat, vomiting, diarrhea, mydriasis.

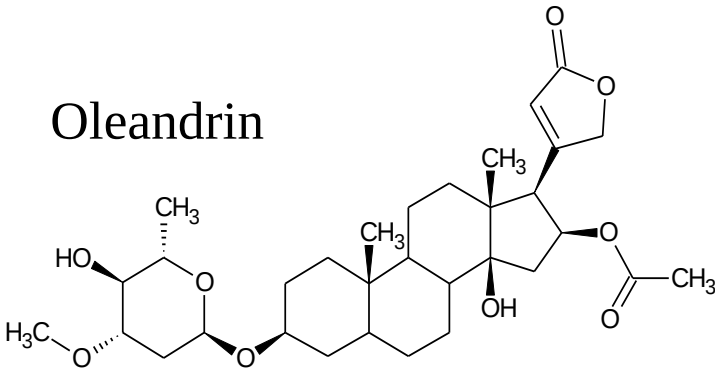
H. purpurascens, ↑ *H. odoratus* ↓

Cardiac Glycosides

Nerium oleander, Apocynaceae,

Rose laurel
Leaves: about 1.5% cardenolides
Oleandrin, gitoxigenin

Oleandrin



Intoxication (leaves, seeds):
nausea, vomiting, bradycardia
hyperkalemia
ventricular fibrillation

Saponins

Steroidal saponins: Liliaceae: genera *Alium*, *Smilax*, *Asparagus*
Agavaceae: genera *Agave*, *Yuca*
Dioscoridaceae: *Dioscoridea*
Solanaceae, Fabaceae, Scrophulariaceae

Triterpenoid saponins: Cucurbitaceae, Fabaceae, Primulaceae,
Ranunculaceae, Rosaceae…

Saponins - generalities

Soluble in water, foaming in water

Cause hemolysis

Toxic for fish and amphibians

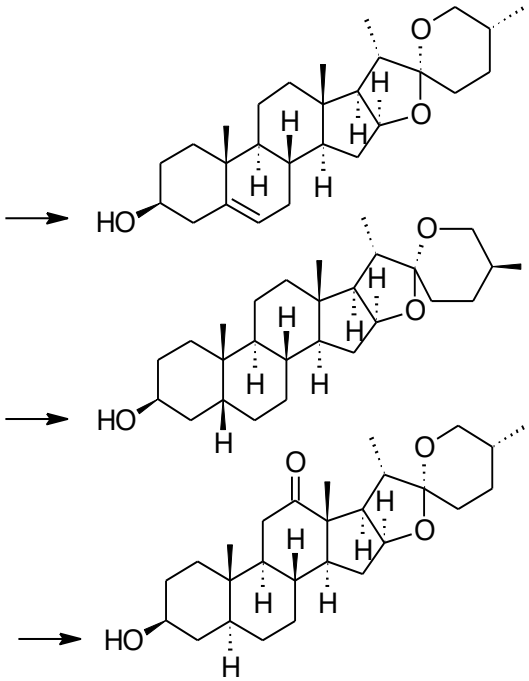
Poorly absorbed in the gastrointestinal tract – low toxicity *per os*

Anti-inflamatory, anti-tussive, secretolytics.

Saponins

Monodesmosides – saccharide part attached at C-3 of aglycone
Bidesmosides - two sacharide moieties (C-3 and additional one)

Steroidal aglycones:

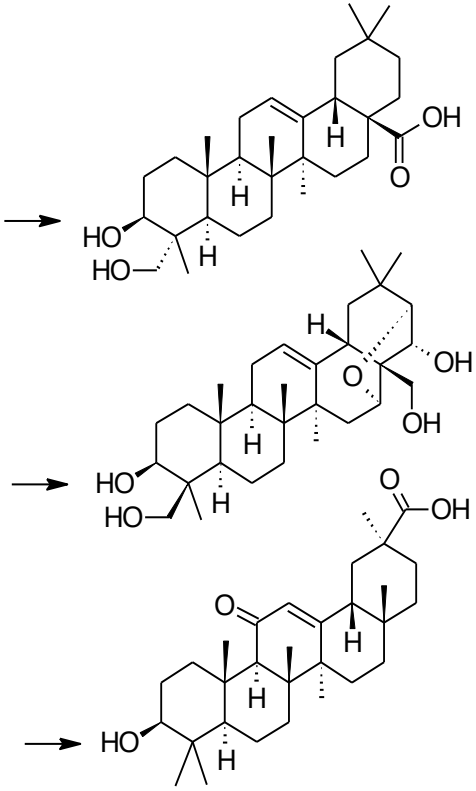


Diosgenin

Sarsapogenin

Hecogenin

Triterpenoid aglycones:

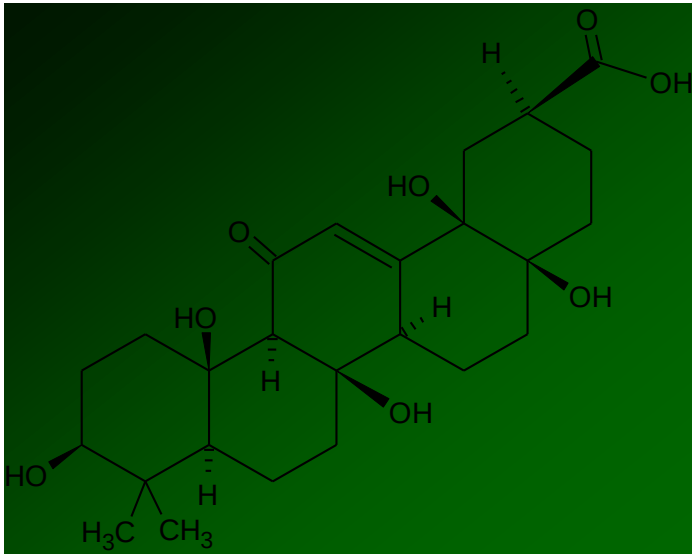


Hederagenin

Aescigenin

Glycyrrhetic acid

Glycyrrhetinic acid
and the apparent mineralocorticoid excess

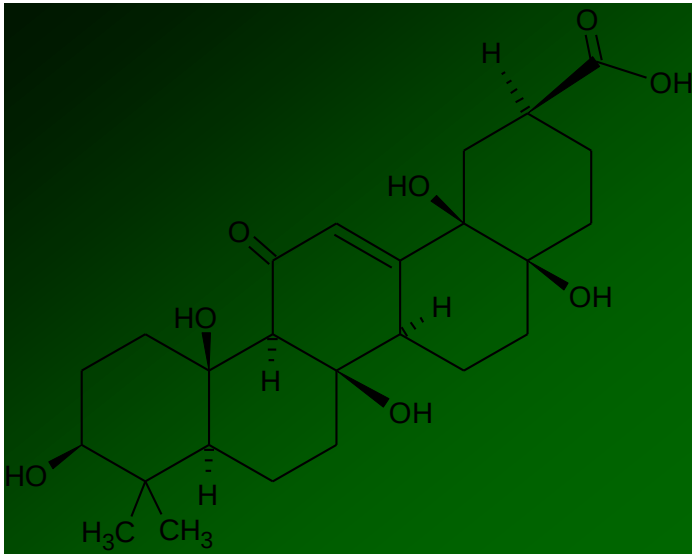


- Glycyrrhetinic acid,

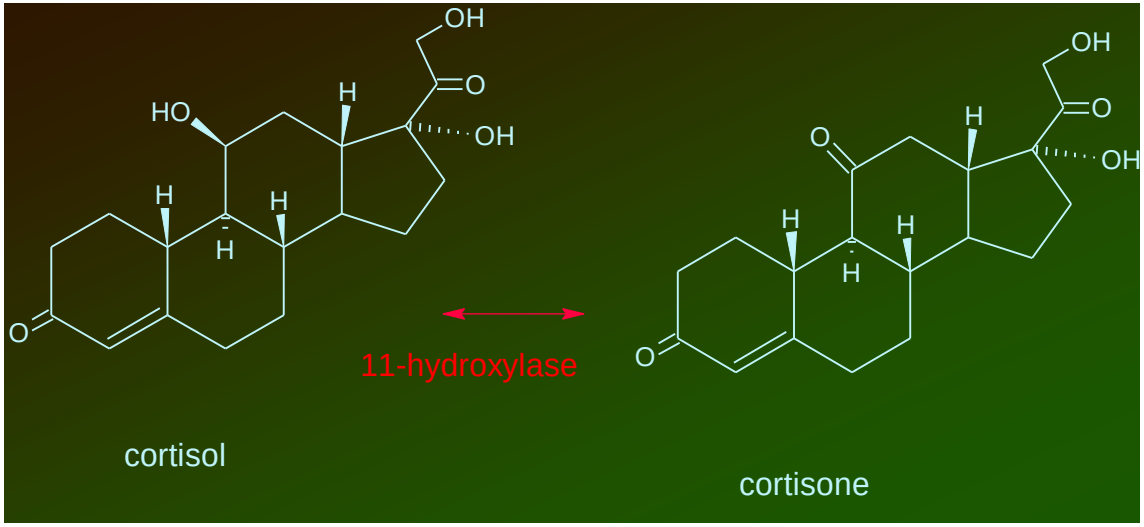
Licorice (*Glycyrrhiza glabra*)

Use: pastry, candies,
herb teas, soft drinks
pastis
chewing gums
chewing tobaco

Glycyrrhetinic acid
and the apparent mineralocorticoid excess

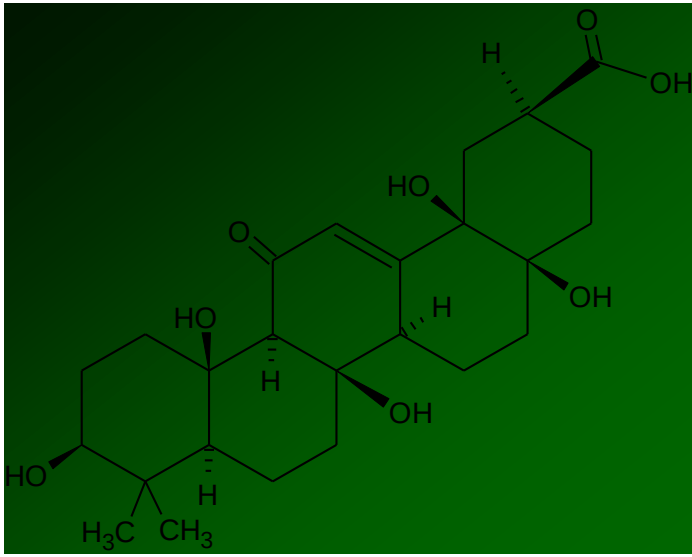


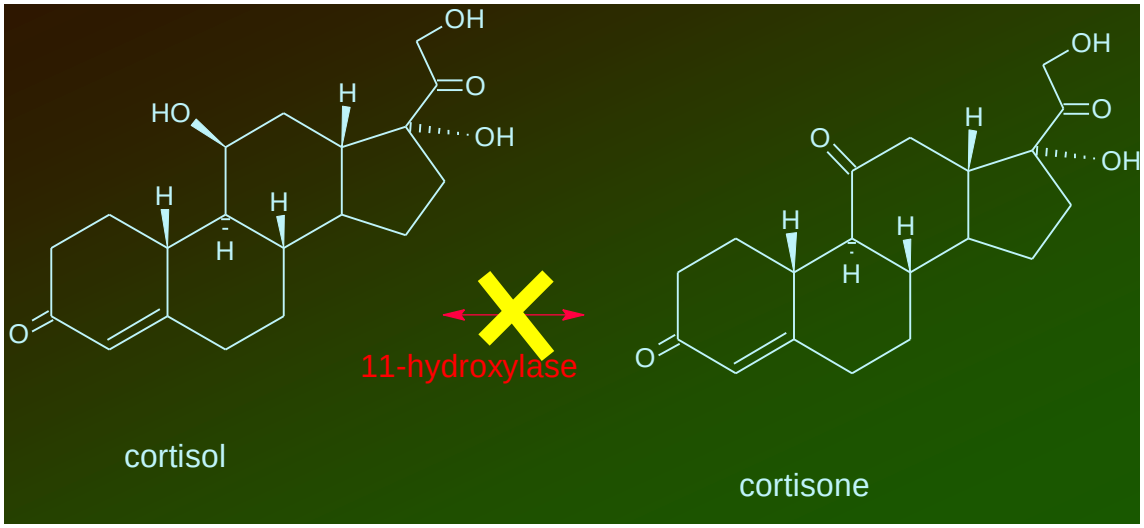
- Glycyrrhetinic acid, abundant in licorice



cortisol – cortisone shuttle
protects mineralocorticoid sensitive tissues from mineralocorticoid activity of cortisol

Glycyrrhetinic acid
and the apparent mineralocorticoid excess

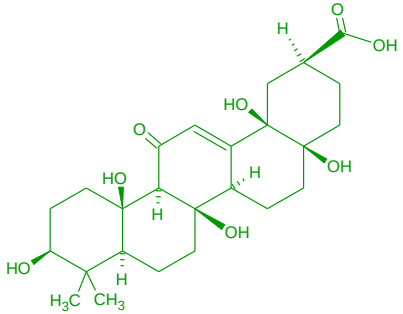




- Glycyrrhetinic acid,

Inhibition of 11-β hydroxysteroid dehydrogenase may result in AME: Sodium and water retention, loss of potassium, high blood pressure, alkalosis, higher risk of left ventricular hypertrophy and coronary artery disease

Glycyrrhetinic acid



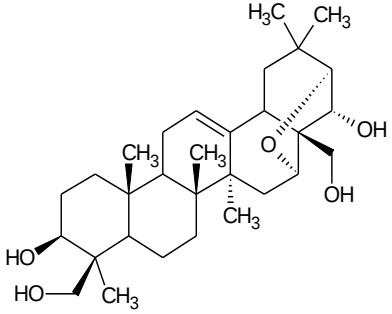
-regular licorice intake lead to a dose-dependent increase of the blood pressure.

Sigurjónsdóttir H.A. et al.: J. Human Hypertension: **15**, 2001, pp 549-552

-in healthy subjects, only the highest doses of licorice (814 mg/day of glycyrrhizin) led to untoward effects. These were favoured by subclinical disease or oral contraceptives.

Bernardi, M et al:. Life Sciences: **55**, 1994, pp 863-872

Saponins



Aesculus hippocastanum
Hippocastanaceae
Common chestnut

Drug: seed.

40-50% starch and other sugars
6-8% lipids
flavonol glycosides
saponins

Aescines: triterpenoid saponins

Anti-inflamatory, anti-edema, anti-exudative, veno-protective

Saponins

Primula veris Primulaceae
Primrose

Drug: flos primulae,
radix primulae

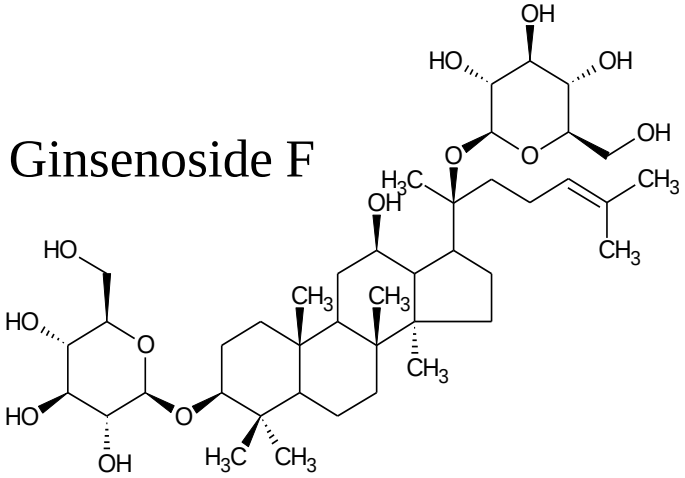
Flavonoids
Primulic acid
Saponins (2% - flower, 10% - root)

Bronchosecretolytic, expectorant,

Treatment of bronchial catarrh,
Mouthwashes

Saponins

Ginsenoside F



Panax ginseng Aralicaceae
P. notoginseng

Small herbaceous plant, palmilobate leaves, white flowers, red berries

Drug: dried root.

Composition: Polysaccharides, glycopeptides, vitamins, sterols, essential oil – sesquiterpenes and polyalkines.
Saponins (about 20) – ginsenosides

Use: CNS stimulant, neuroprotective, “adaptogen“,
treatment of asthenia, modulation of immune response,

Saponins

Calendula officinalis Asteraceae
Marigold

Drug: flowers or capitulums

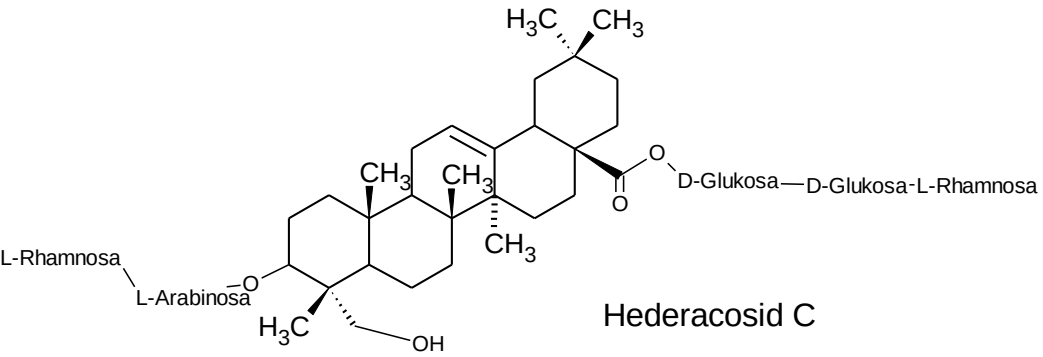
Flavonoids (isorhamnetin, quercetin)
Carotenes (lycopene)
Essential oil (sesquiterpenoids)
Triterpenoids (faradiol, arnidiol etc.)
Triterpenoid saponins

Anti-bacterial, anti-inflamatory

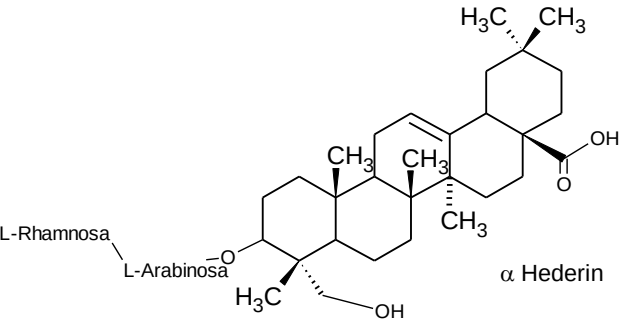
Used topicaly: lotions, creams, soaps

Saponins

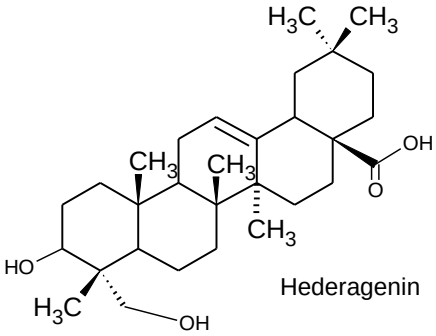
Hedera helix Hederaceae
Common Ivy



Hederacosid C



α Hederin



Hederagenin

Saponins

Hedera helix Hederaceae
Common Ivy

- Drug: leaves, wood

- Flavonoids
- Polyalkynes
- Saponins (5-8%)
  Bidesmosides, monodesmosides

Expectorant, spasmolytic,
Fungistatic (*Candida albicans*)
Anti-bacterial

Cyanogenic glycosides

-Cyanogenesis was recorded in more than 2500 plant species

-Families *Rosaceae*, *Fabaceae*, *Poaceae*, *Araceae*, *Euphorbiaceae*, *Passifloraceae* etc

-Glycosides of 2-hydroxynitriles: cyanogenic glycosides

-Readily cleaved by β-glucosidases → cyanohydrines → HCN

-Acute intoxication: asthenia, vomiting, hypotension, tachycardia

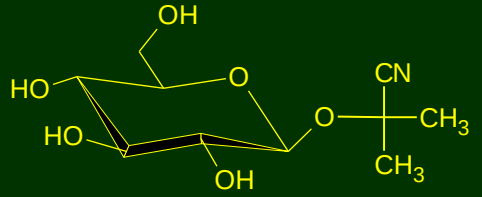
-Chronic intoxication: TPO inhibition, thyroid gland disorders, atrophy of optic nerves, polyneuropathy

- Certain insects

  accumulate cyanogenic glycosides from nutrition sources

  Protection agaist predators

Cyanogens in cassava

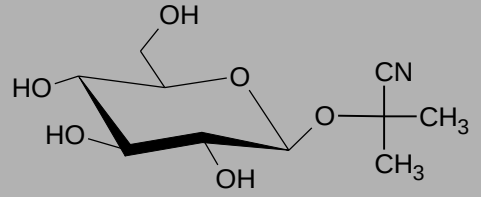


linamarin

- **Cassava** (*Manihot esculenta*) is an important dietary staple for more than 500 million people in developing countries.
- People eat 60% of the **cassava** produced and one third of the harvest feeds animals.
- All cultivars of **cassava** contain the cyanogenic glucoside, linamarin, but in different concentrations.
-
- The roots of those cultivars with high cyanogenic content are processed to reduce the level of linamarin, because linamarin is hydrolysed in the intestinal tract of both men and animals by microbial flora and HCN is released.

Kamalu B. P.: International Journal of Food Sciences and Nutrition **46**, 1995, pp. 65-93

Cyanogens in cassava



linamarin

WHO safe value: CN <10 ppm (cassava flour)

Indonesian legislation: CN <40 ppm

Real cassava products:

	CN average	CN range	% over 40
roots	19	10 - 80	26
flour and chips	54	27 - 200	33
starch	5	1 - 10	0

Djazuli M., Bradbury J.H.: Cyanogen content of cassava roots and flour in Indonesia. *Food Chemistry* 65 (1999) 523-525

Cyanogens in cassava

- Technologically it is possible to diminish the cyanogen content in cassava by grinding and fermentation. The care given to this laborious procedure is decisive for the influence of this foodstuff on human health in individual cultures.

Padmaja G.: *Critical Reviews in Food Science and Nutrition* **35** (1995), 299-339

- In conditions near to insufficient iodine intake, consumption of cassava may be the crucial epidemiogenetic factor.

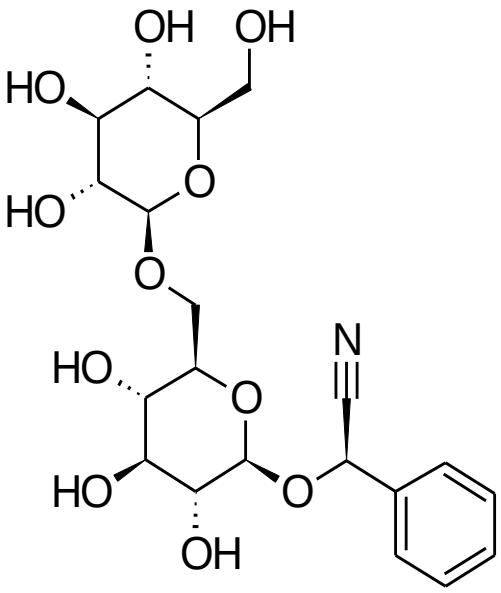
  The Bororos, a nomadic tribe in the Central Africa whose diet is based rather on milk products, have considerably lower goiter prevalence when compared to the local rurals, whose nutrition is based on cassava (17% vs. 76%, respectively).

Biassoni P. et al.: *European Journal of Endocrinology* 138 (1998), 681-685

Cyanogenic glycosides

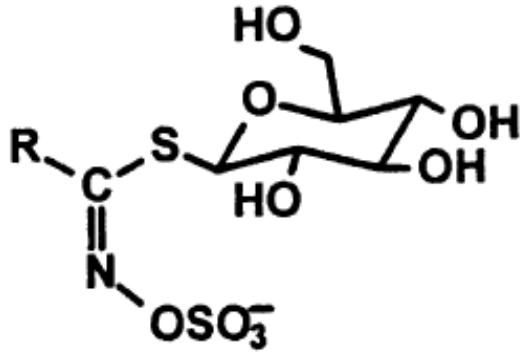
Rosaceae*:* *Prunus spp.*

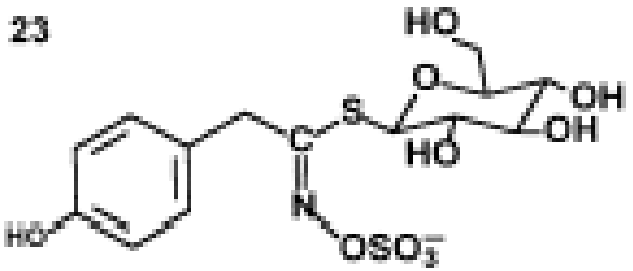
Amygdalin found in seeds: apricots, almonds, cherries, plums etc.



Glucosinolates

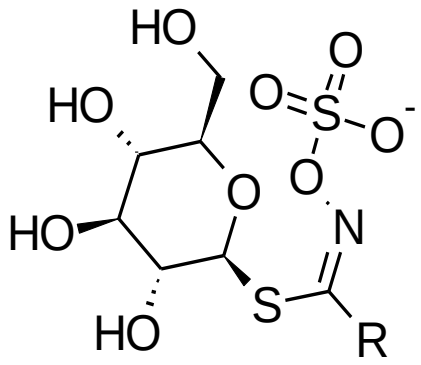
Bataceae, ***Brassicaceae,***
Bretschneideraceae, ***Capparaceae***
Caricaceae, *Euphorbiaceae*,
Gyrostemonaceae, *Limnanthaceae*,
Moringaceae, *Pentadiplandraceae*,
Phytolaccaceae, *Pittosporaceae*,
Resedaceae, *Salvadoraceae*
Tovariaceae, *Tropaeolaceae*





J.W. Fahey, A.T. Zalcmann, P. Talalay: The chemical diversity and distribution of glucosinolates and isothiocyanates among plants, Phytochemistry 56 (2001) 5-51

Glucosinolates



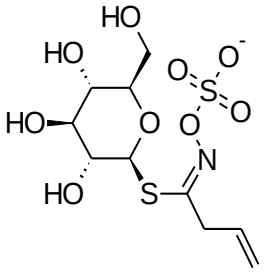
Sugar part: glucose

S-glucosides

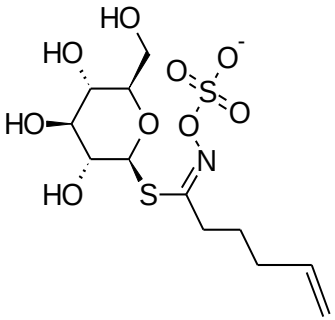
Over 200 different aglycones (aliphatic, aromatic)

Prone to hydrolysis

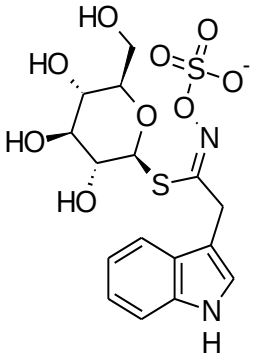
Products of hydrolysis responsible for metabolic effects



Sinigrin



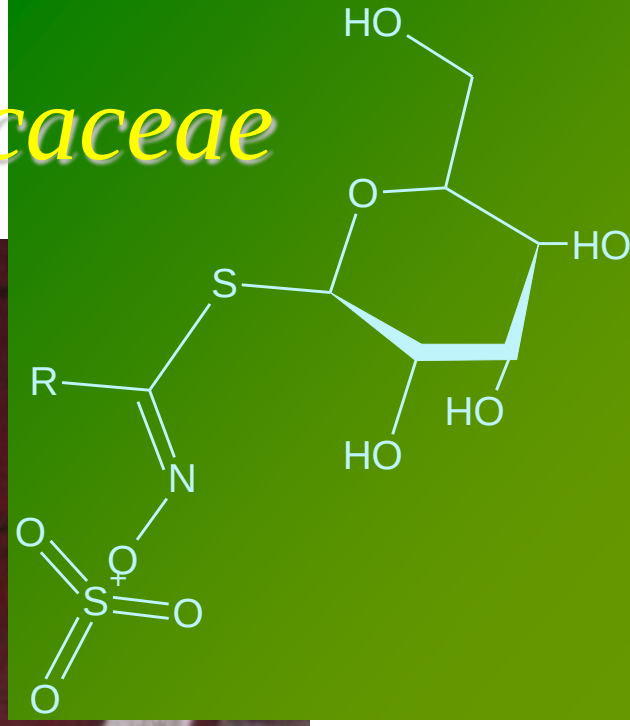
Gluconapin



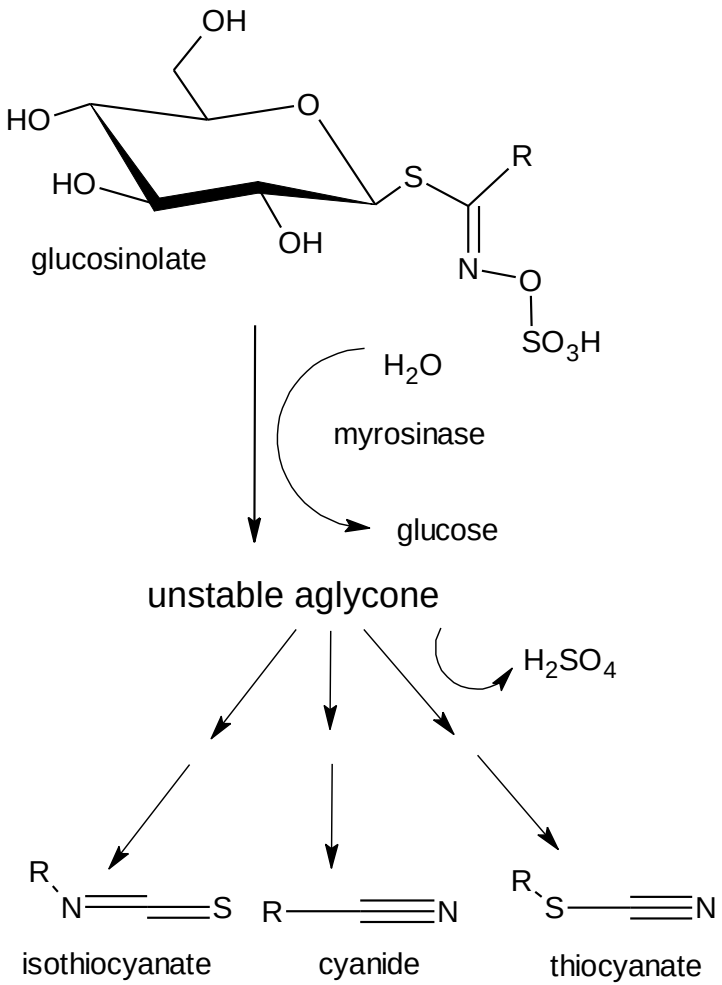
Glucobrassicin


Brassica oleracea

Glucosinolates in the *Brassicaceae*



Glucosinolates



- about 1% of dry weight in some tissues of the *Brassica* vegetables although the content is highly variable

Fahey J. et al.: *Phytochemistry* **56**: (2001), 5-51

Consuming cabbage and kale is a risk factor for the presence of thyroid nodules.

Obradovic L : Medicinski Pregled 53, January - February 2000, pp. 64-67

Endemic thyreopathy caused by iodine deficiency

Goiter - Central Carpathian area, 1920-30-ies.

S. Vome

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

8. Phenolics

– generalities
– selected types of phenolics

Lecturer: Oldřich Lapčík

Phenolics

A vast group of substances

Aromatic ring substituted with at least one hydroxy group, free or engaged in another function: ether, ester or glycoside.

Only plants and microorganisms synthesize the aromatic nucleus (except of estrogens)

Two main biosynthetic pathways:

-Shikimate pathway
-Polyketide pathway

Phenolics

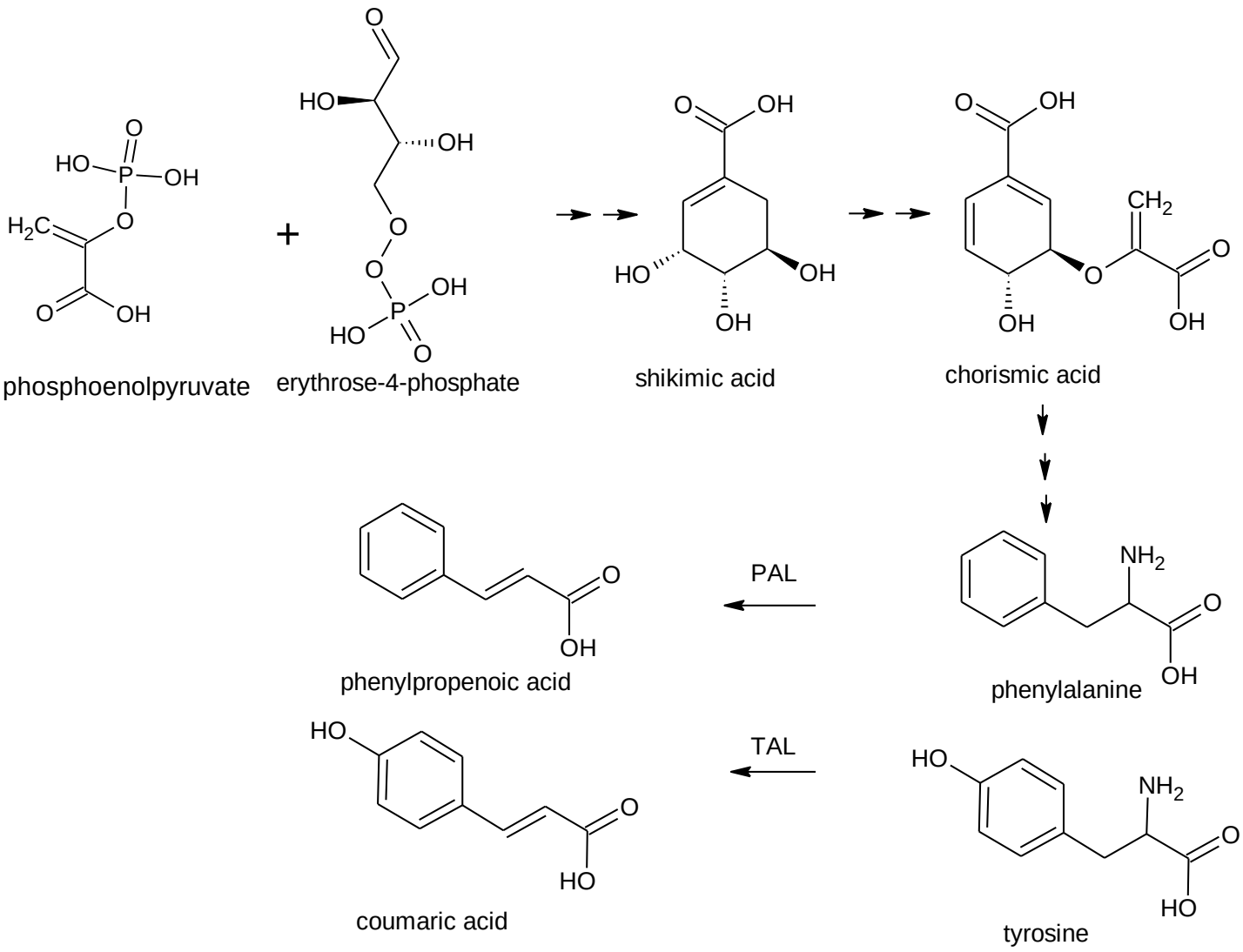
Phenylpropanoids

-Phenols and phenolic acids
-Coumarins
-Lignans
-Flavonoids: Flavonoids, Isoflavonoids, Anthocyanins
-Tannins

Polyketides

-Quinones and xanthones
-Orcinols and floroglucinols

Shikimate pathway - phenylpropanoids



Simple phenols and phenolic acids

Generalities

-Soluble in polar organic solvents

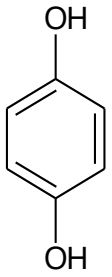
-Soluble in NaOH or Na_2HCO_3

-Unstable in alkaline conditions, prone to oxidation

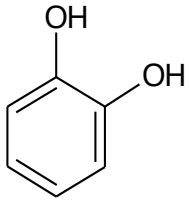
-Cinnamates tend to isomerize in aqueous solutions

-Extraction from fresh plant material should be preferred (acidified water-ethanol etc.)

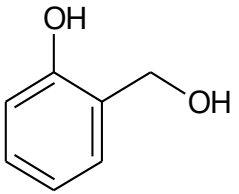
Examples of simple phenols and phenolic acids



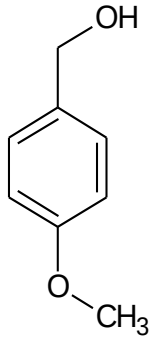
hydroquinone



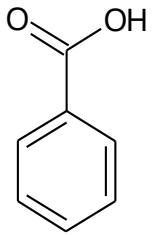
catechol



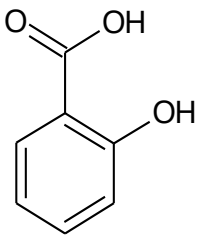
salicylic alcohol



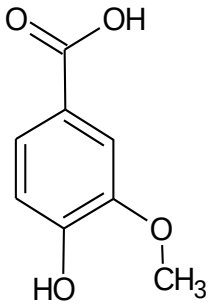
anis alcohol



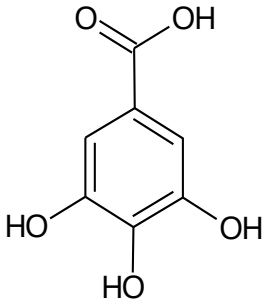
benzoic a.



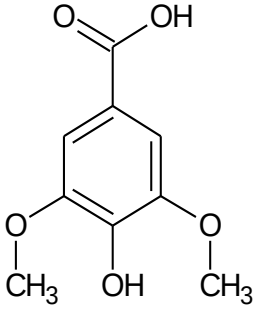
salilcylic a.



vanilic a.

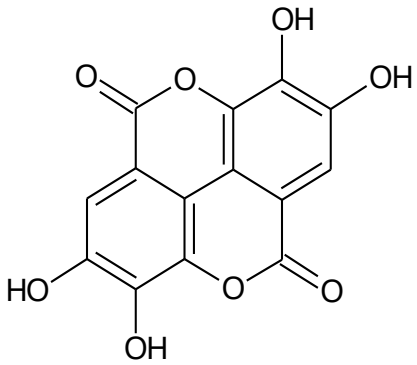


gallic a.

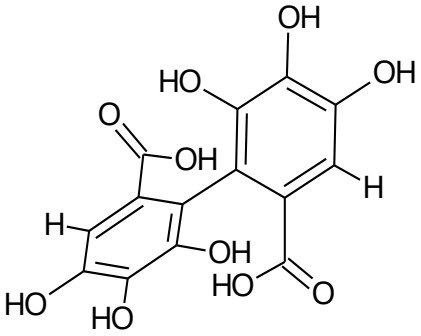


syringic a.

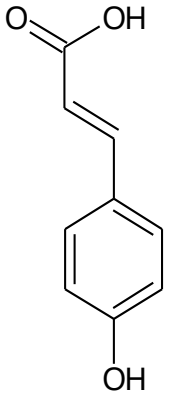
acids:



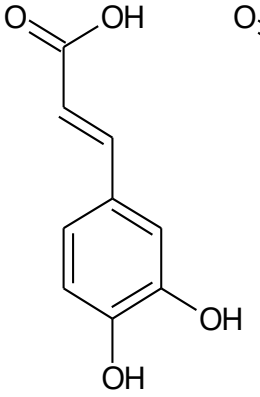
ellagic a. (lactonized form)



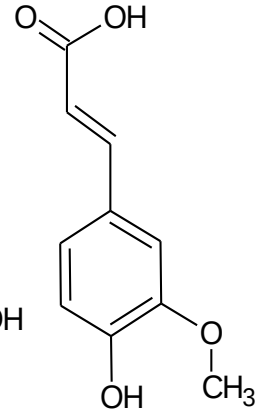
ellagic a.



p-coumaric a.

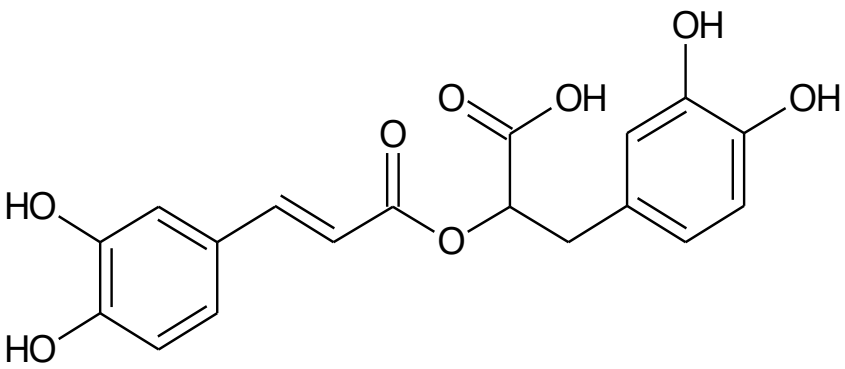


caffeic a.

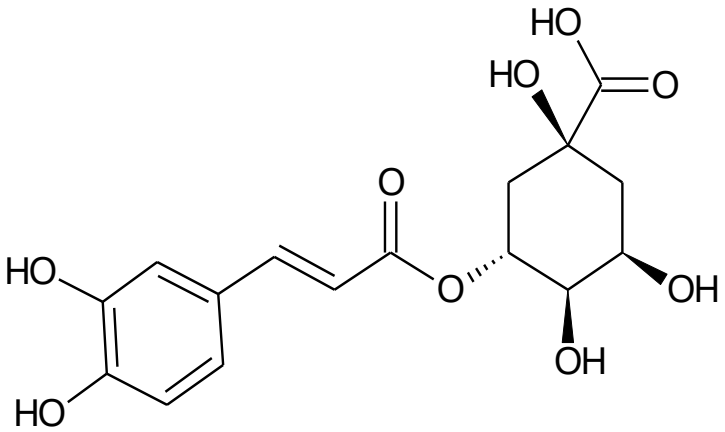


ferrulic a.

Simple phenols and phenolic acids



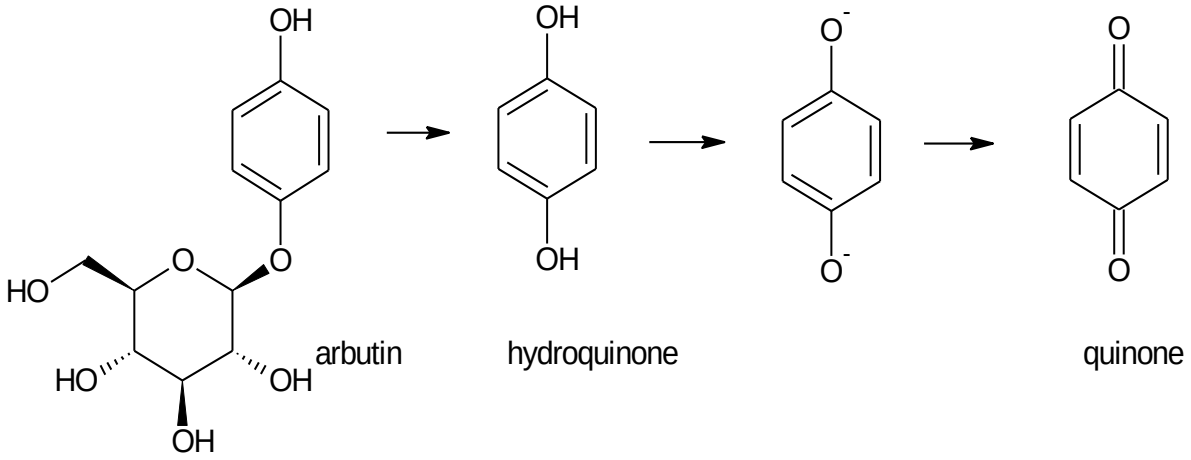
rosmarinic acid



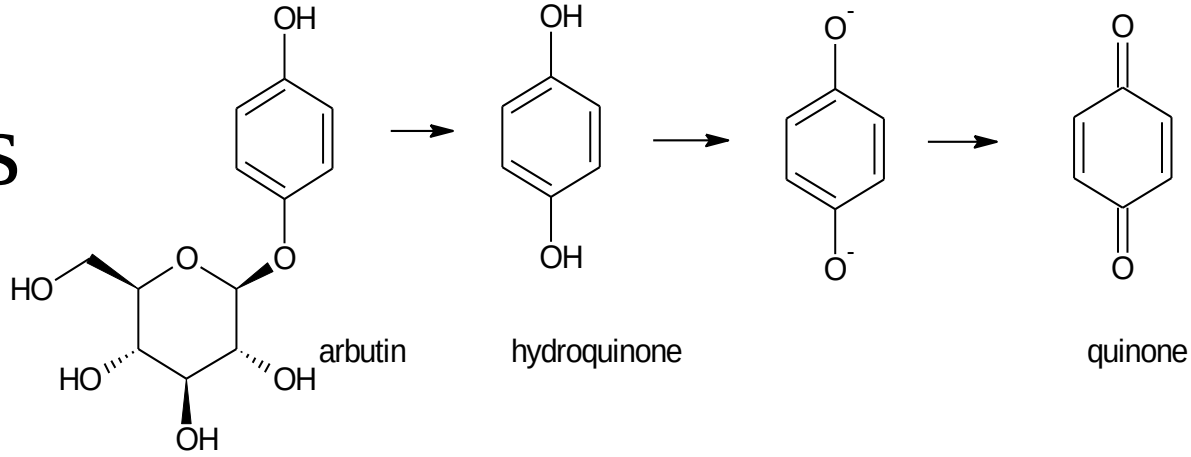
chlorogenic acid

Simple phenols

Oxidation of hydroquinones to quinones



Simple phenols



Arcostaphylos uva-ursi
Ericaceae

Bearberry

Edible berries

The leaves:
- arbutin (6-10%)
- methylarbutin
- ursolic acid

Used in urologic teas

Phenolic acids

Rosmarinus officinalis Lamiaceae
Rosemary

Dried flowering tops:
-essential oil (10-25 g/kg)
terpenoids: carnosol, borneol
- flavonoids (glycosides of luteolin)
-phenolic acids:
rosmarinic acid
caffeic acid

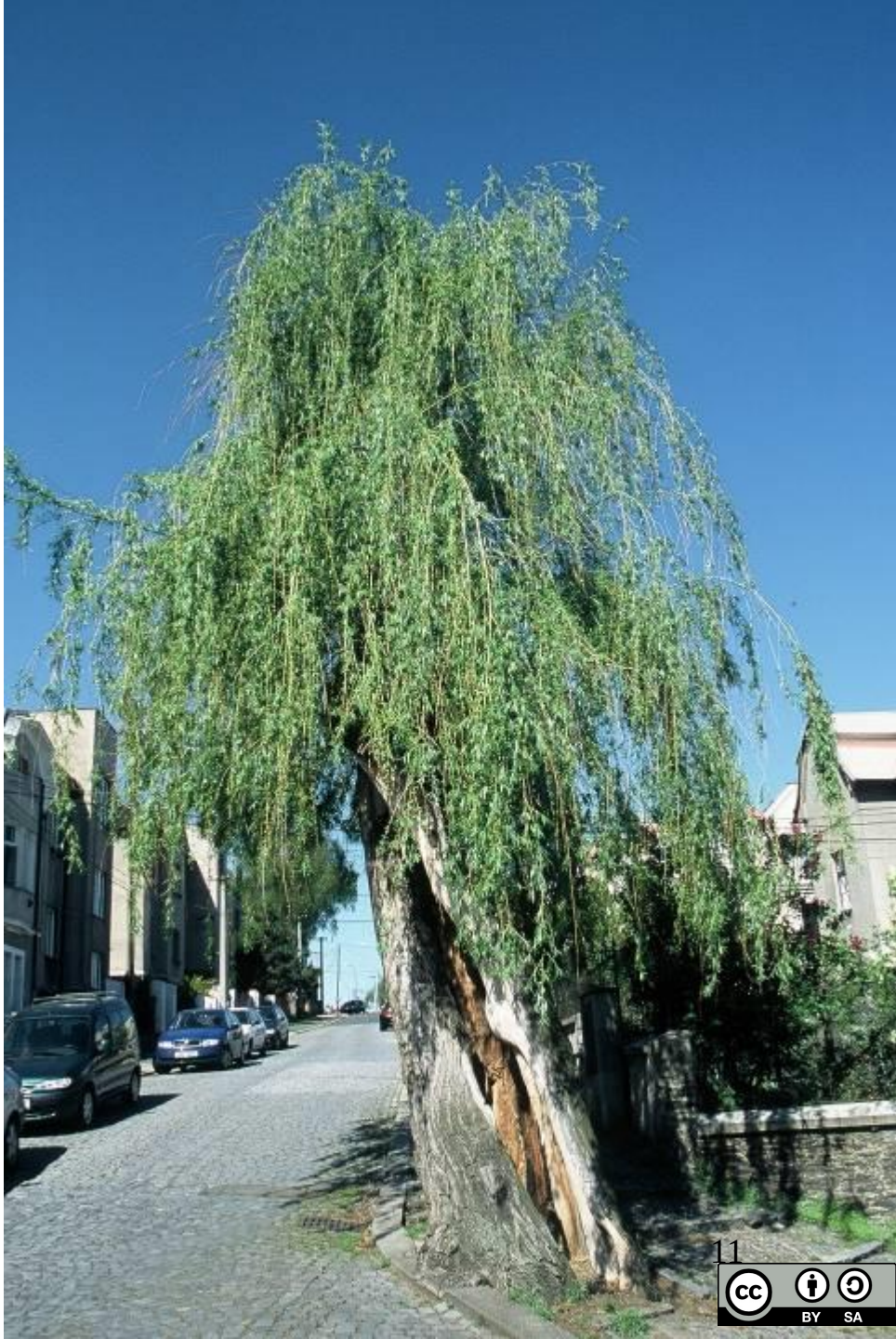
Choleretic, diuretic, spasmolytic
Anti-inflammatory

Phenolic acids

Salix spp. Salicaceae
Willow

Bark:
- flavonoids
- glycosides of phenols
  and of phenolic acids

-Salicin, salicortin



Phenolic acids

Myroxylon balsamum Fabaceae
(Peruvian balsam)

A tree growing in Central America

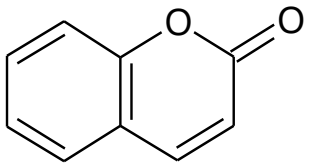
The bark is beaten, then the exudate is collected
- benzoic acid esters, caffeic acid esters (50-60%)
- alcohols, free cinnamic and benzoic acids

Antiseptic, healing properties
Used topically:
burns, frostbites, cracks, erythema, pruritus, dermatitis.

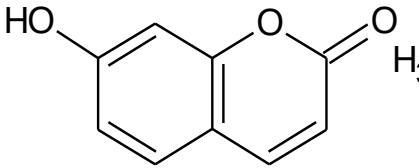
Coumarins - generalities

- Soluble in polar organic solvents
- Lactones – hydrolysable in alcaline conditions
- Characteristic UV spectra, often fluorescent compounds
-
- Furanocoumarins – photosenzitizers

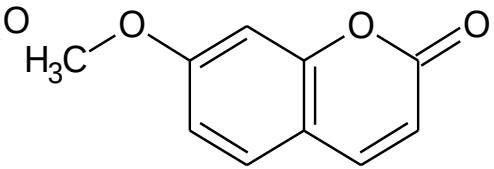
Coumarins



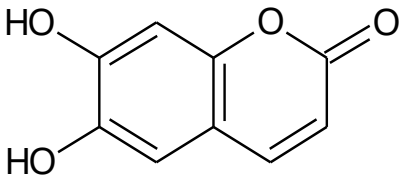
coumarin



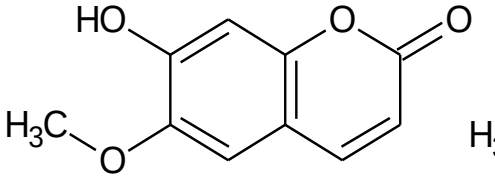
umbelliferone



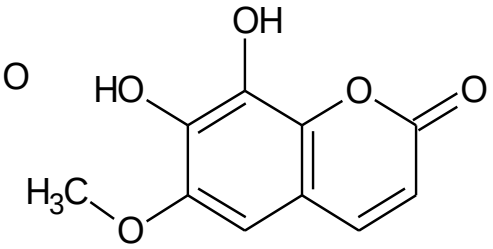
herniarin



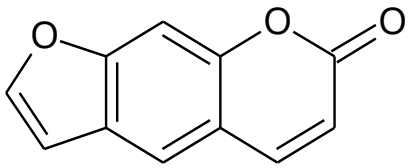
aesculetin



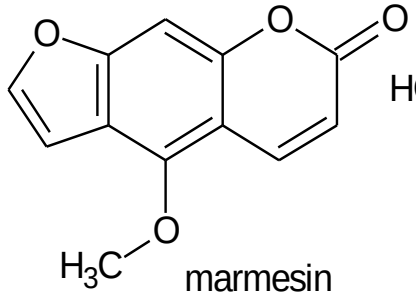
scopoletin



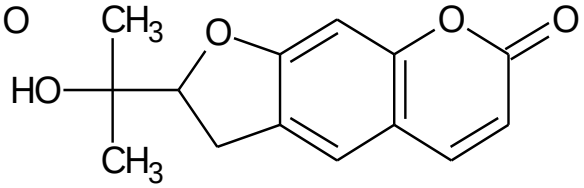
fraxetin



psoralen



marmesin



bergapten

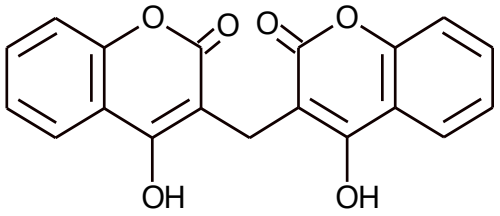
Coumarins

Meliotus officinalis Fabaceae
Sweet clover

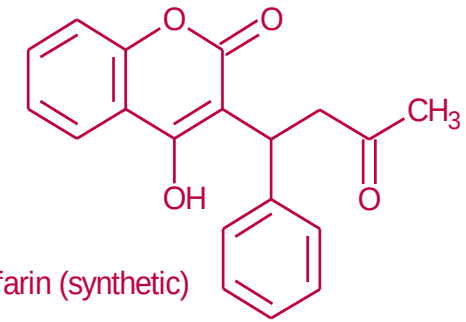
A plant growing in Europe
Up to 1,5 m height

Flowering tops:
-saponins
-flavonoids
-phenolic acids
-coumarin
-dicoumarol

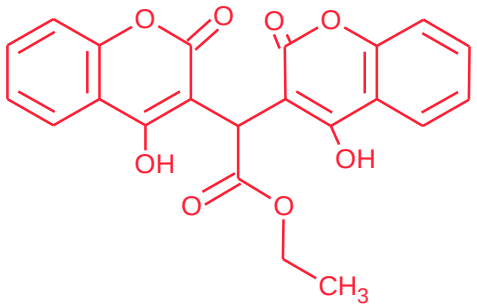
Anticoagulant coumarins -
- Vitamin K antagonists



dicoumarol (*from M. officinalis*)



warfarin (synthetic)



pelentan (synthetic)

Coumarins

Melilotus officinalis Fabaceae
Sweet clover

A plant growing in Europe
Up to 1.5 m heigh

Flowering tops:
-saponins
-flavonoids
-phenolic acids
-coumarin
-dicoumarol

Coumarins

Galium odoratum Rubiaceae
Asperula odorata

Sweet woodruff

- coumarin (1%)
- iridoids (asperuloside)

- sedative, spasmolytic
- spice
- flavoring of liquors

Furanocoumarins

Apiaceae (Daucaceae)
Angelica archangelica
Heracleum sp.,
Pastinaca sativa

Rutaceae
Ruta spp.
R. graveolens: Rue plant

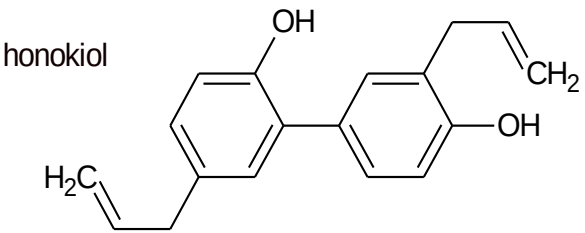
Lignans

- Products of condensation of 2 phenylpropanoic units
- C18 skeletons – 2x benzene ring + 6 non-aromatic carbons
- Different structutres

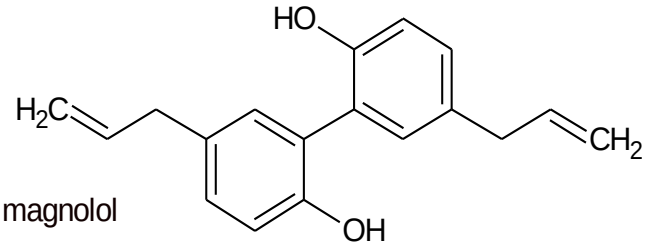
- Antioxidants, interactions with enzymes, binding to receptors

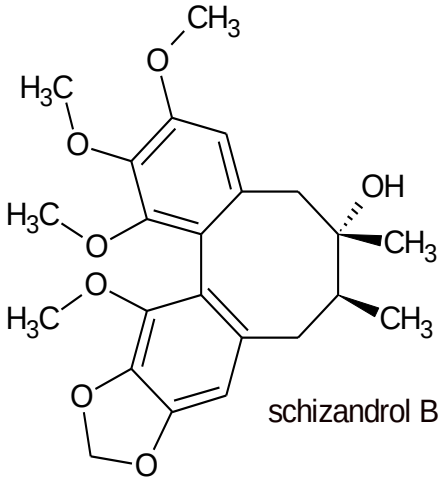
Lignans

honokiol

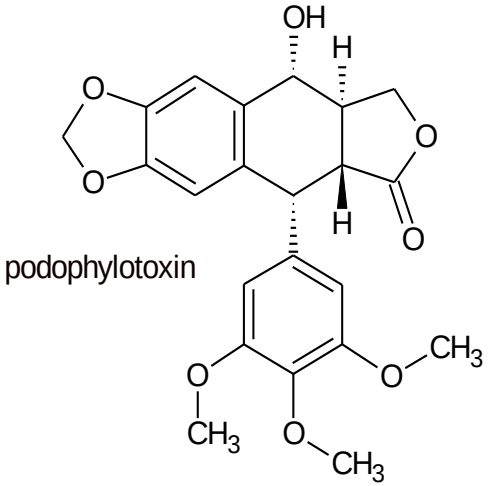


magnolol

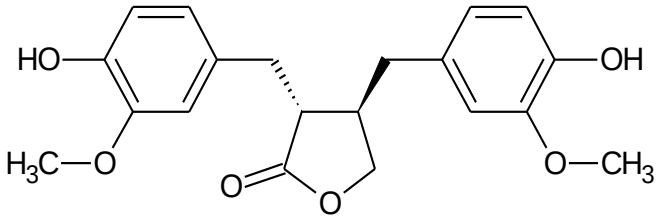




schizandrol B

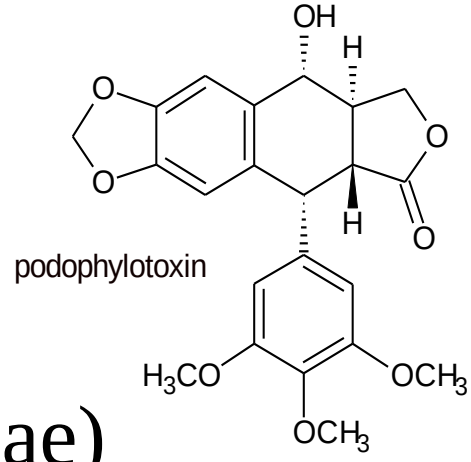


podophylotoxin



matairesinol

Lignans



podophylotoxin

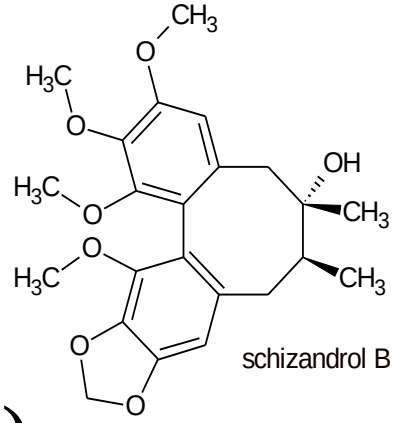
- *Podophyllum peltatum* (Berberidaceae)
- English name: May apple

Rhizome extracts contain
up to 20% of podophylotoxin

Cytostatic, toxic.
Interaction with tubulin

Semisythetic derivatives of p. are used in chemotherapy

Lignans



- *Schizandra sinensis* (Schizandraceae)
- English name: Schizandra

Fruits rich in vitamin C

Seeds rich in lignans

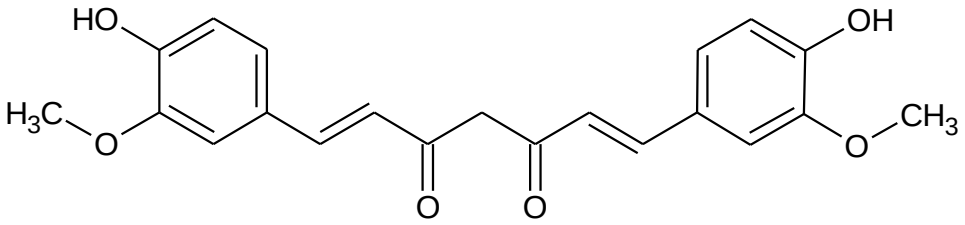
Antioxidants

Hepatoprotective

Tonics

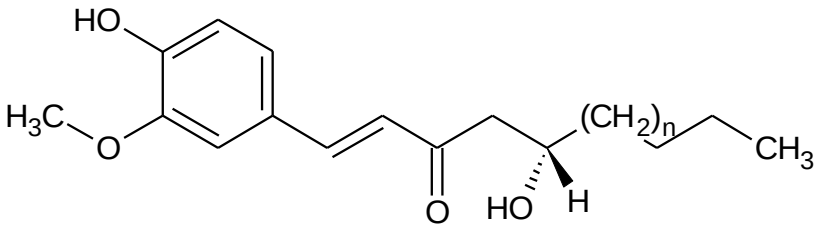
Diarylheptanoids (curcuminoids)

- Zingiberaceae



curcumin

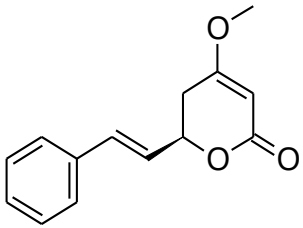
- *Curcuma longa, C. domestica*
-
- *Zingiber officinale*



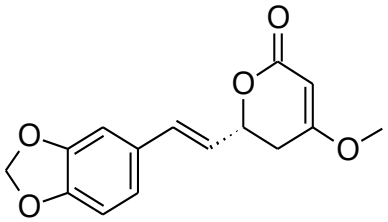
gingerol

- Zingiberaceae
- *Curcuma longa*

- Piperaceae
- *Piper methysticum*



kawain



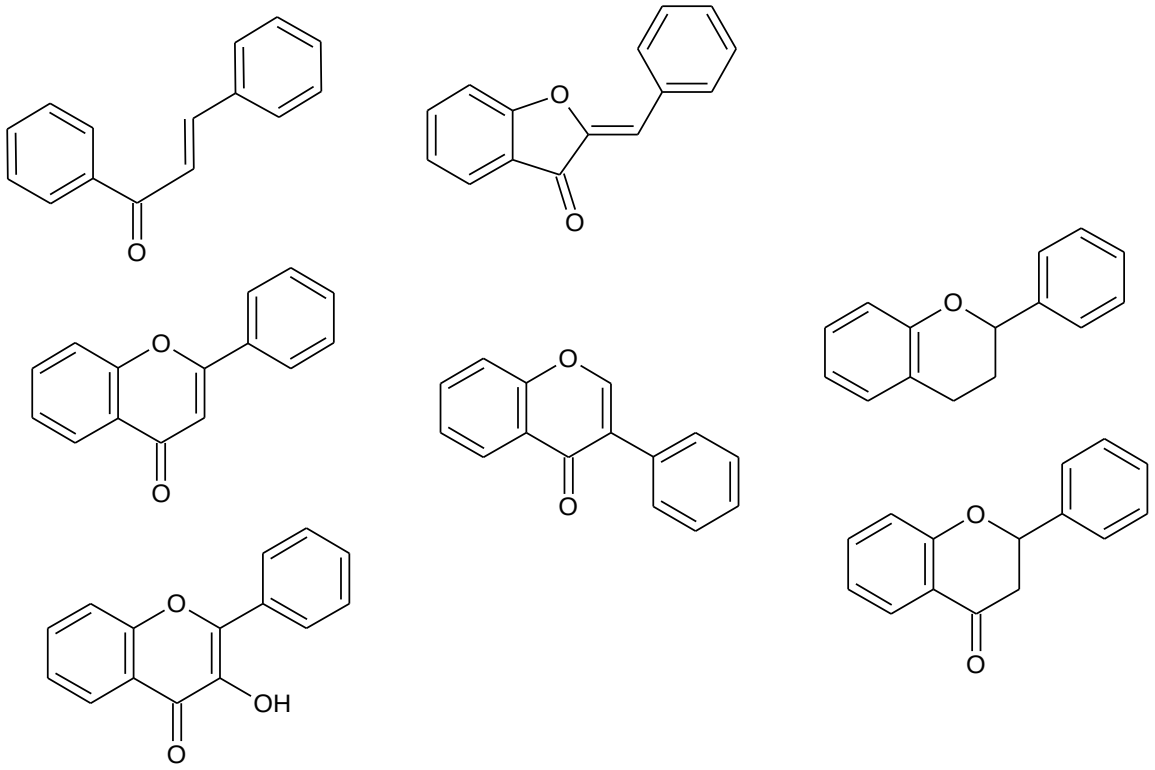
methysticin

Flavonoids

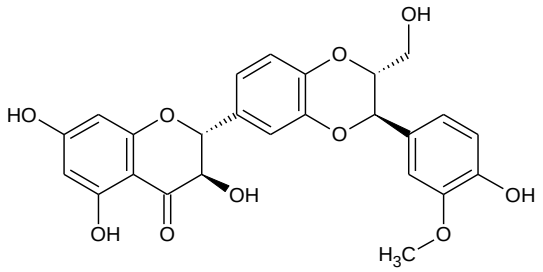
C15 skeleton

- phenylpropane C9 + polyketide 3 x C2

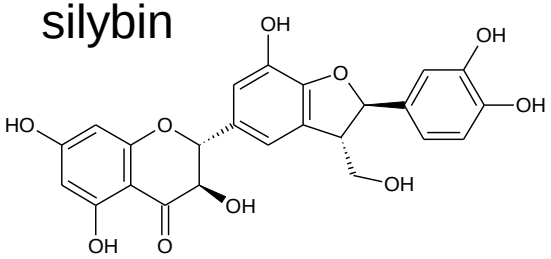
Flavonoids



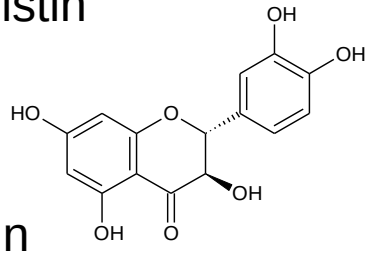
Silybum marianum, Asteraceae
Blessed Milk Thistle



silybin



silychristin

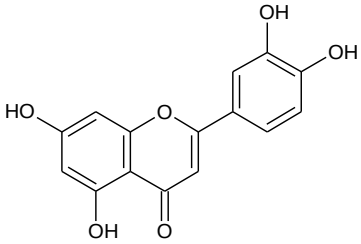


taxifolin

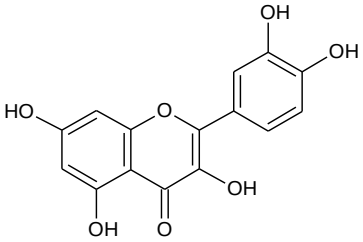
Centuries-long reputation

Hepatoprotective
Antiniflammatory

Equisetum arvense, Equisetaceae
Field horsetail



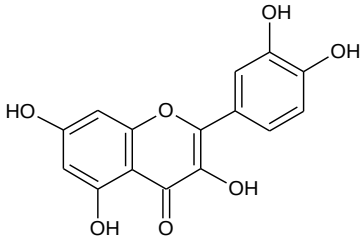
luteolin



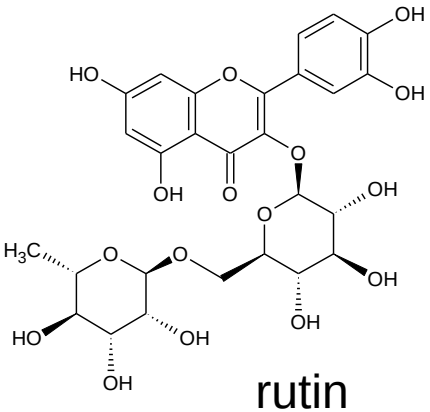
quercetin

Fagopyrum esculentum, Polygonaceae
Buckwheat

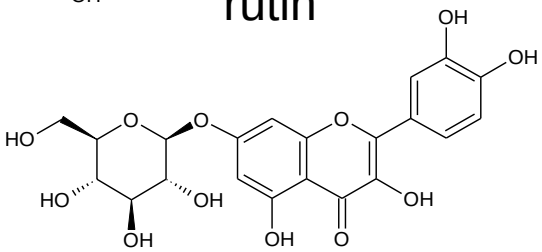
Sophora japonica, Fabaceae
Japanese pagoda tree



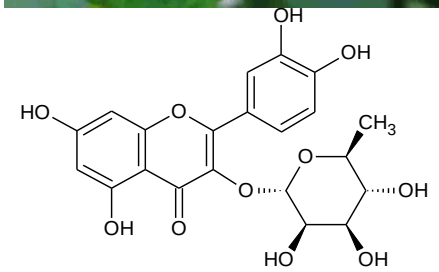
quercetin



rutin



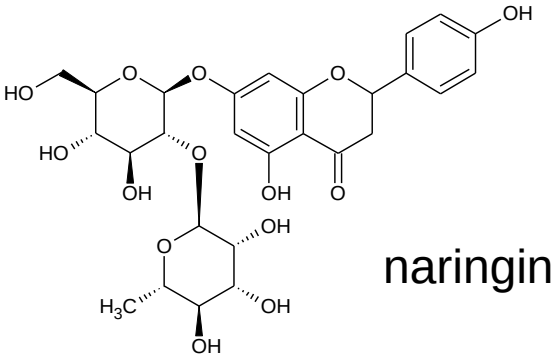
quercimeritrin



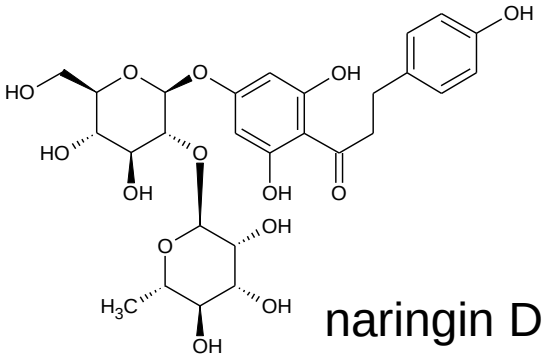
quercitrin

Antioxidants, venoprotectives

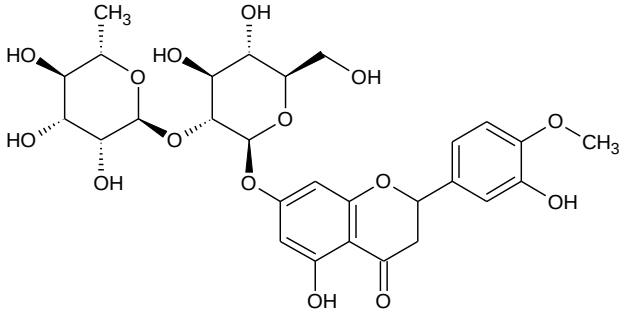
Citrus paradisum, Rutaceae
Citrus aurantium, Rutaceae



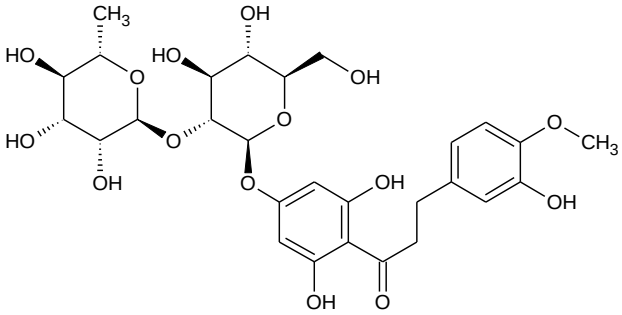
naringin



naringin DHC



neohesperidin

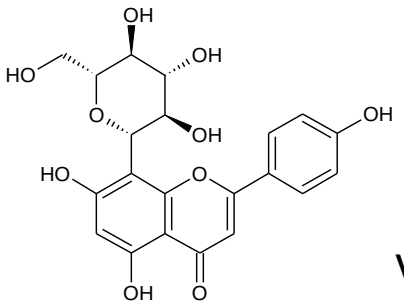


neohesperidin DHC, E_{959}

natural bitter substances

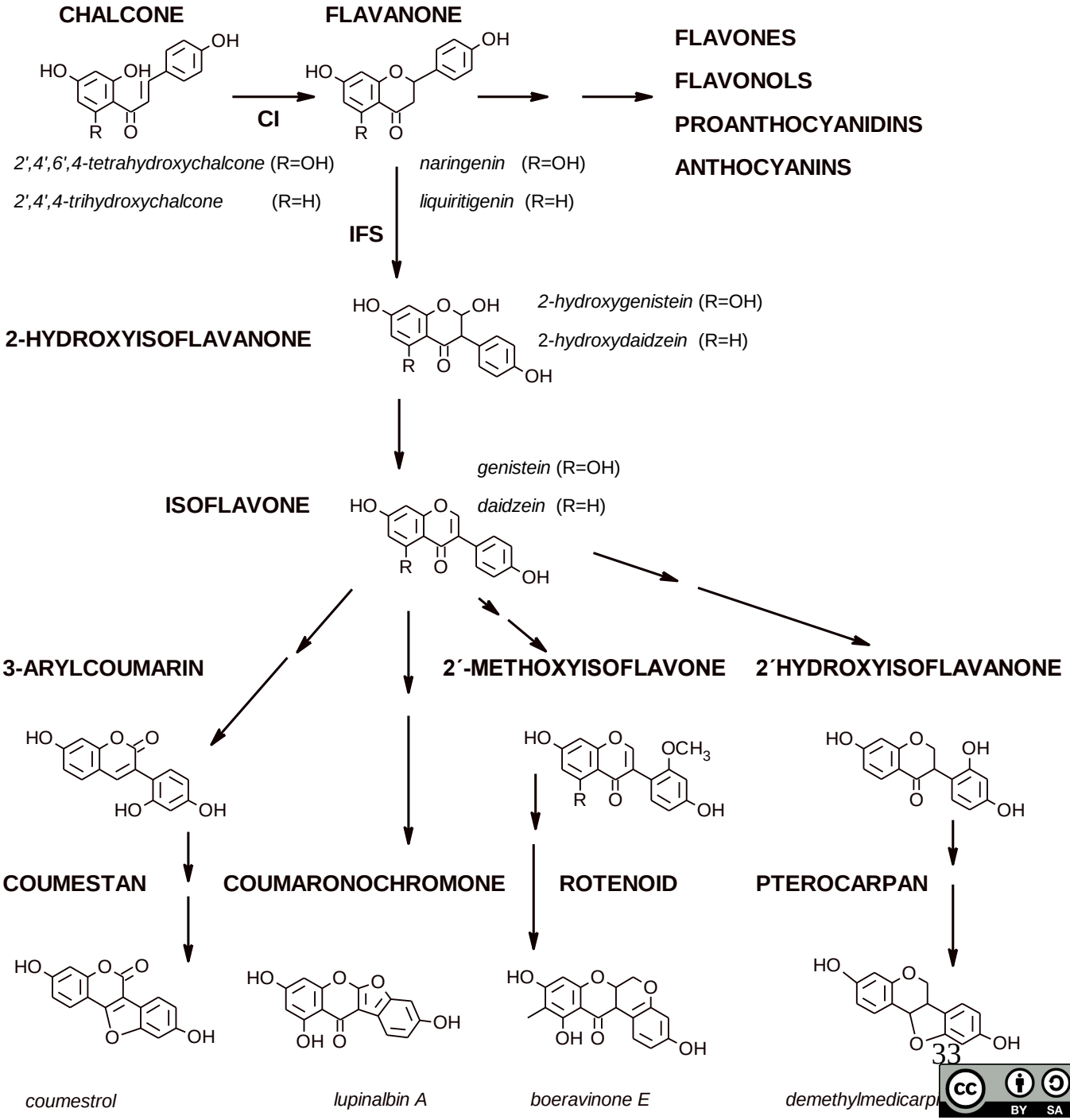
semisynthetic intensive sweeteners

Panicum miliaceum, Poaceae
Millet



vitexin

Isoflavonoids



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

9. Phytoestrogens

Lecturer: Oldřich Lapčík

PHYTOESTROGENS:

STRUCTURAL TYPES
and
BOTANICAL SOURCES

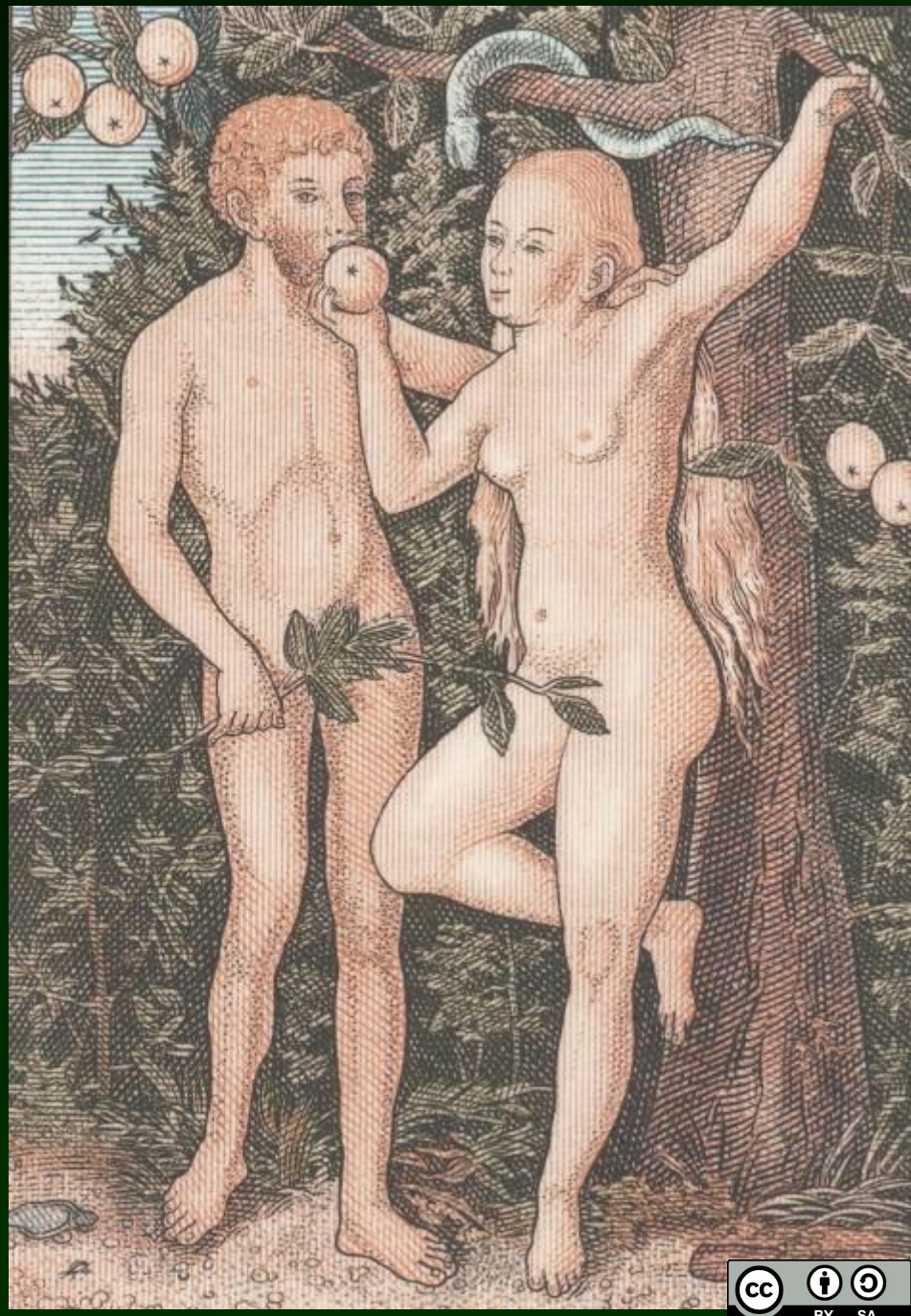
Oldřich Lapčík
Department of Chemistry of Natural Compounds
Faculty of Food and Biochemical Technology
University of Chemical Technology, Prague

Estrogen

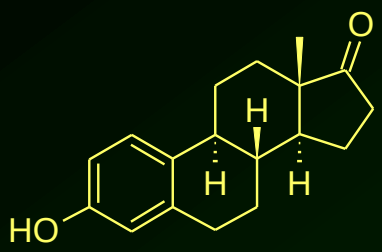
- Oistros –
  mad desire

- Oestrus –
  gad fly, hornet

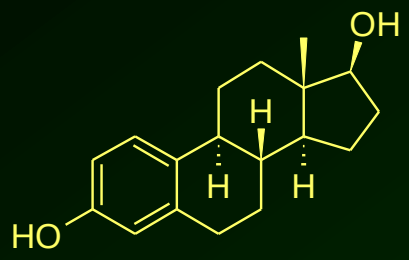
- Genero, genere –
  to make, to elicit



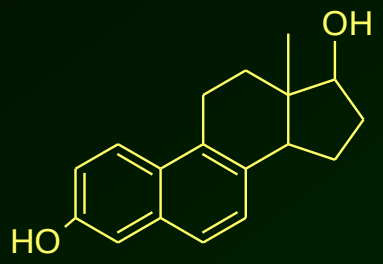
Estrogen



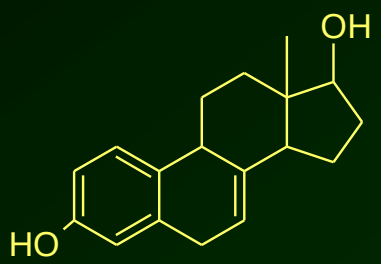
Estron



Estradiol



Dihydroequilenin



Dihydroequilin

- E. Allen, E.A. Doisy (1923) An ovarian hormone: preliminary report on its localization, extraction and partial purification, and action in test animals. JAMA 81: 819.

- Adolf. F. J. Butenand – 1929 isolation of estron (Nobel prize 1939)

Functions of estrogens

- CNS: behavior
- Sexual organs: control of fertility (F)
- Non-sexual target organs:
  regulation of multiple metabolic pathways
  maturation of conective tissue

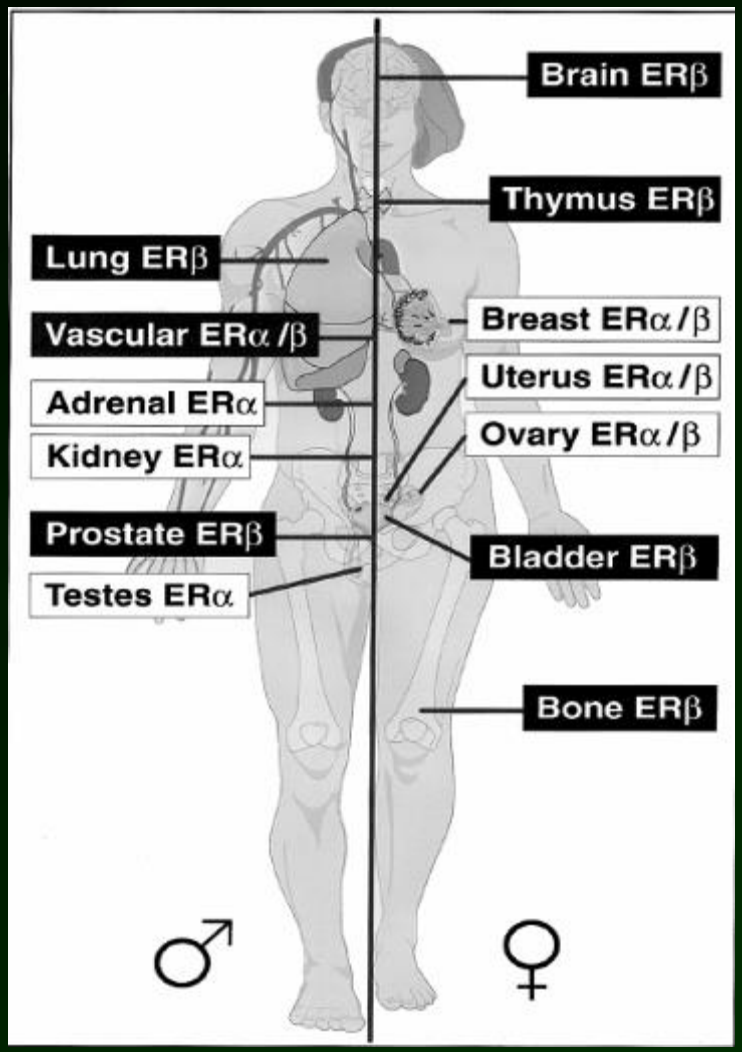
Receptor systems:

nuclear receptors - ERα, ERβ

membrane bound ERs

Sugiyama et al. Trends Endocrinol Metab. 21: 545-552, 2010.

Tissue distribution of ERs



- Breast – epithel: ERα
  – stroma: ERβ
- Uterus – ERα, ERβ
- Ovary – ERα, ERβ
- Prostate – ERα
- Testes – ERβ
- Liver – ERα

Gustafsson J.A. J. Endocrinol **163:** 379–383, 1999
Sugiyama et al. Trends Endocrinol Metab. 21: 545-552, 2010.

Identification of phytoestrogens

- In vivo
  - Keratinisatin of vaginal mucosa (OVX)
  - Uterus weight (OVX)
  - Expression of ER dependent genes (e.g.. vitellogenin)

- In vitro
  - Binding studies - uterine cytosol (OVX); receptors: ERα, ERβ
  - Cell cultures – HeLa, MCF7, MC3T3, Ishikawa
  - Reporter genes (e.g. luciferase, GFP)

- In silico – docking studies

Wang C.C. et al.: J. Chromatography B 777: 3-29, 2002
Sugiyama H. et al. BBRC 379: 139-144, 2009.

- Bennets H.W., Underwood E.J., Shier F.L.: A specific problem of sheep on subterranean clover pastures in Western Australia. *Aust. Vet.J.* **22:** 2-12, **1946**

- **1954**: A review of 53 estrogenically active plant species (R.B. Bradbury and D.E. White)

- **1985: More than** 300 estrogenic plants (Price K.R., Fenwick G.R.)

- **2018: ???**

Are there estrogens in plants?

Hughes C. (1996) J. Clin. Endocrinol. Metab. 81: p 2405.

- …. any putative estrogen should be capable of inducing estrus in lower mammals.
- ..estrogen…. must act on the brain, rather than the vagina!
- …..there is hardly any occurrence of truly estrogenic compounds in plants…

- …. we must conclude that these compounds are not estrogens at all, and we have been in error in characterizing them as such!

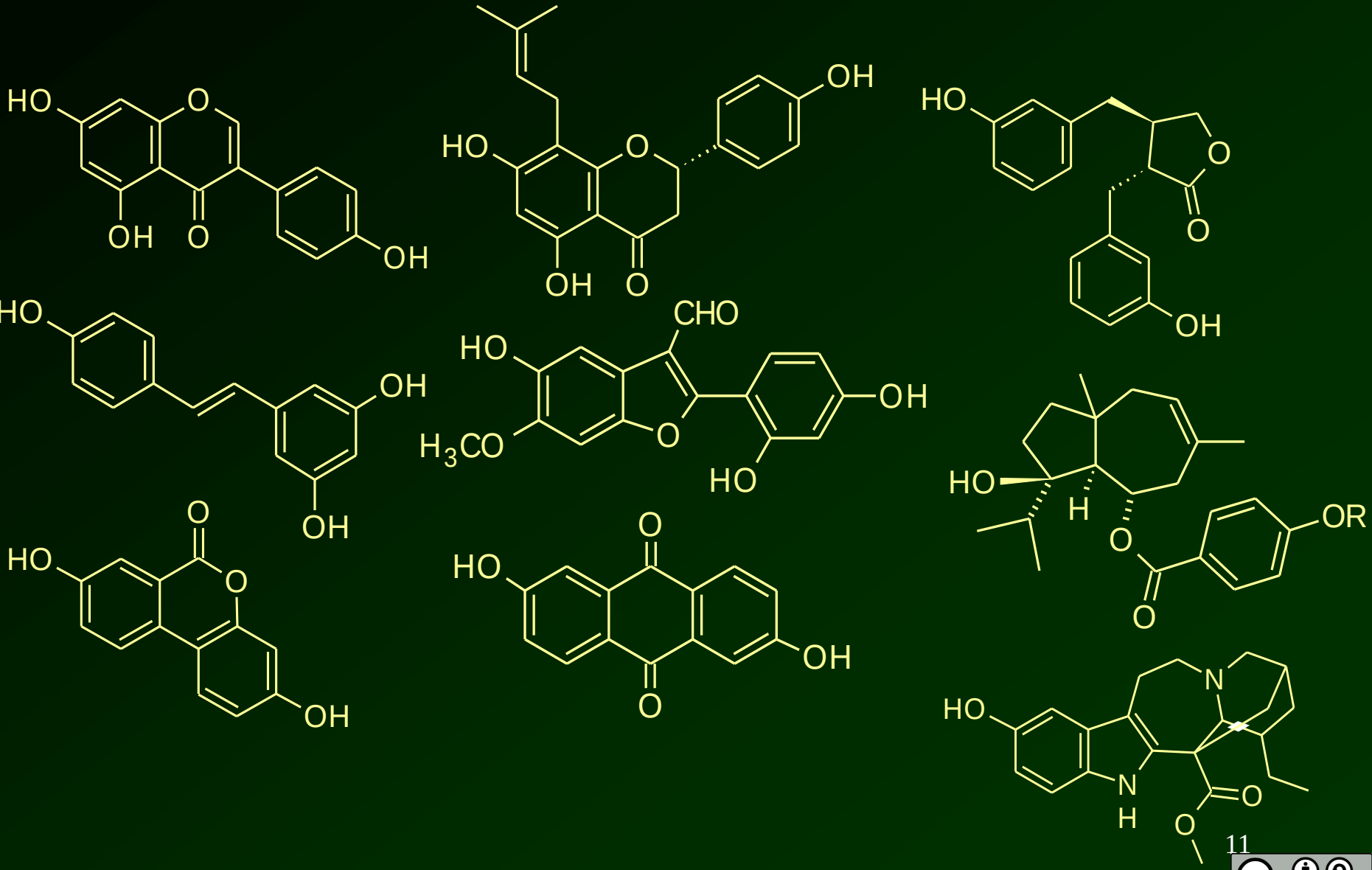
Yes, there are phytoestrogens

- Lieberman S. (1996) J. Clin. Endocrinol. Metab. 81: 2405.

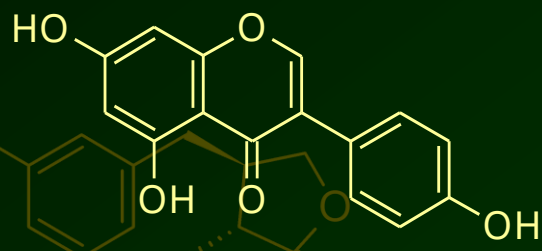
- “The identification of an estrogen using the cornification end point as the standard is purely phenomenological; the results need not be biased by interpretation. Historically, most estrogens were identified as such by this simple assay; few were detected by their effects on the brain. ……

  it does not matter what you call it as long as you are right.“

Phytoestrogens: structural features



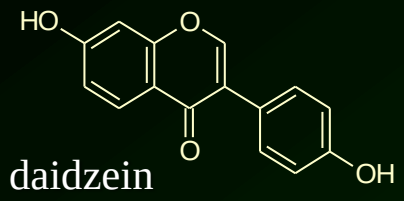
Phytoestrogens: structural features



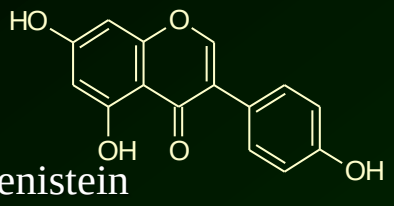
- Aromatic ring (at least one, usually two)
- Relative flatness and rigidity of the molecule
- Two or three charged groups, usually hydroxyls, the distance between them similar to that between C3 and C17 –hydroxyls in 17-β estradiol
- Molecular weight not far from endogenous estrogens

Mazur and Adlercreutz , Nutrition 16:654-687,2000

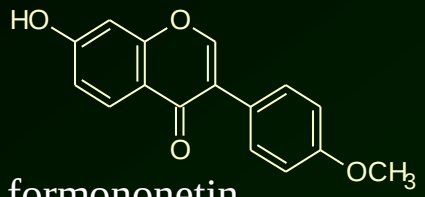
Isoflavonoids



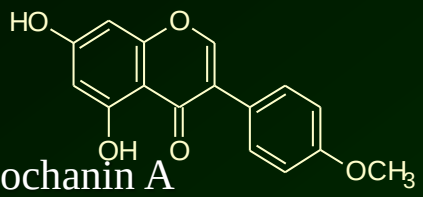
daidzein



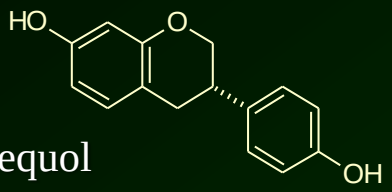
genistein



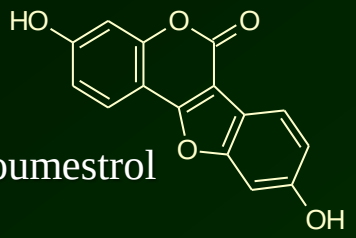
formononetin



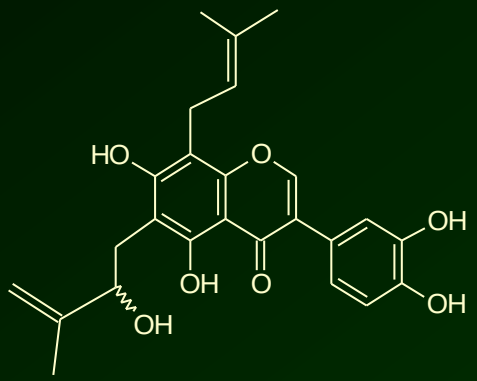
biochanin A



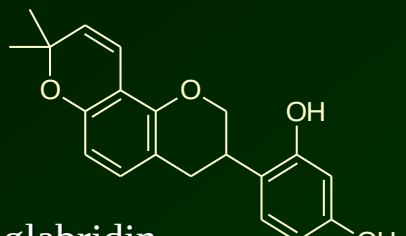
equol



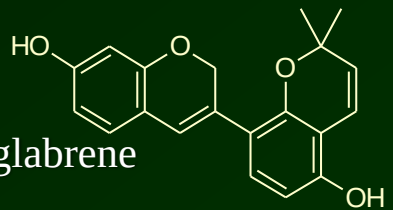
coumestrol



isoerythrosenegalensin



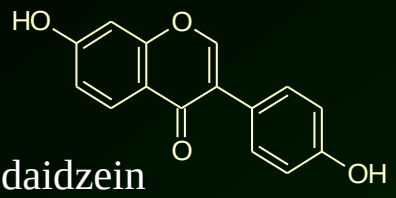
glabridin



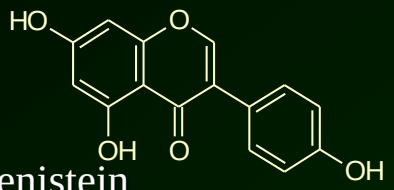
glabrene

- 3- phenylchromans
- More than 1200 compounds
- Estrogenically active:
  several dozens
- Occurence: ~ 60 families
- Dominant source: Fabacae
  (Leguminosae)
- Additional sources: Iridaceae

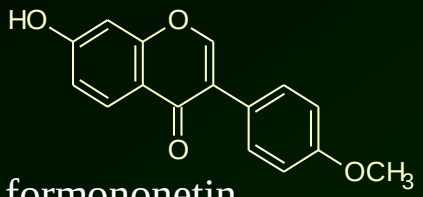
Isoflavonoids



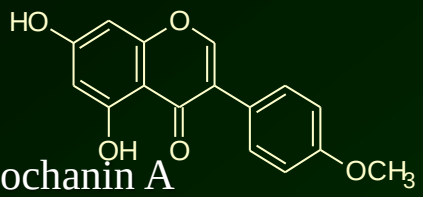
daidzein



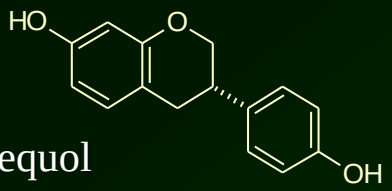
genistein



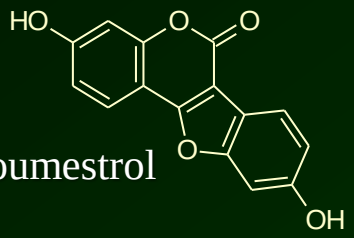
formononetin



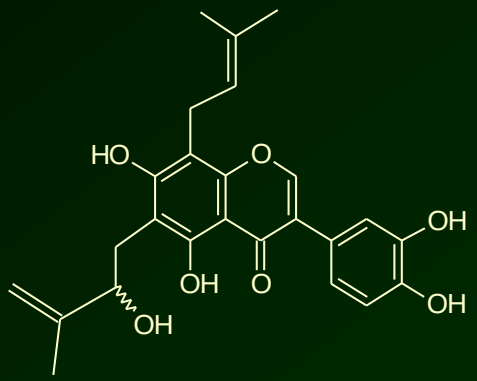
biochanin A



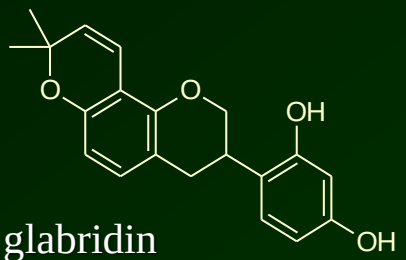
equol



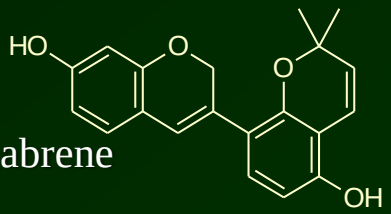
coumestrol



isoerytrosenegalensin



glabridin

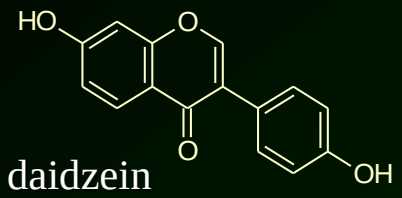


glabrene

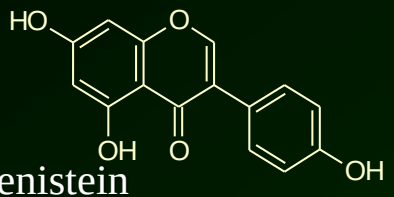

Trifolium pratense


Glycine max.

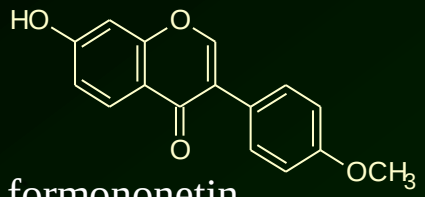
Isoflavonoids



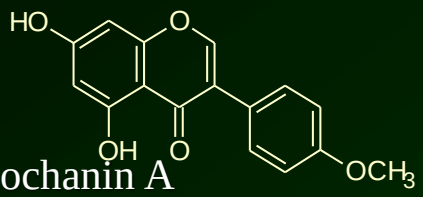
daidzein



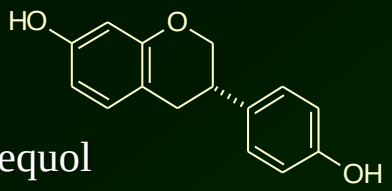
genistein



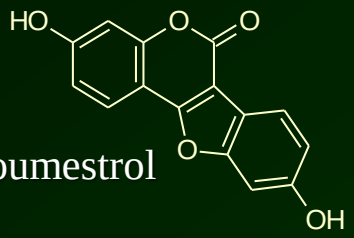
formononetin



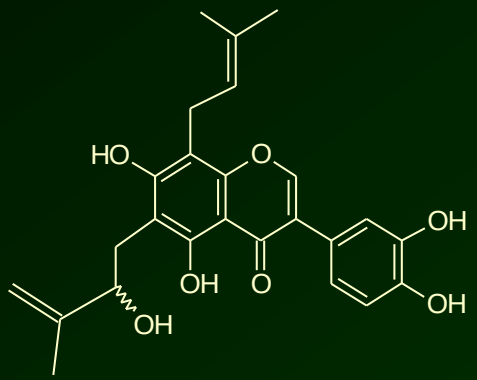
biochanin A



equol

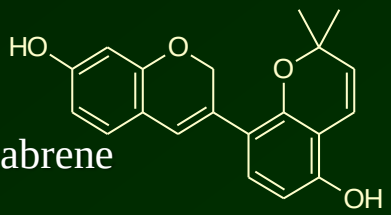


coumestrol



isoerytrosenegalensin

glabridin

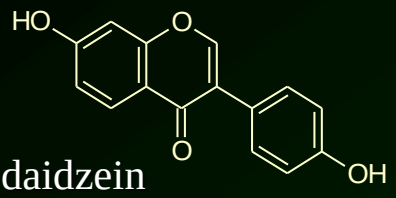


glabrene

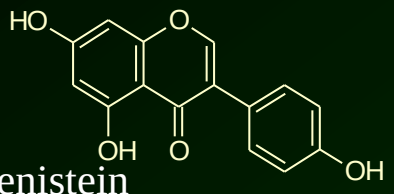

Glycyrrhiza glabra


Milletia pachycarpa

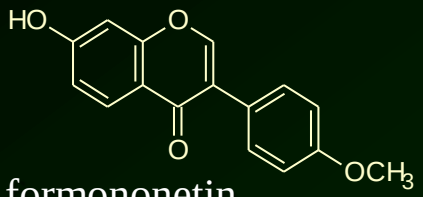
Isoflavonoids



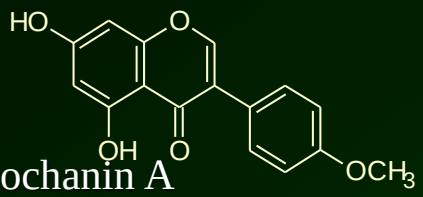
daidzein



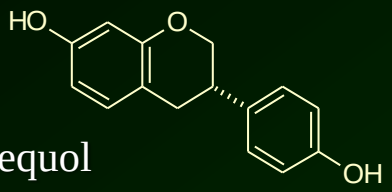
genistein



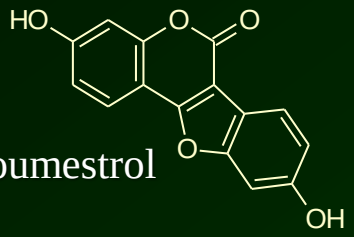
formononetin



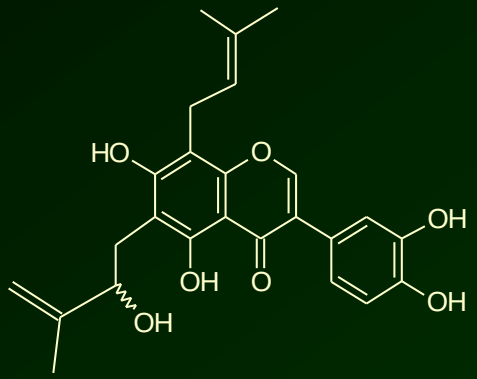
biochanin A



equol

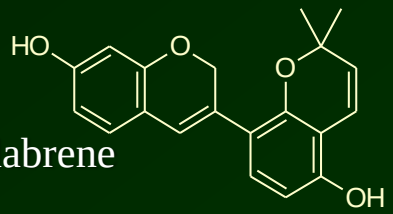


coumestrol



isoerytrosenegalensin

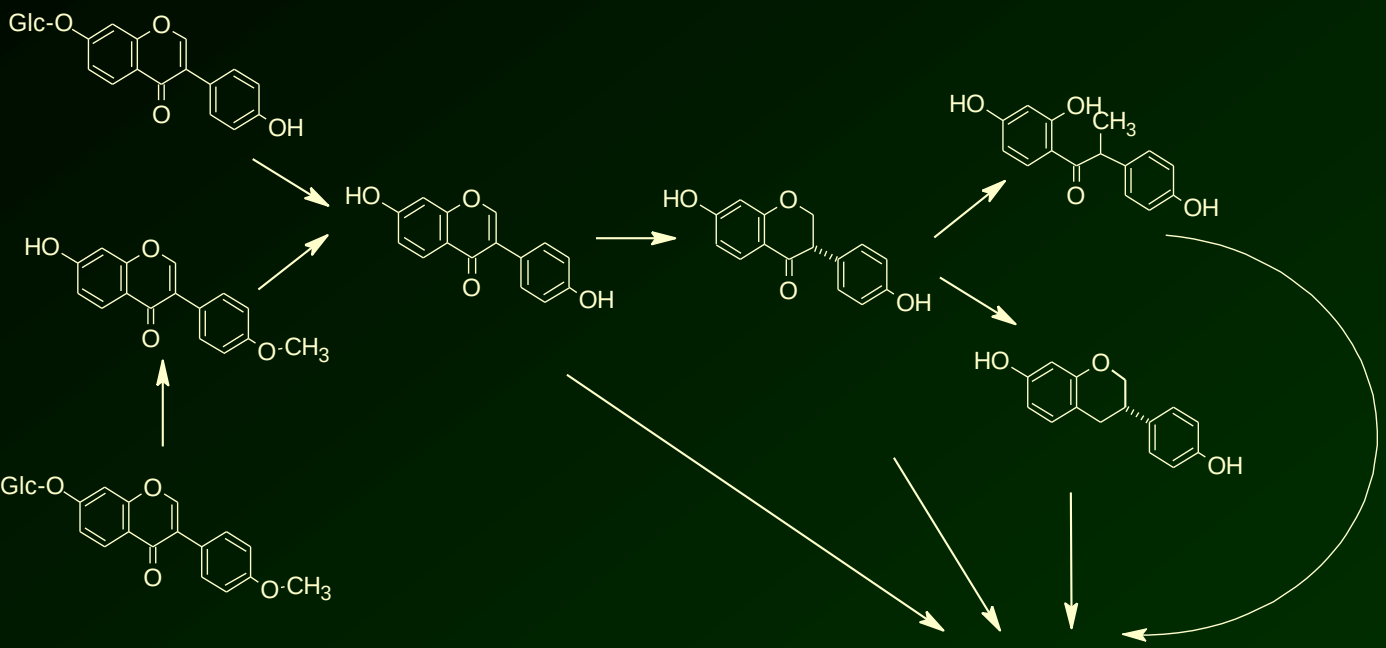
glabridin



glabrene

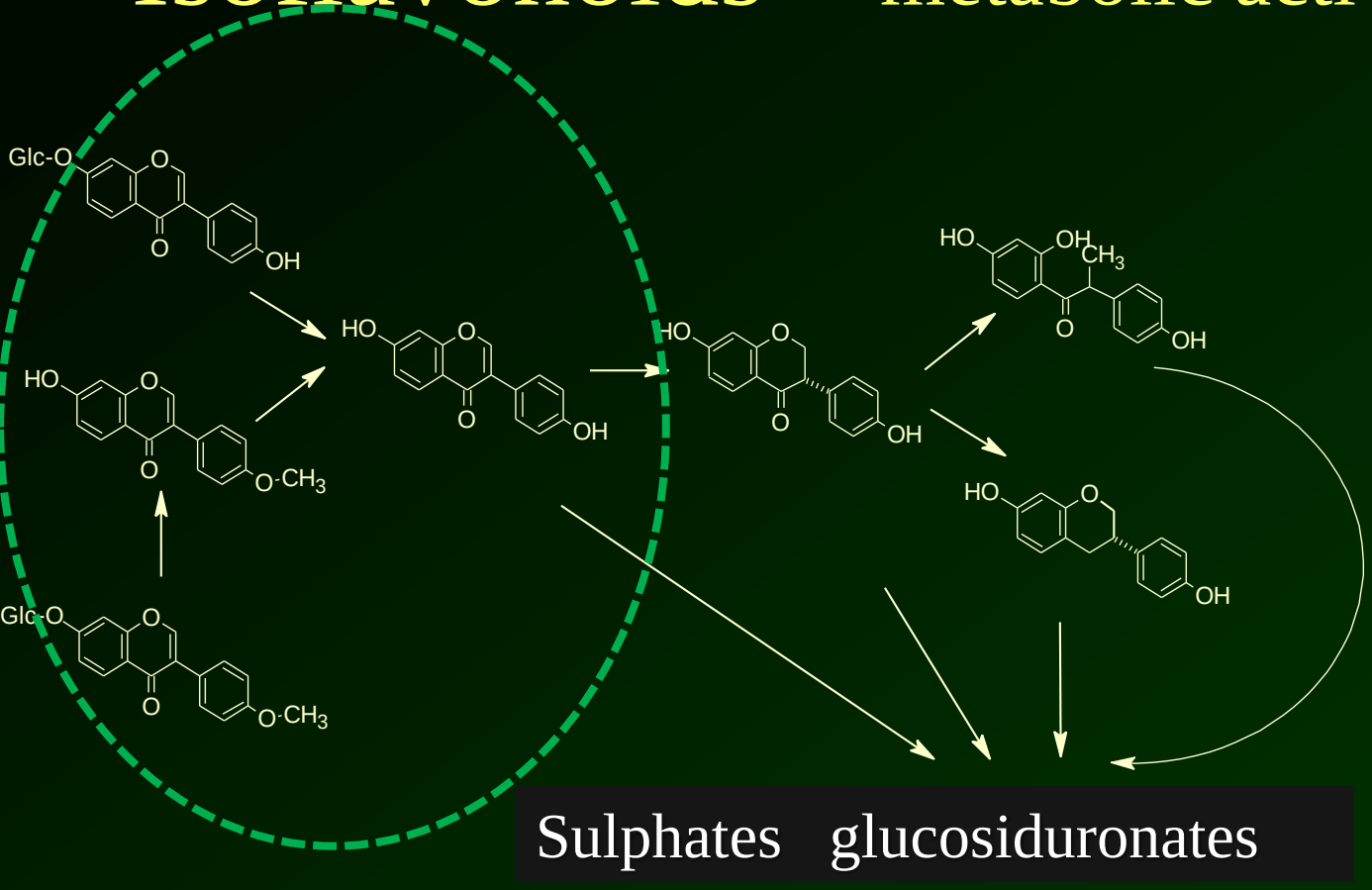

Iris sp. (Iridaceae)

Isoflavonoids – metabolic activation



Sulphates glucosiduronates

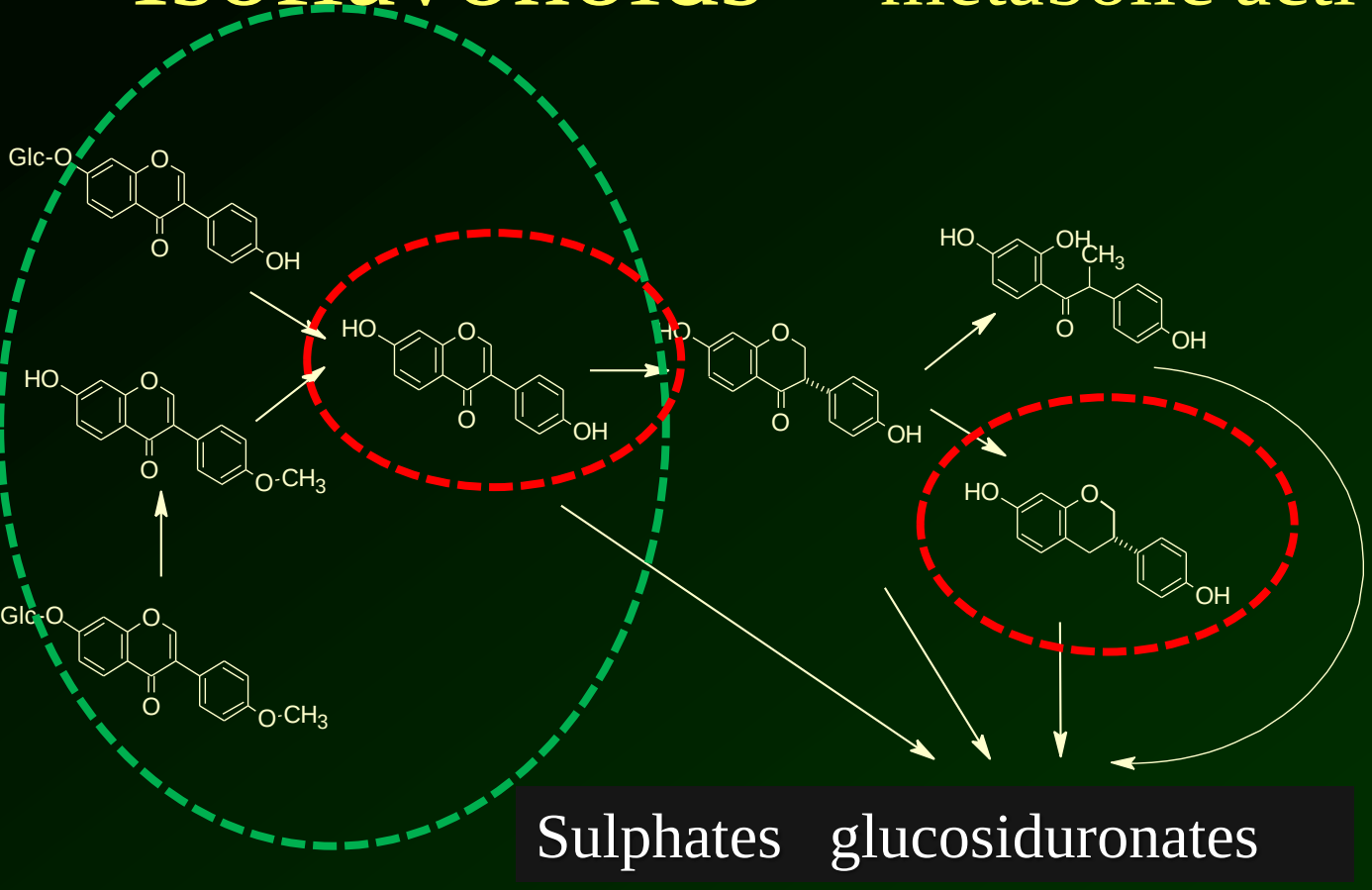
Isoflavonoids – metabolic activation



Sulphates glucosiduronates

Plant source

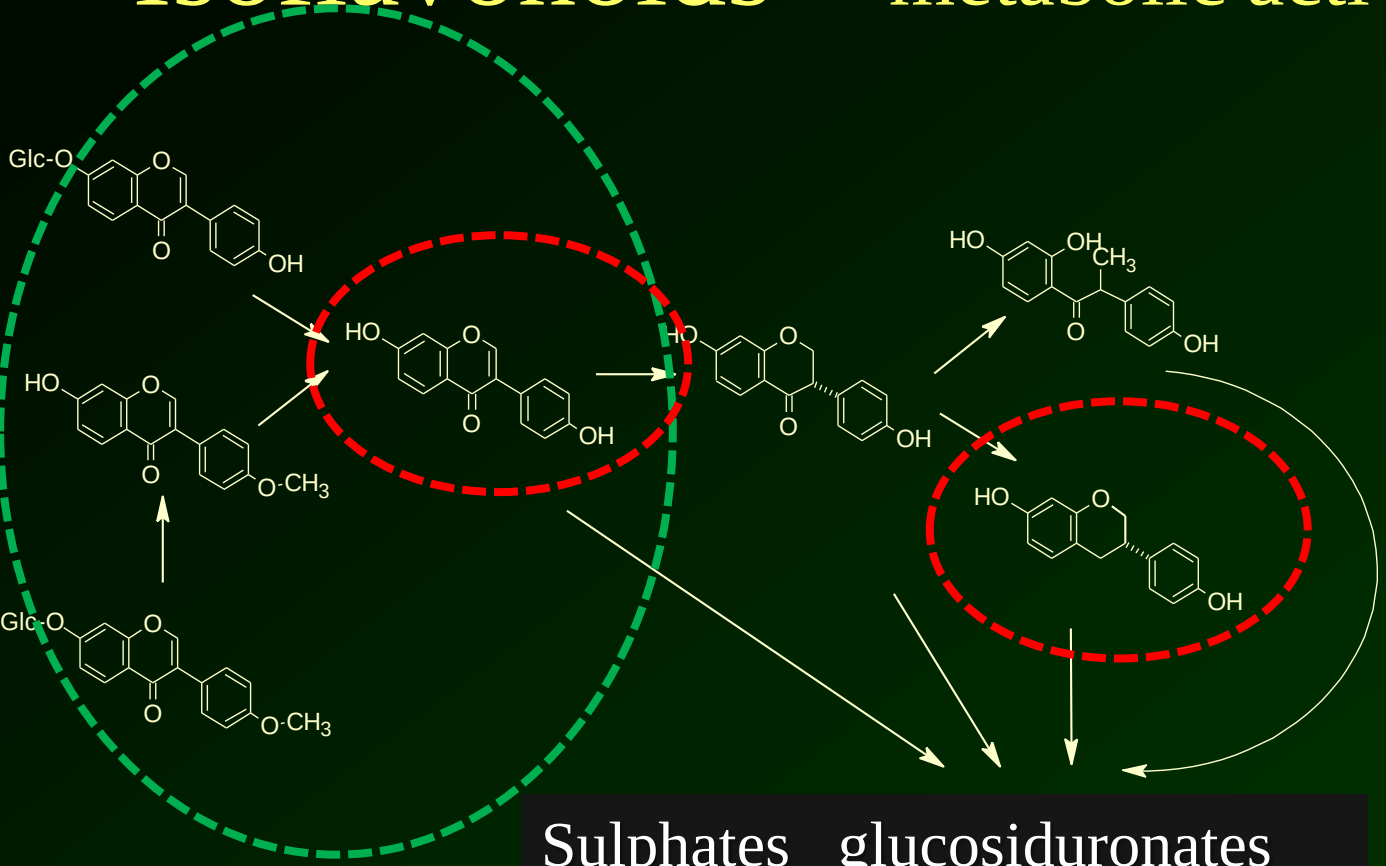
Isoflavonoids – metabolic activation



Sulphates glucosiduronates

Plant source >> active metabolites

Isoflavonoids – metabolic activation

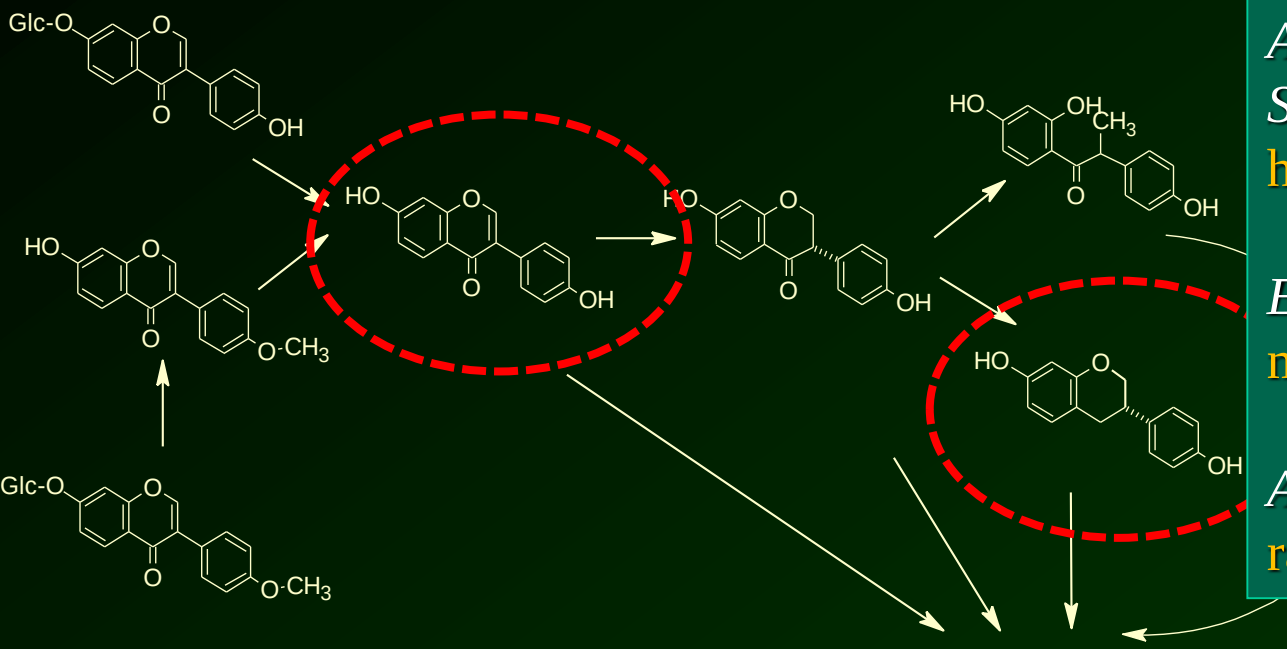


Sulphates glucosiduronates

Plant source >> active metabolites

>> inactive metabolites >> excretion

Isoflavonoids – metabolic activation



Eggerthella spp.
Adlercreutzia equolifaciens.
Slackia isoflavoniconvertens
human

Enterorhabdus musicola
mouse

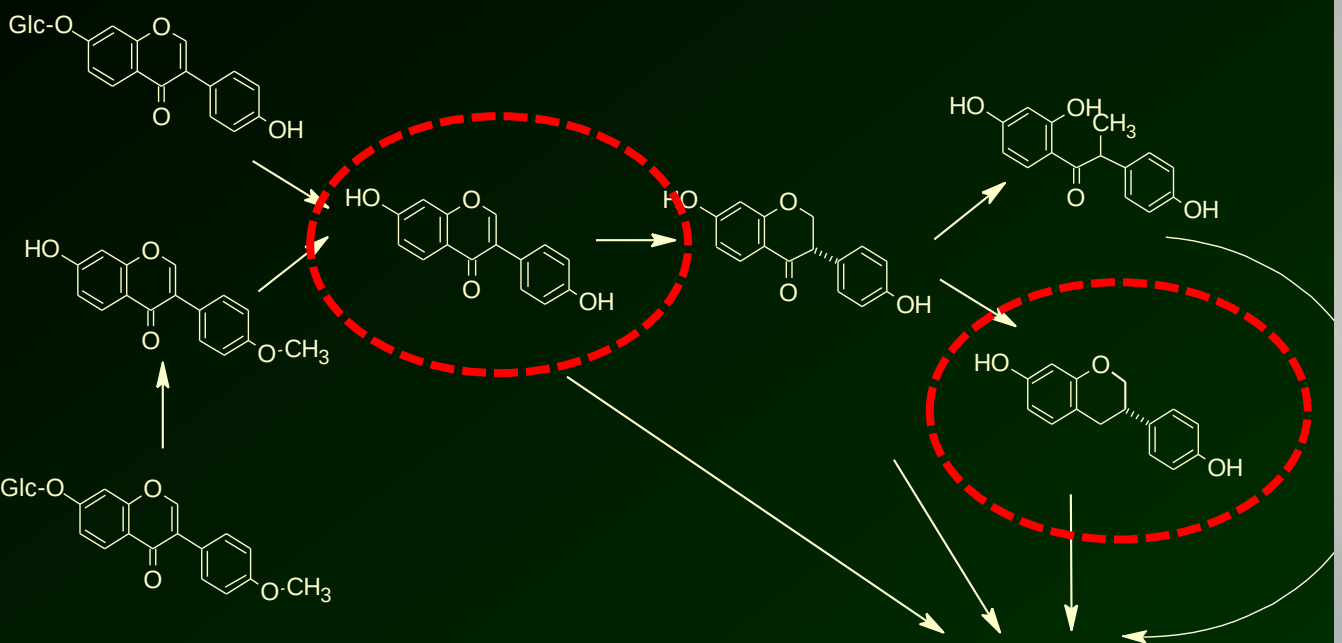
Asaccharobacter celatus
rat

Sulphates glucosiduronates

Maruo T. et al: *Int. J. Syst. Evol. Microbiol. (2008), 58, 1221–1227*

Braune A.: *Polyphenol Communications* 2010, 22-23.

Isoflavonoids – metabolic activation



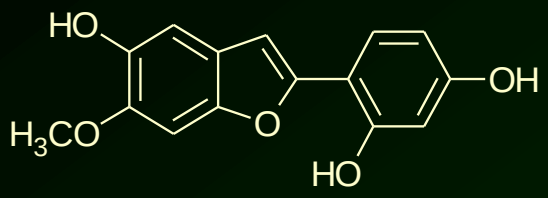
Sulphates glucosiduronates

Herman Adlercreutz

Maruo T. et al: *Int. J. Syst. Evol. Microbiol. (2008), 58, 1221–1227*

Braune A.: *Polyphenol Communications* 2010, 22-23.

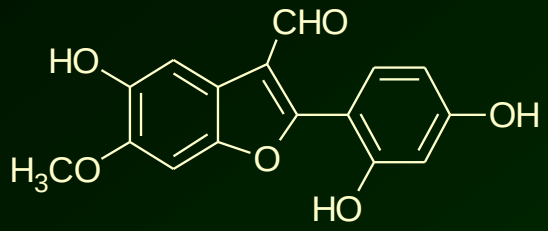
Arylbenzofurans



Ebenfuran I

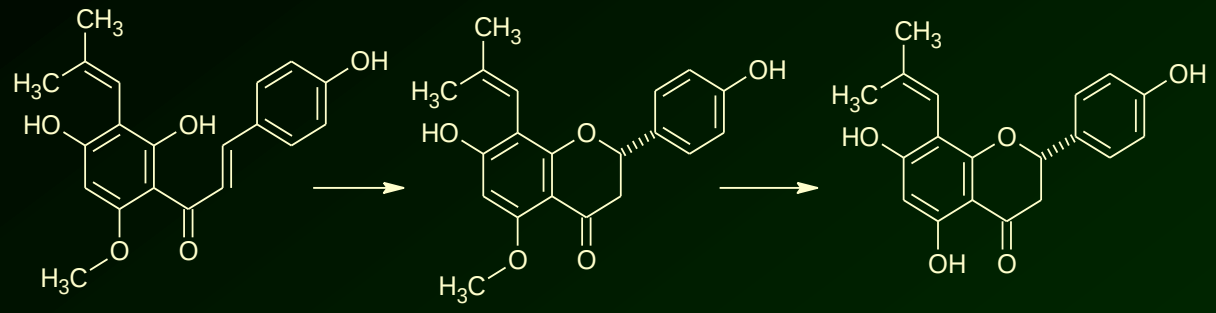
Onobrychis ebenoides

Ebenfuran II



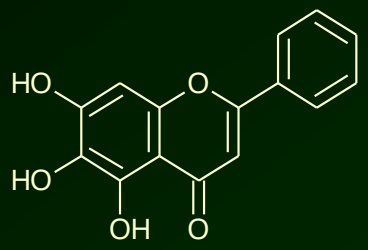
Fabaceae – *Onobrychis, Hedysarum, Erythrina, Cicer*
Moraceae – *Morus* sp.
Hepaticae – *Corsinia* sp.

Flavonoids



Eubacterium limosum - 90% conversion of IXH to 8 PN

Possemiers S. et al., J. Agric. Food Chem. 2005, 53, 6281-6288

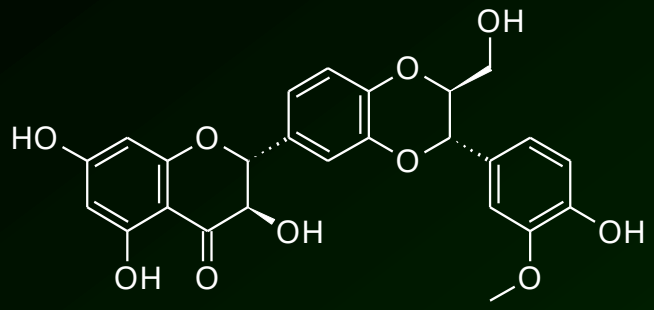


baicalein

Humulus lupulus

Scutellaria bai

Flavonoids

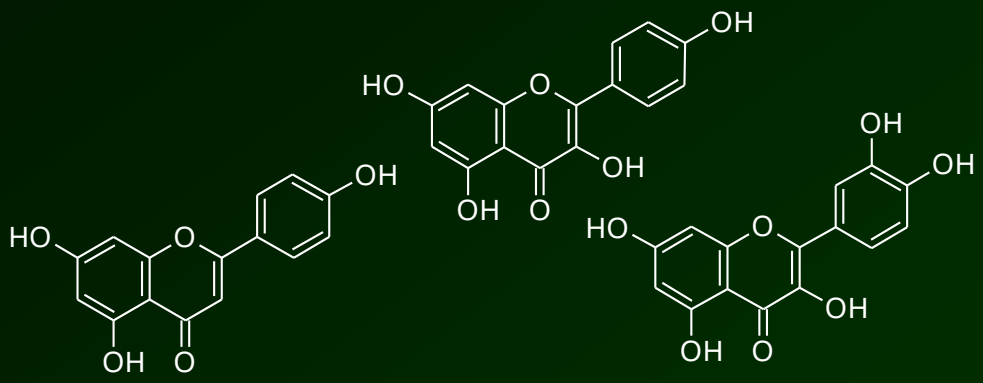


silybin

antiosteoporotic (OVX)
ERβ-ligand
(docking studies)

Plíšková M. *et al:* Toxicology (2005) 215, 80–89

El-Shitany et al.: Phytomedicine (2010) 116-125



apigenin kaempferol quercetin

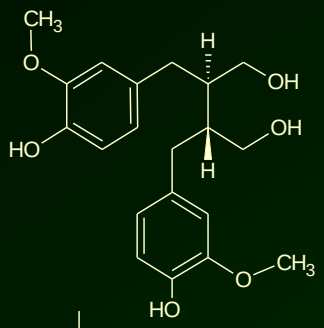
common flavonoids
multiple sources

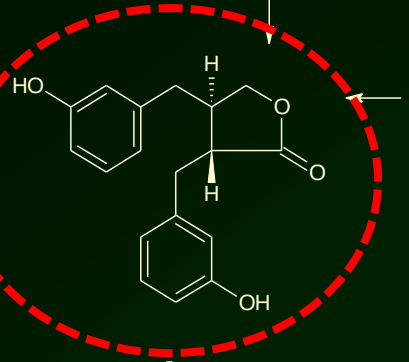
unclear mechanism
of action
in estrogen-dependent tissues

Lignans

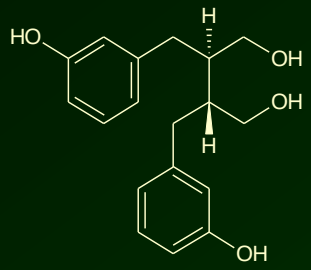
matairesinol

secolariciresinol





enterolactone



enterodiol

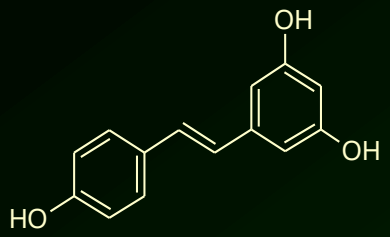
Linum ussitatissimum

	Secolariciresinol mg/kg	Matairesinol mg/kg
Linseed	3600 - 5000	10-13
Sesam	1	6
Sunflower seed	6	0
Groundnuts	3	0
Rye	1.3	1.7
Cranberries	15	0
Pumpkin	38	0.4
Broccoli	4	0

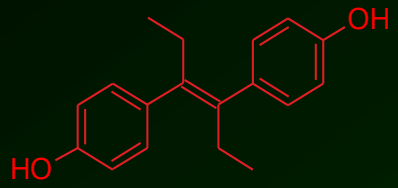
Stilbenoids

-Popularized in connection with “French paradox“
-found in wine (and many other sources)

- Weak ER ligand
-Active in other signaling systems
(ArHR, antioxidant…)



resveratrol



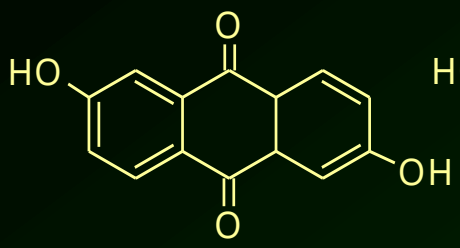
diethylstilbestrol
(xenoestrogen)

	mg/kg dry weight	mg/kg
Cabbage	9	0,5
Kale	15	2,6
Broccoli	15	1,8
Carrot	4	0,4
Red beet	8	1,8
Onion	12	1,1

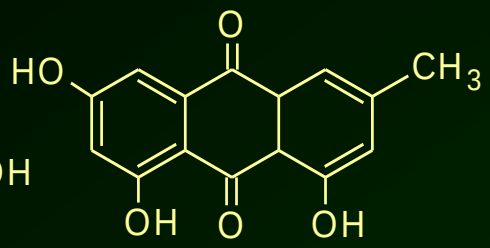
Gehm B.D. et al.: Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. PNAS 94 (1997) 14138-14143

Šmidrkal et al.: Resveratrol. Chem listy 95 (2001) 6002-609

Anthraquinones



2,6 dihydroxyanthraquinone



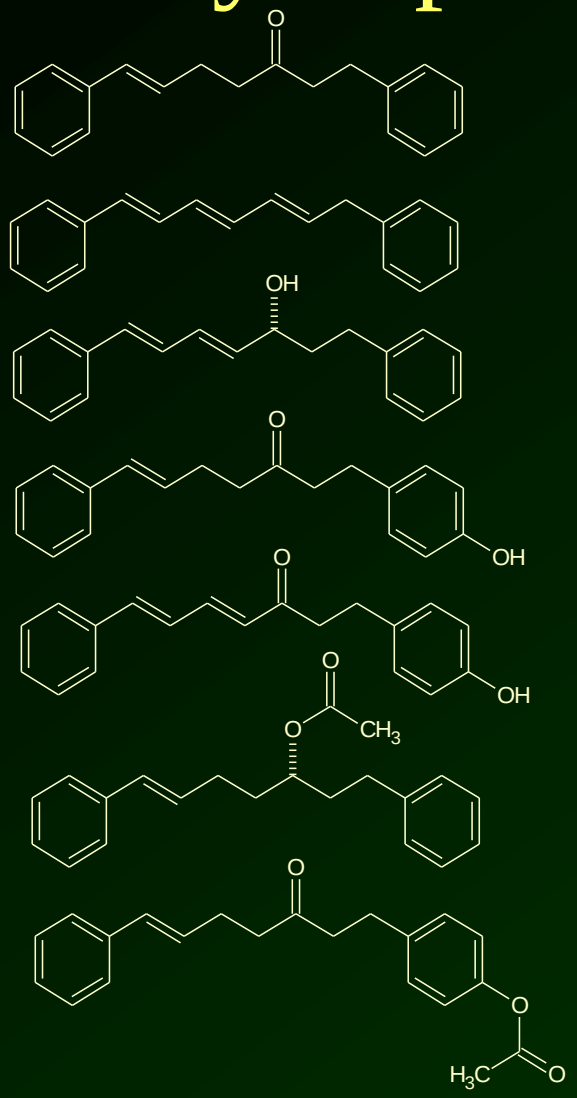
emodin

Polygonum cuspidatum

- Stimulation of estrogen-dependent cell lines MCF7 and MT3T3 by *Polygonum* sp., *Cassia* sp., *Rheum* sp., *Aloe* sp. extracts
- Affinity of 2,6 dihydroxy anthraquinone and emodin to ERα and ERβ ~ μM – comparable to daidzein

Matsuda H. et al.: Phytoestrogens from the Roots of *Polygonum cuspidatum* (Polygonaceae): Structure-Requirement of Hydroxyanthraquinones for Estrogenic Activity. Bioorg. Med. Chem. Lett. 11 (2001) 1839–1842

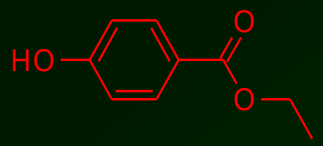
Diarylheptanoids



Suksamrarn A. et al.:Diarylheptanoids, new phytoestrogens from the rhizomes of *Curcuma comosa* Bioorg. Med. Chem 16 (2008) 6891-6902

4-hydroxy benzoic acid esters

ferutinin



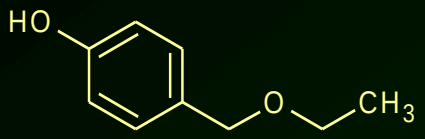
ethylparaben
(xenoestrogen)

- afiinity to ERα higher than to Erβ
- negative influence on fertility and sexual behavior in mice (F, M)
- active against osteoporosis in OVX rats
- etnopharmacology – contraception, abortives

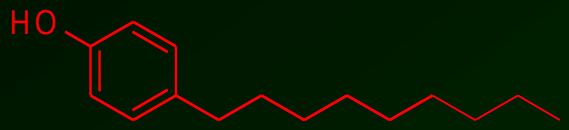
Ferula hermonis, Apiaceae

Appendino G. et al.: J. Nat. Prod. 2002, 65, 1612-1615
L´Huliere A. et al.: J. Nat. Prod. 2005, 68, 468-471
Ferretti M. et al.: J. Anat. (2010) 217, 48–56

Alkyloxyphenols

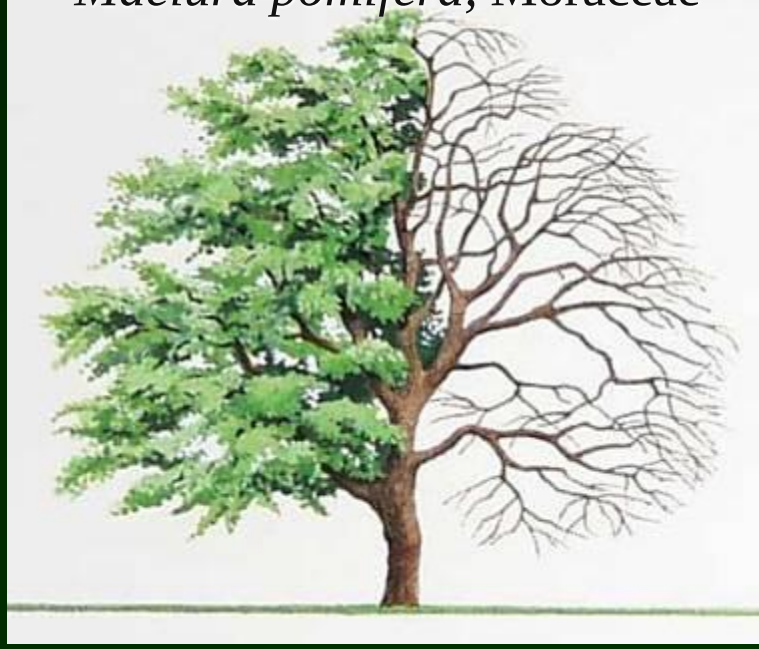


4-ethoxymethylfenol



nonylfenol (xenoestrogen)

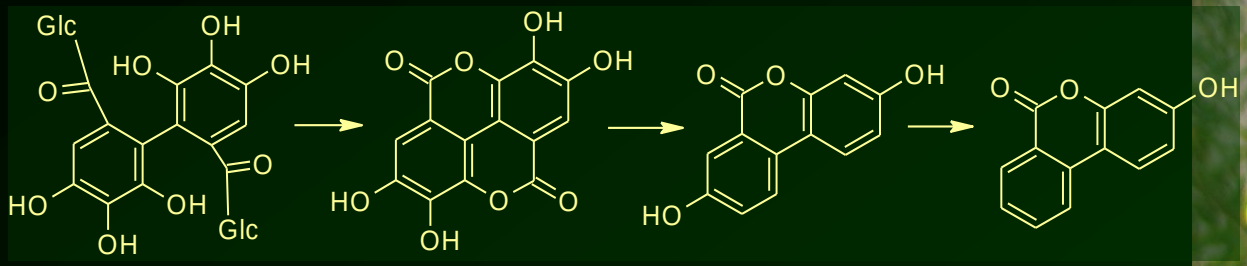
Maclura pomifera, Moraceae



- estrogen-like activity of *M. pomifera* extracts
- stimulation of MCF7 and HeLa cells
- binding to ERα and ERβ

Pearce V. et al.: 4-Ethoxymethylphenol: a novel phytoestrogen that acts as an agonist for human estrogen receptors. J. Steroid Biochem. Molec. Biol. 84 (2003) 431–439

Ellagic acid and urolithins



ellagitanin ellagic acid urolithin A urolithin B

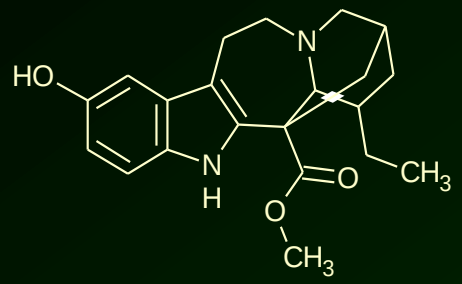
Punica granatum, Punicaceae

- Stimulation of MCF7 cells (ellagic a., urolithin A)
- Urolithin A: ERα binding comparable with genistein
- -"- ERβ -"- daidzein
- mixed activity: ER agonist/antagonist

Larrosa M. et al.: J. Agric. Food Chem. 54 (2006) 1611-1620.

Strati A. et al: Clin. Biochem (2009) 42, 1358-1362

Alkaloids



10-hydroxycoronaridine

Tabernaemontana spp. (Apocynaceae)

Tabernaemontana divaricata

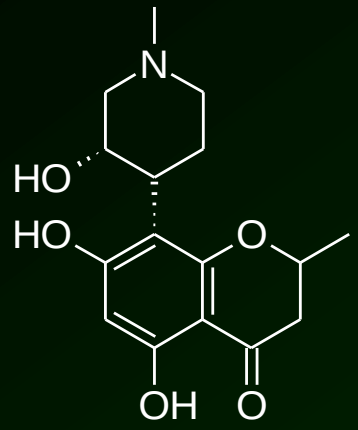
Tabernaemontana penduliflora

T. heyneana

Masuda K. et al: Isolation of 10-hydroxycoronaridine from *Tabernaemontana penduliflora* and its estrogen-like activity. Planta Medica 66 (2000) 169-171

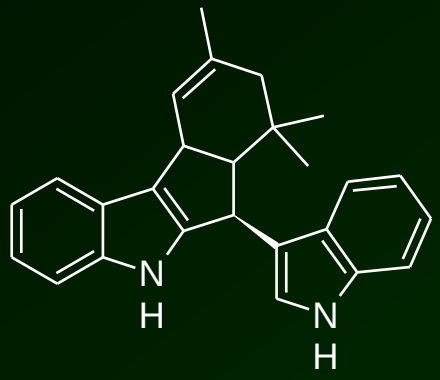
Srivastava S. et al.: A new alkaloid and other anti-implantation principles from *Tabernaemontana heyneana*. Planta Medica 67 (2001) 577-579

Alkaloids



rohitukine
Dysoxylum binectariferum
(Meliaceae)

contraceptive



yuehchukene
Murraya paniculata
(Rutaceae)

contraceptive

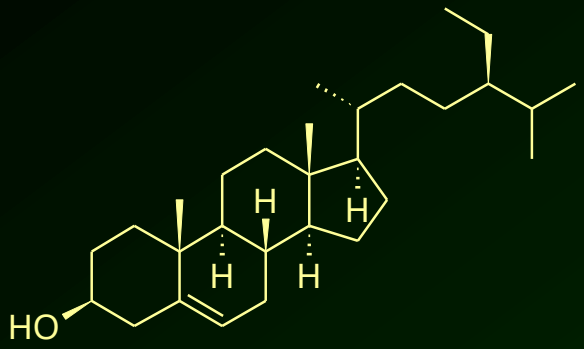
Murraya paniculata (Rutaceae)

Keshri. et al: Contraception (2007) 76, 400-407

Mohanakumara P. et al.: Fitoterapia (2010) 81, 145-148

Bergmann J., Lennart V. (1994) Pure Appl. Chem. (1994) 66, 2331-2334

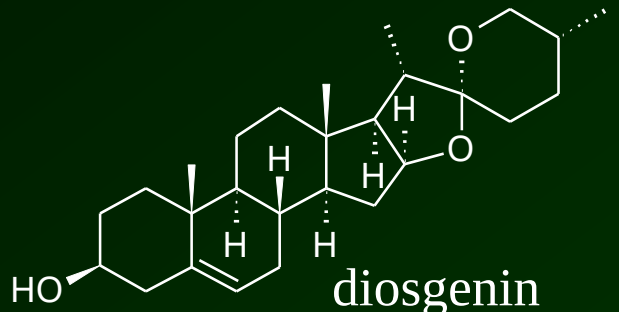
Non-phenolic steroids and terpenes



β-sitosterol

common plant steroid

ocassionally referred to as phytoestrogen
no clear evidence



diosgenin

aglycon part of a saponin
abundant in *Dioscorea* sp. (yam)
ocassionally referred to as estrogen
no clear evidence

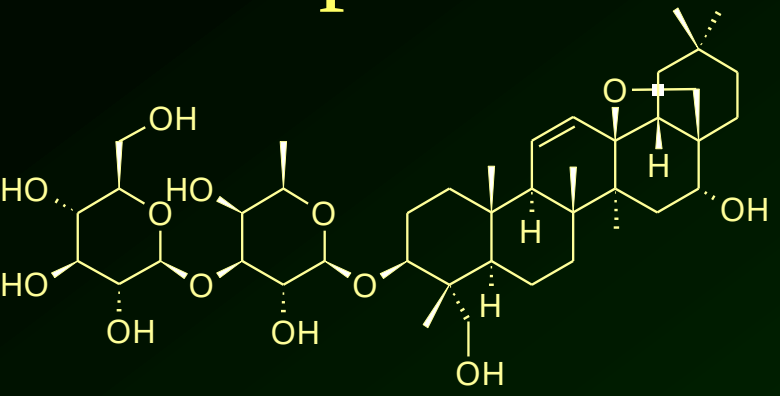
Hassan A et al.: Naturwiesenschaften (1964) 409-410

Awad A.B and Fink C.: J. Nutr (2000) 2127-2130

Mac Latchy D. and Kraak G.: Toxicol. Appl. Pharm. (2010) 305-312

Wu W.H et al.: J. Am. Coll. Nutr. (2005) 24, 235–243

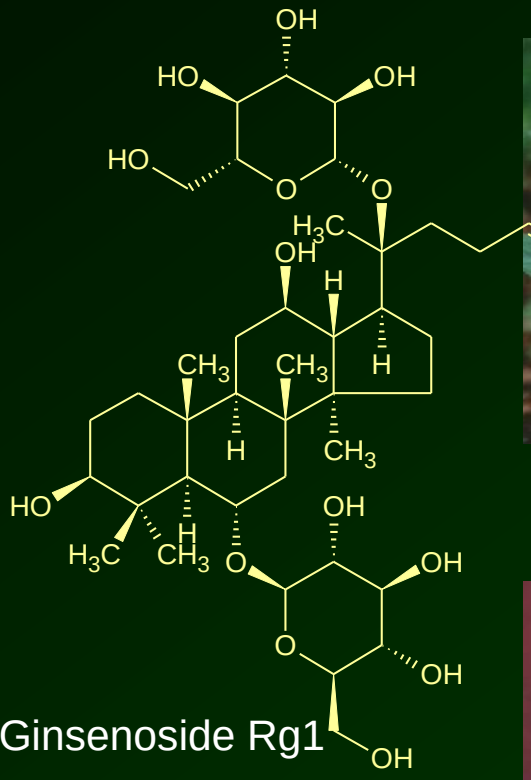
Non-phenolic steroids and terpenes



Saikosaponin D

Bupleurum falcatum
(Umbelliferrae)

Stimulation of ERα+
cell lines (MCF7, HeLa)



Ginsenoside Rg1

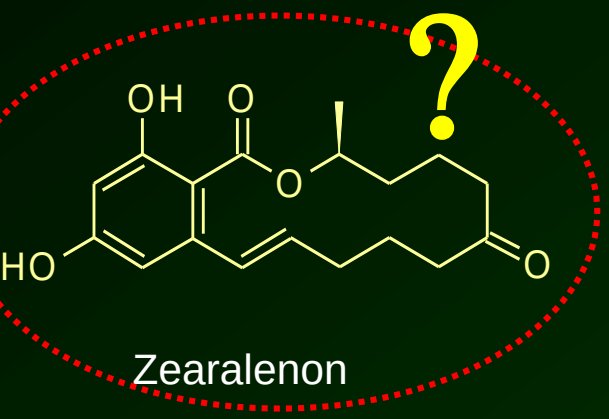
Panax ginseng (Araliaceae)

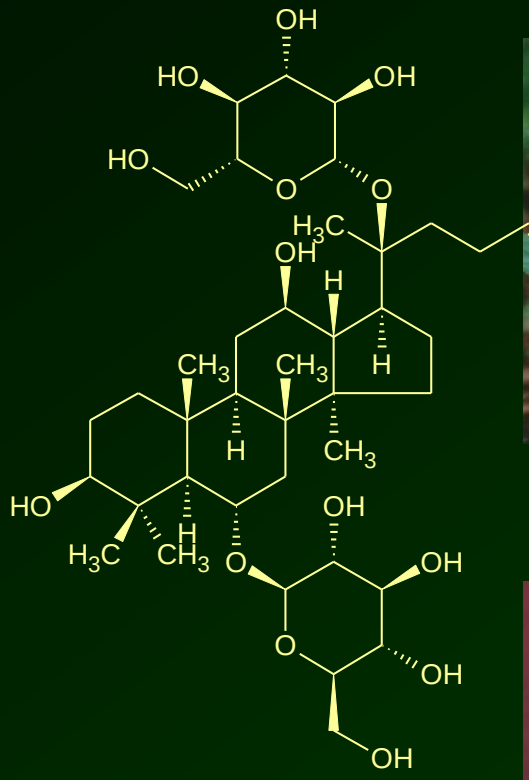
Lau W.S. et al: Ginsenoside Rg1 exerts estrogen-like activities via ligand-independent activation of ERα pathway. J. Steroid Biochem Molec. Biol. 108 (2008) 64–71

Wang P. et al.: Eur. J. Pharmacol. (2010) 626, 159-165

Non-phenolic steroids and terpenes

Saikosaponin D





Ginsenoside Rg1

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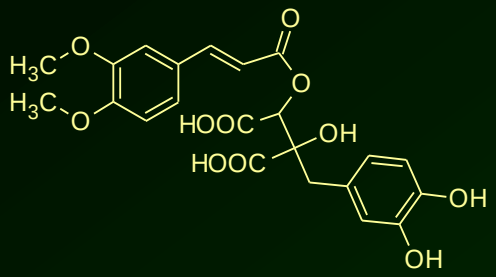
Wang P. et al.: Eur. J. Pharmacol. (2010) 626, 159-165

Unknown structures

actaeolactone



cimicifugic acid

Nuntanacorn P. et al.: J. Nat. Prod. 69 (2006) 314-318.

Actaea racemosa syn.
Cimicifuga racemosa, Ranunculaceae

- *Actaea racemosa*:

- treatment of menopausal symptoms
- unclear effect on ERs
- unknown active principles
- additional signaling systems: μ endorphin receptor
  serotonin receptor

Rhyu M.R. et al: J. Agric. Food Chem. 54 (2006) 9852-9857.

Burdette J.E. et al.: J. Agric. Food Chem. 51 (2003) 5661-5670.

Phytoestrogens: health and economic concerns

- 50-70 ies – Veterinary medicine, negative connotations
  fertility of animals
- since 80-90 ies – Human medicine, positive expectations
  menopause, PMS
  prevention of cardiovascular diseases
  -“- neoplasia
- late 90-ies – marketing tool

Practical use of phytoestrogens

- Menopause
- PMS

- cosmetics and obscure applications
  - breast
  - vagina
- etnopharmacological applications
  - fertility control - + i –


Iris germanica

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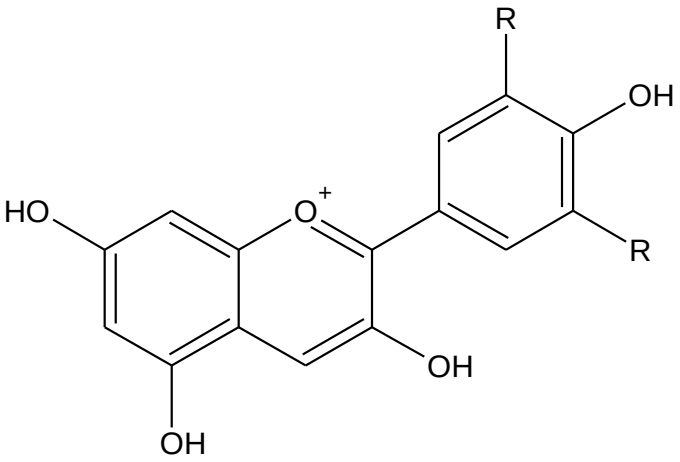
MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

10. Anthocyanins, tannins and quinones

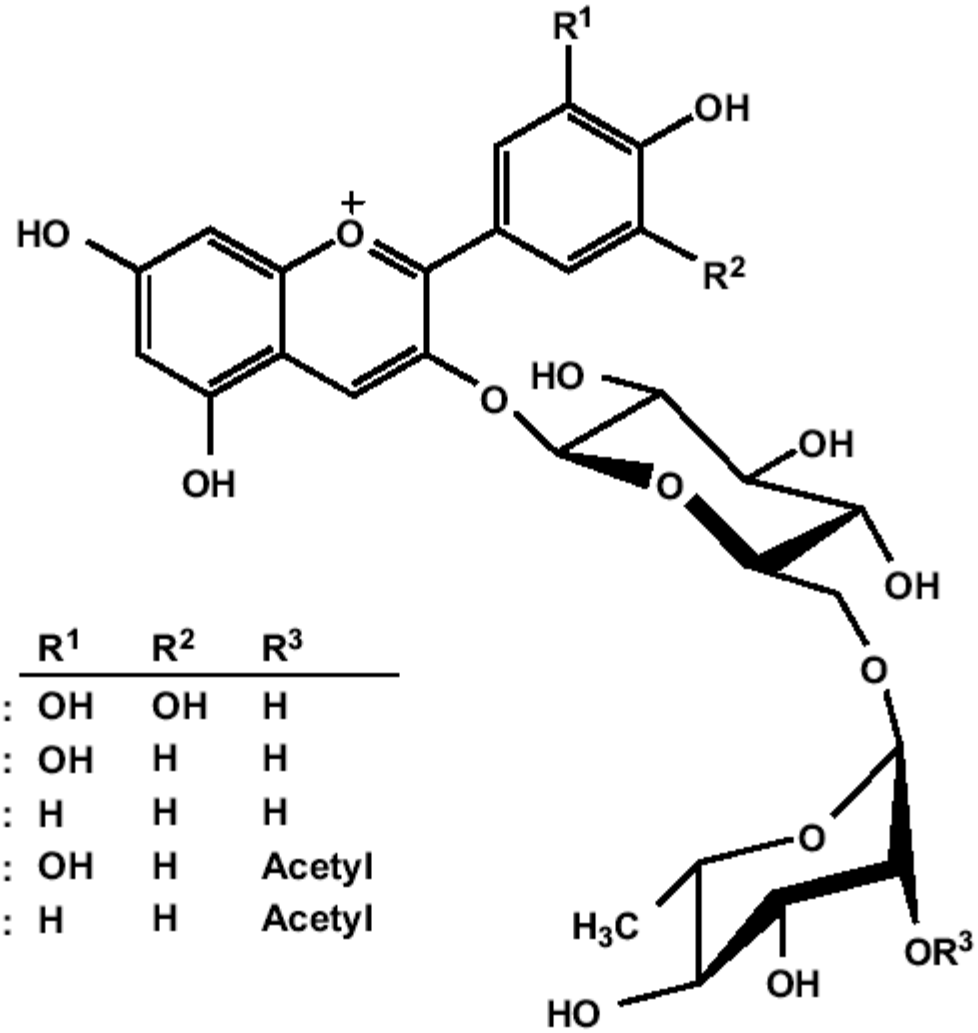
Lecturer: Oldřich Lapčík

Anthocyanins



Aglycones as well as glycosides
Unstable in alkaline solutions
Sensitive to oxidation

Antioxidants, venoprotective,



	R1	R2	R3
1:	OH	OH	H
2:	OH	H	H
3:	H	H	H
4:	OH	H	Acetyl
5:	H	H	Acetyl

Fig. 1. The anthocyanins identified in 45 tulip samples. **1** = delphinidin 3-rutinoside; **2** = cyanidin 3-rutinoside; **3** = pelargonidin 3-rutinoside; **4** = cyanidin 3-(2‴-acetylrutinoside); **5** = pelargonidin 3-(2‴-acetylrutinoside).

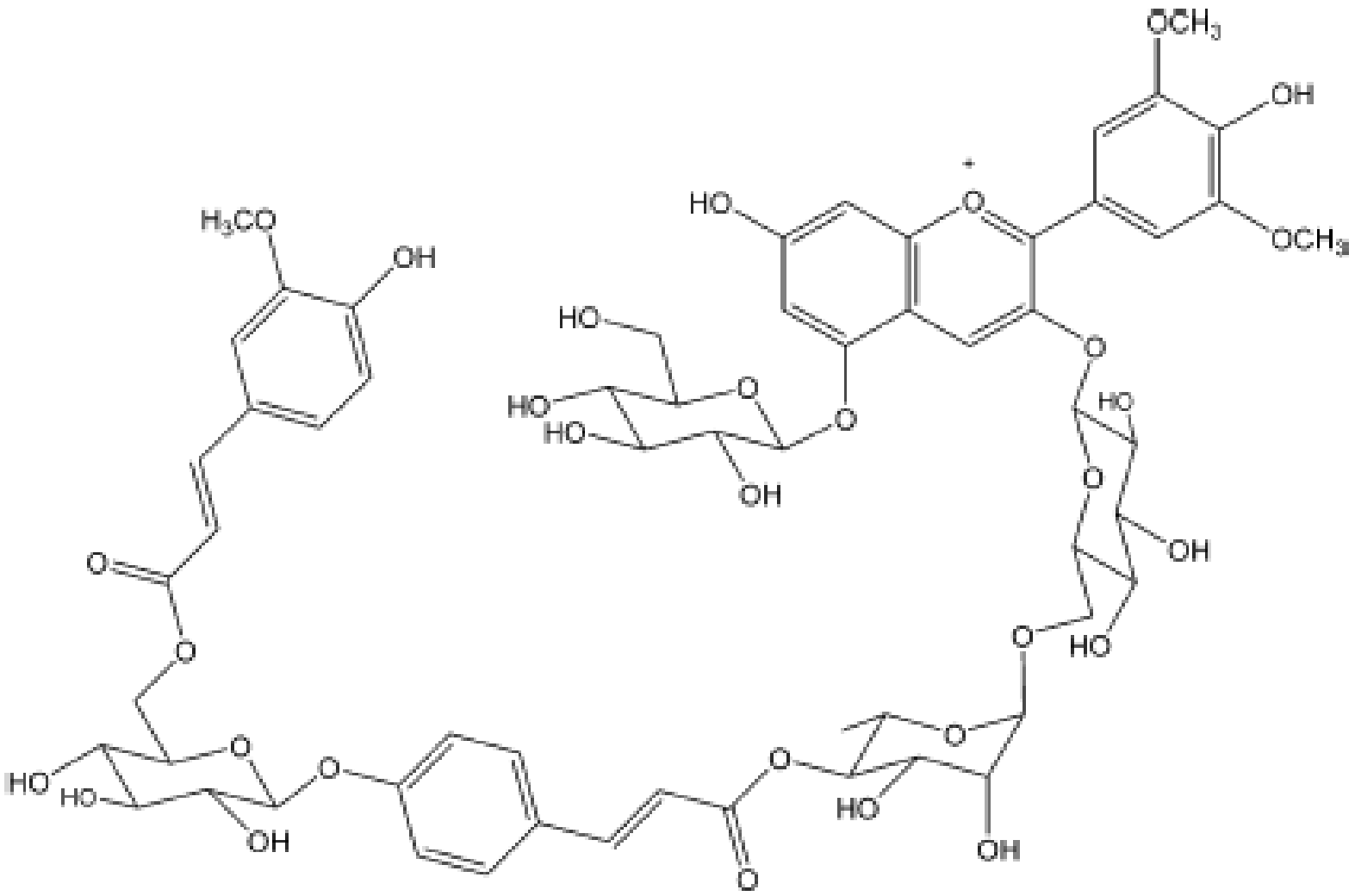
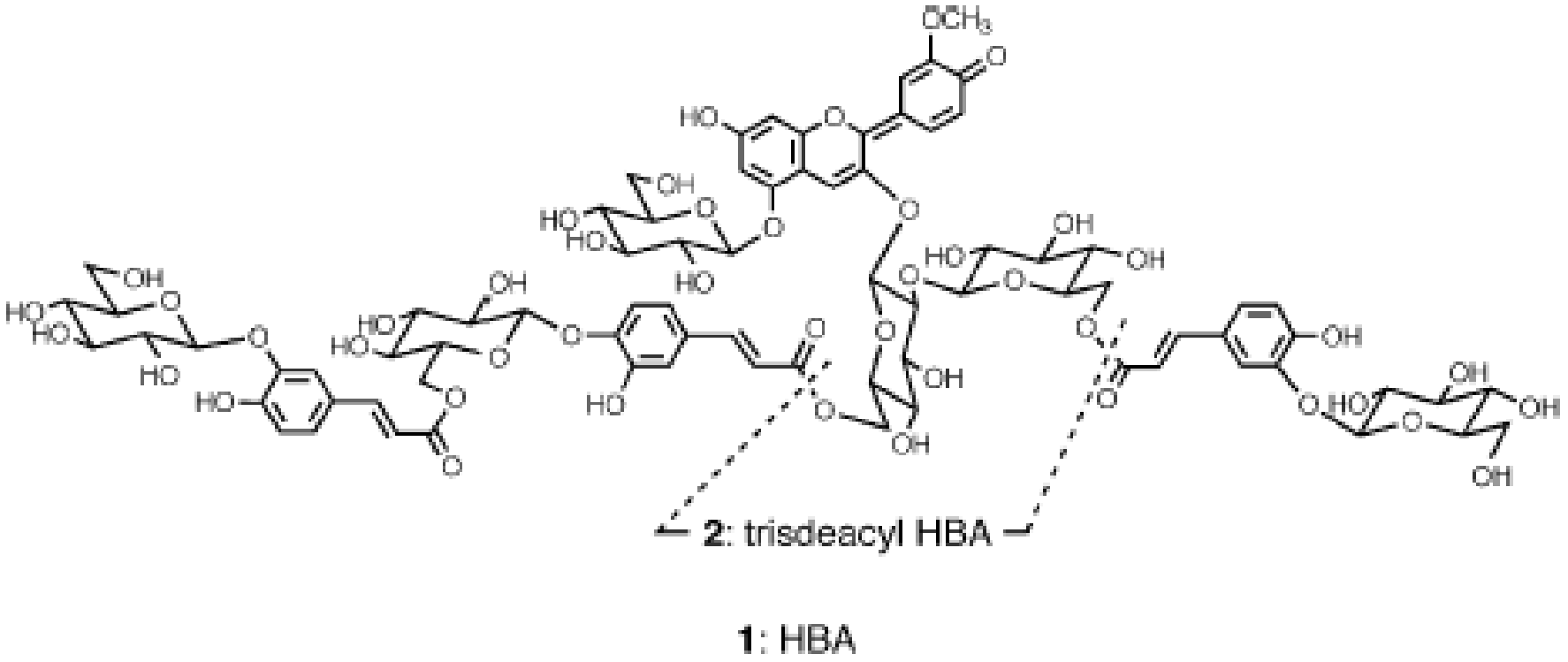
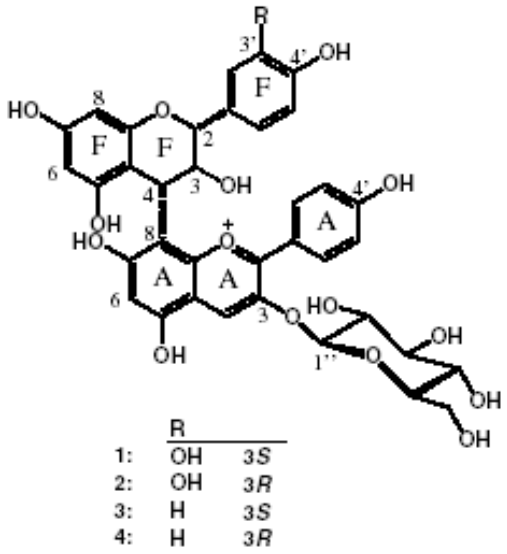


Fig. 6. *Petunia hybrida* cv 'Festival' new anthocyanin (Gonzalez et al., 2001).

Heavenly blue anthocyanin



T. Fossen et al. / Phytochemistry 65 (2004) 1421–1428



	R	
1:	OH	3*S*
2:	OH	3*R*
3:	H	3*S*
4:	H	3*R*

Fig. 2. The structures of the flavan-3-ol(4α → 8)pelargonidin 3-*O*-β-glucopyranosides (**1**–**4**) isolated from strawberry extract. The letter A denotes the aglycone ring systems belonging to the anthocyanidin substructure, whereas the letter F denotes the aglycone ring systems belonging to the flavanol substructure.

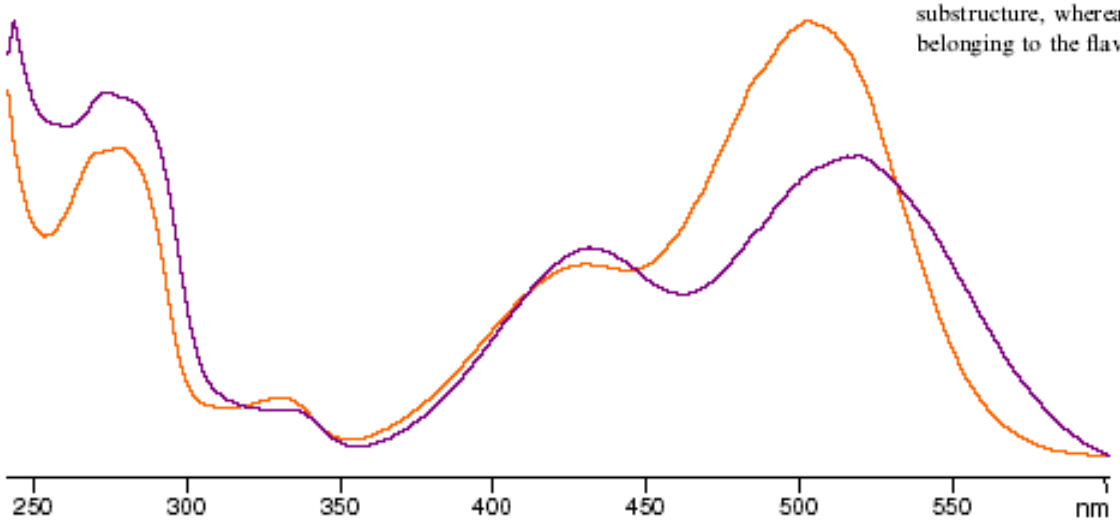
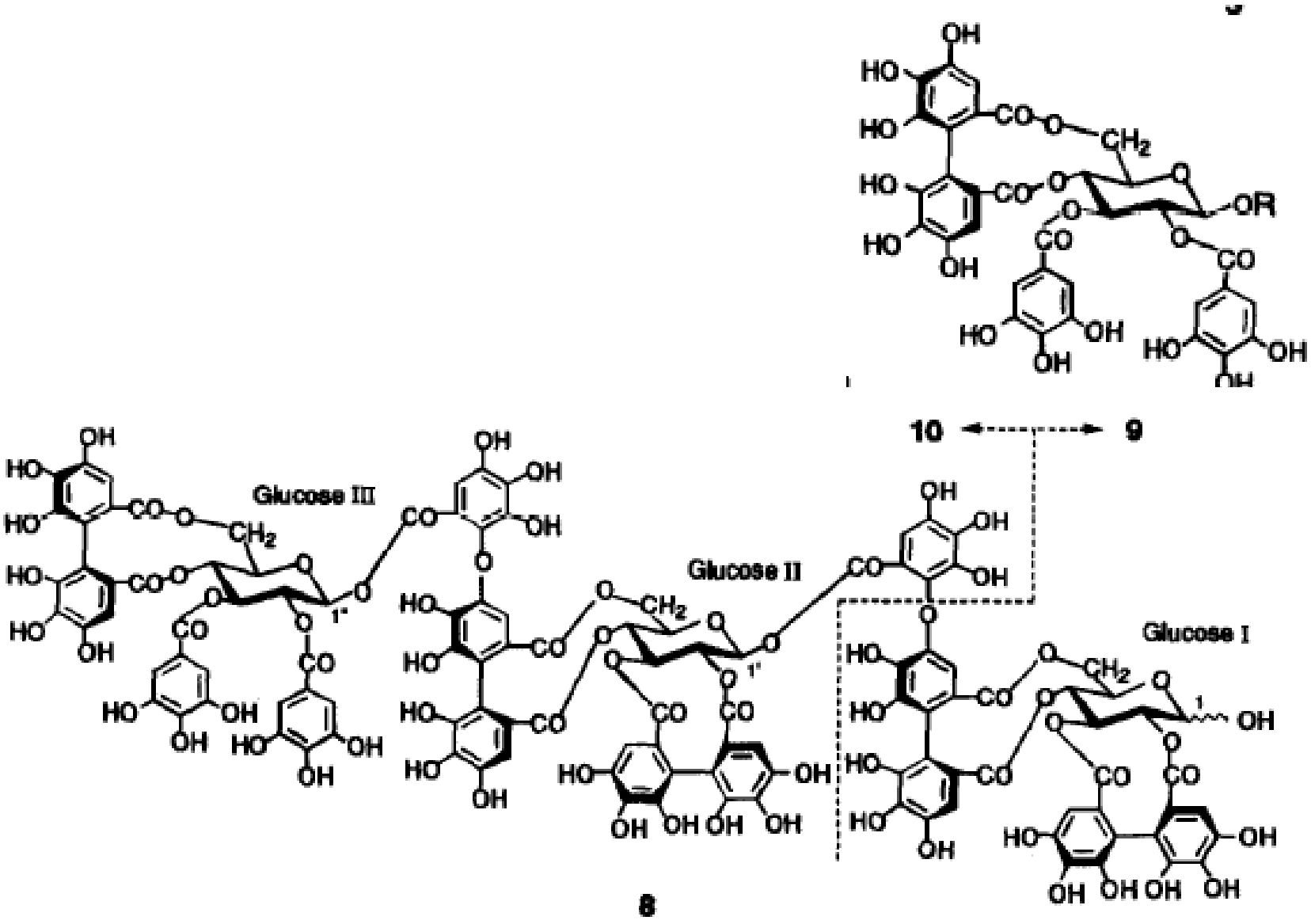


Fig. 1. The UV–Vis spectra of epiafzelechin(4α → 8)pelargonidin 3-*O*-β-glucopyranoside (**4**) (purple) and pelargonidin 3-glucoside (**5**) (orange) isolated from strawberry extract.

Tannins



ZHE-XIONG JIN et al., DIMERIC AND TRIMERIC ELLAGITANNINS FROM CORYLUSHETEROPH YLLA, Phytochemistry, Vol. 48, No. 2, pp. 333-338, 1998

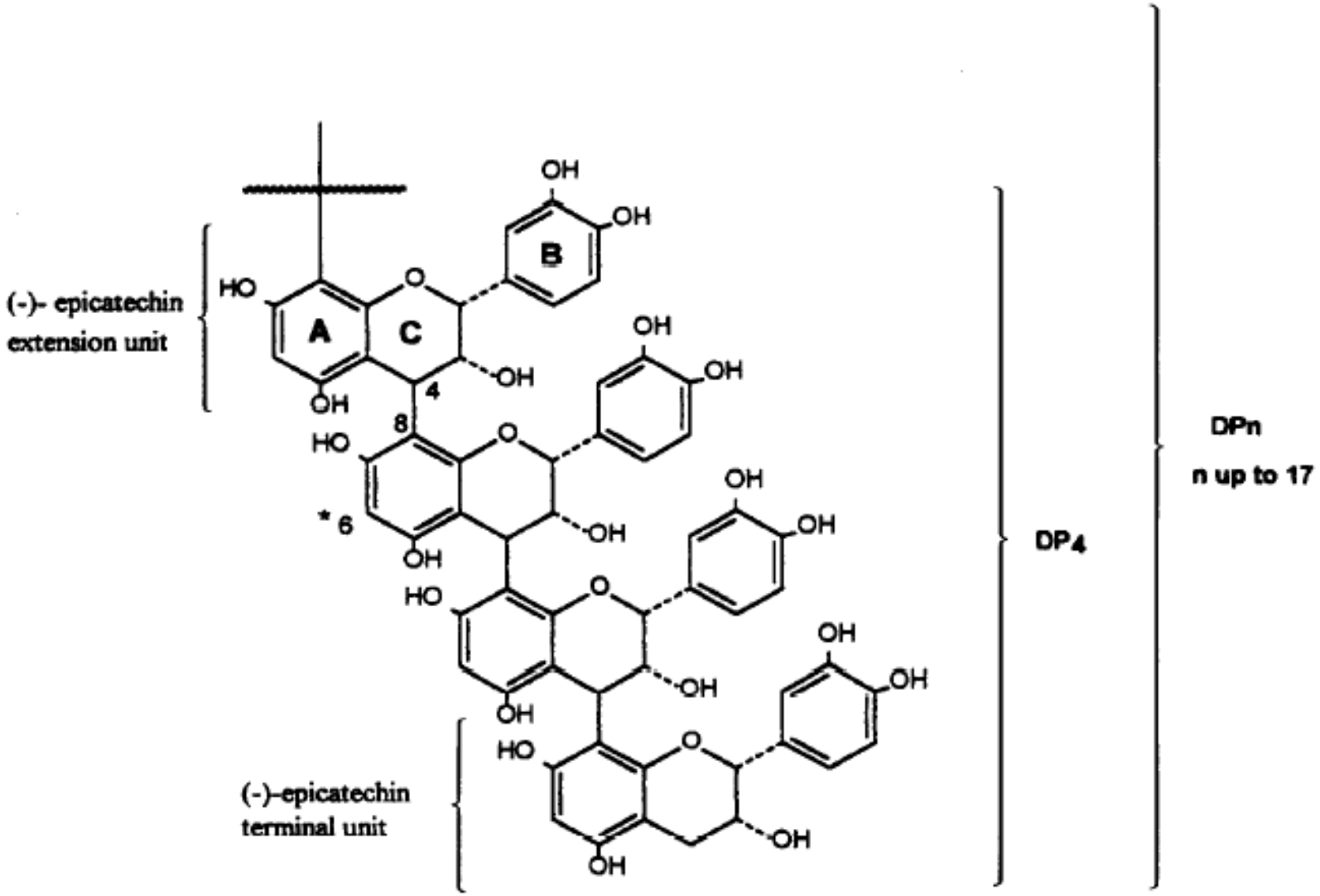


Fig. 4. Structures of cider apple pulp and skin polymeric procyanidins (* eventuality of C_4–C_6 linkages can not be excluded).

Sylvain Guyota et al: Characterization of highly polymerized procyanidins in cider apple (Malus sylvestris var. kermerrien) skin and pulp. Phytochemistry 44 (1997) 351-357.

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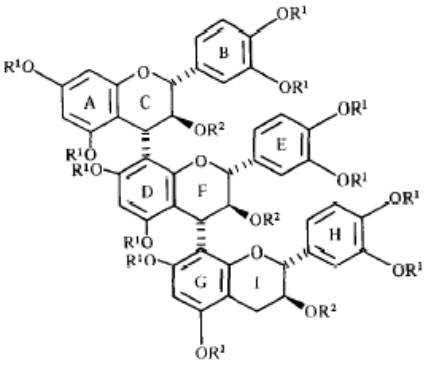
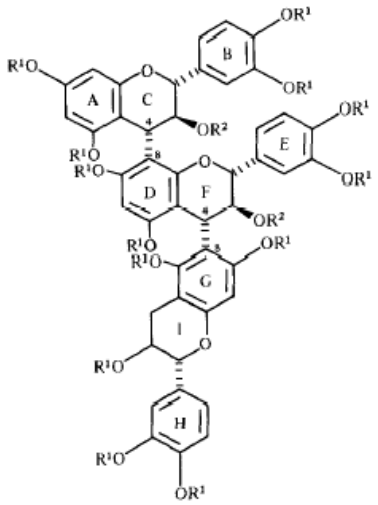
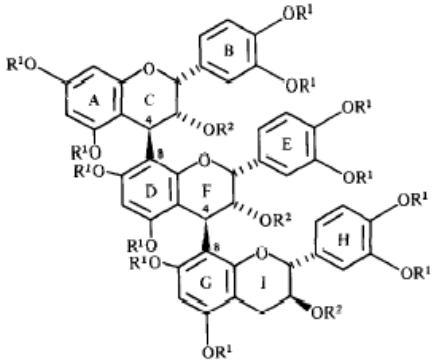


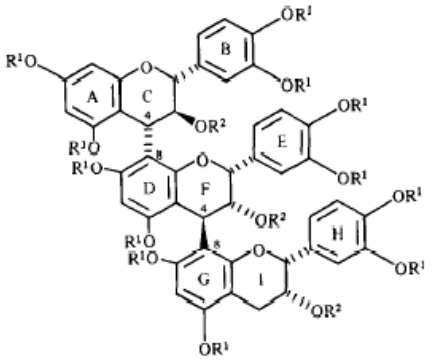
15 $R^1 = R^2 = H$
16 $R^1 = Me$, $R^2 = Ac$
17 $R^1 = R^2 = Ac$



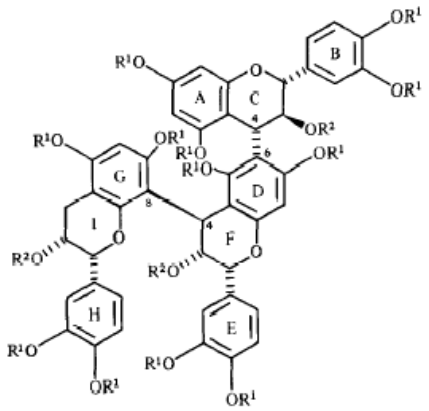
18 $R^1 = R^2 = H$
19 $R^1 = Me$, $R^2 = Ac$



20 $R^1 = R = H$
21 $R^1 = Me$, $R^2 = Ac$



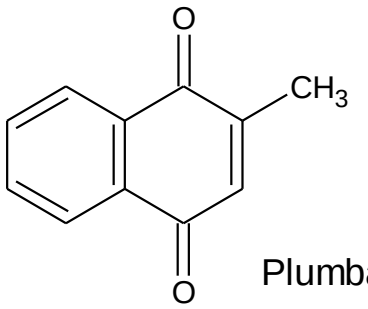
22 $R^1 = R^2 = H$
23 $R^1 = Me$, $R^2 = Ac$



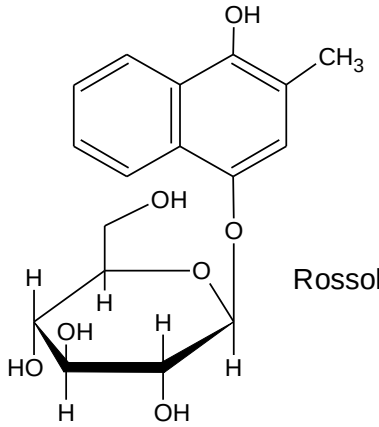
24 $R^1 = R^2 = H$
25 $R^1 = Me$, $R^2 = Ac$

HERBERT KOLODZIEJ:
OLIGOMERIC FLAVAN-3-OLS FROM MEDICINAL WILLOW BARK
Phytochemistry 29 (1990) 955-

Quinones

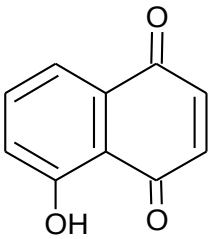


Plumbagin

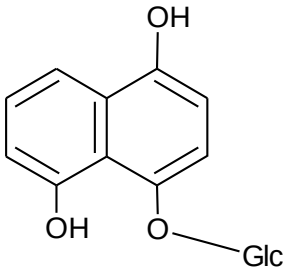


Rossolisid

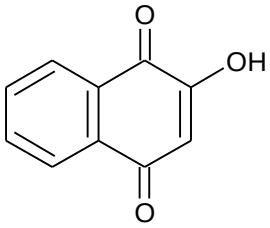
Drosera rotundifolia - Rosnatka okrouhlolistá



Juglon



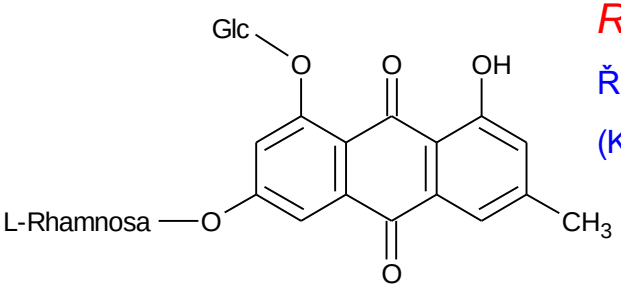
1,4,5 trihydroxynaftelen-glukosid



Lawson

Juglans regia

Lawsonia inermis (Henna)

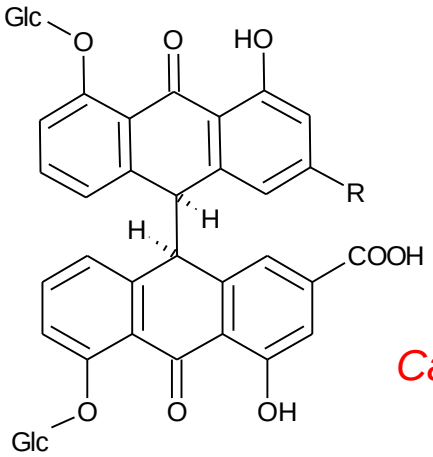


Glucofrangulin

Rhamnus frangula

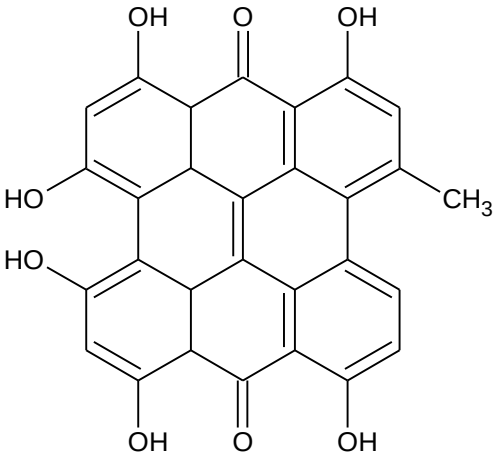
Řešetlák krušina

(Krušina olšová)

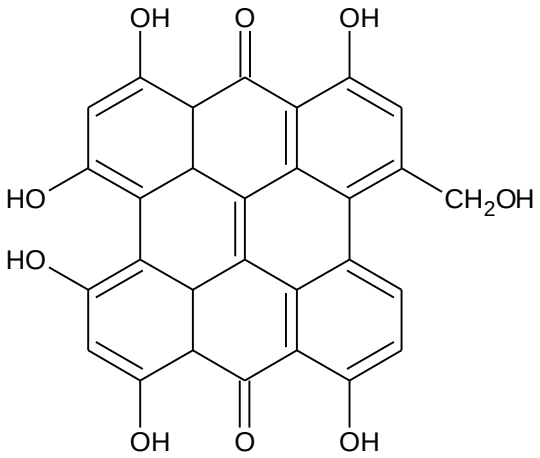


Sennosid

Cassia senna



Hypericin

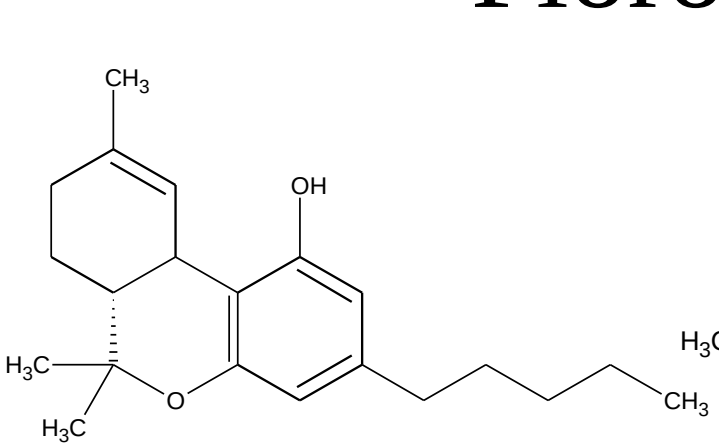


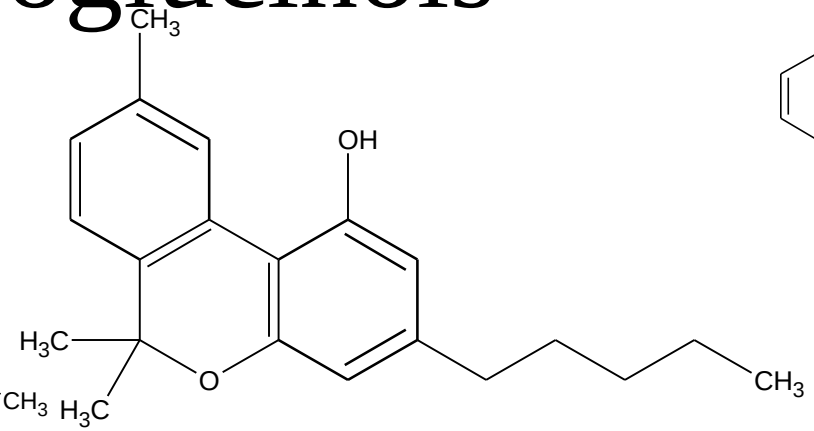
Pseudohypericin

Hypericum perforatum

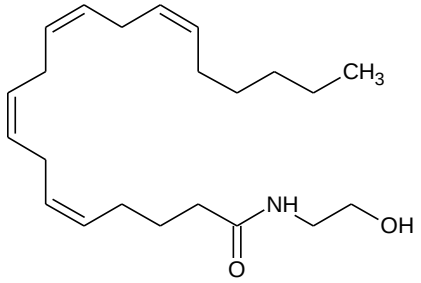
Třezalka tečkovaná

Floroglucinols



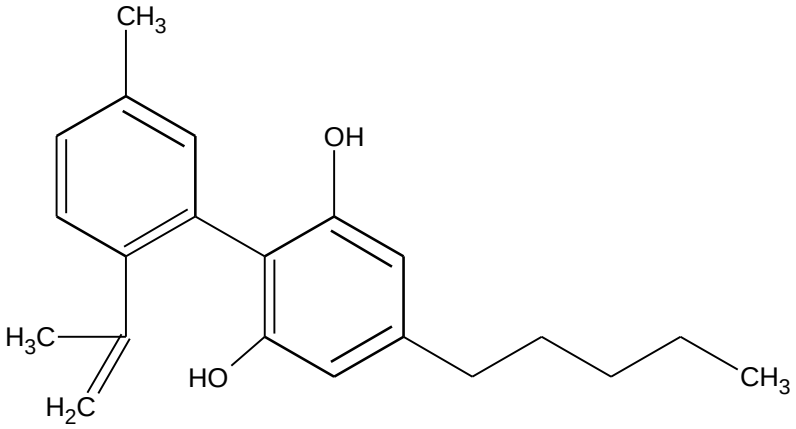


Kanabinol



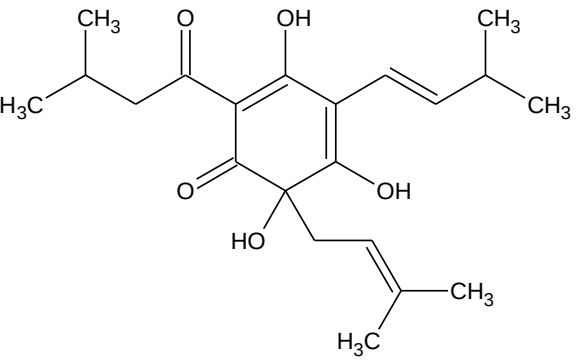
anandamide

THC

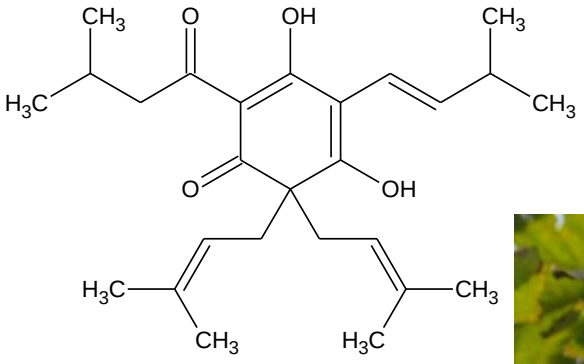


Kanabidiol

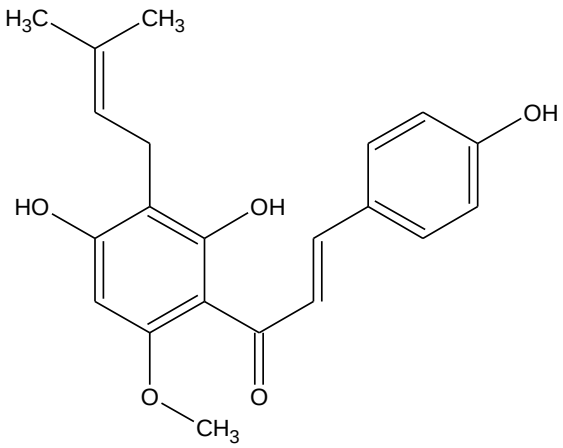
Canabis sativa



Humulon



Lupulon



Xantohumol (prenylchalkon)

Humulus lupulus

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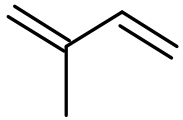
Bioactive Natural Compounds

11. Terpenoids

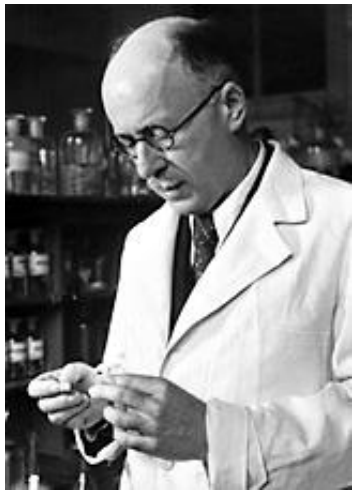
Lecturer: Oldřich Lapčík

Terpenoids

Terpenoids



- 1887 O. Wallach: Biochemical origin of terpenoids
  - condensation of isoprene units followed by further reactions



- 1953 Leopold Ruzicka – The Isoprene Rule:

  Each group of terpenes is formed by head-to-tail condensation of a certain number of isoprene units ... In each group of terpenes, the single precursor leads to different compounds by a series of cyclization, functionalization and rearrangements

  Ruzicka L., Eschenmoser A., Heusser H.: The isoprene rule and the biogenesis of terpenic compounds. Experientia 9., 357, 1953

-

Terpenoids

- **Monoterpenes C10**
- Sesquiterpenes C15
- **Diterpenes C20**
- Sesterpenes C25
- **Triterpenes C30**
  - Steroids (C27, C24, C21, C19, C18)
  - originating from a C30 precursor (squalen)
- **Carotenoids C40**
- Polyterpenes – different degree of polymerization (caoutchouc, gutta-percha)

Essential oils

Definition:

Essential oils are products, generally of complex composition, comprising the volatile principles contained in plants, and more or less modified during the preparation process. Only physical methods may be used for obtaining essential oils: steam distillation and expression.

Composition:

Volatiles, namely monoterpenes, sesquiterpenes, low m.w. alcohols, aldehydes, volatile phenylpropanoids, coumarins etc.

Together with substances found in the intact plant also modified compounds may may be present, e.g. products of hydrolysis or partial decomposition.

Esencial oils

- Distribution: Higher plants
- families – Apiaceae, Asteraceae, Cupresaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae, Zingiberaceae etc.

- Over 17500 essentials oils are known
- One plant species may posses several essential oils from its different anatomic parts

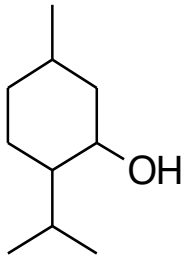
- Function: Attractants, deterrents, phytoalexins, phytohormones etc.

Esential oils – physical-chemical properties

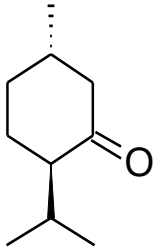
- Liquids, usually of lower density than water
- Certain components may be crystalizable in lower temperatures
- Optically active
- Soluble in organic solvents and in lipids (fat)
- Virtually insoluble in water (but enough to flavouring)

- Chemotypes : composition of essential oils from the same species may vary, sometimes despite their morphologic uniformity.

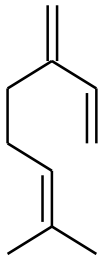
Monoterpenes - examples



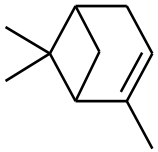
menthol



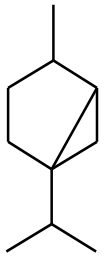
menthon



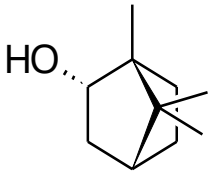
myrcen



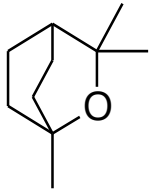
pinen



thujan

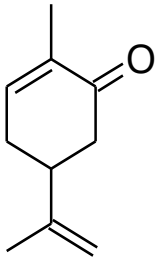


borneol

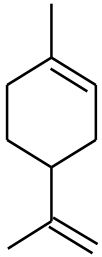


eucalyptol

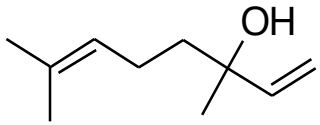
(cineol)



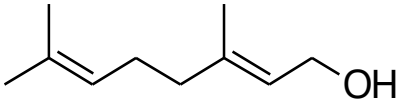
karvon



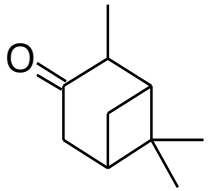
limonen



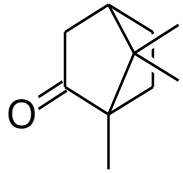
linalool



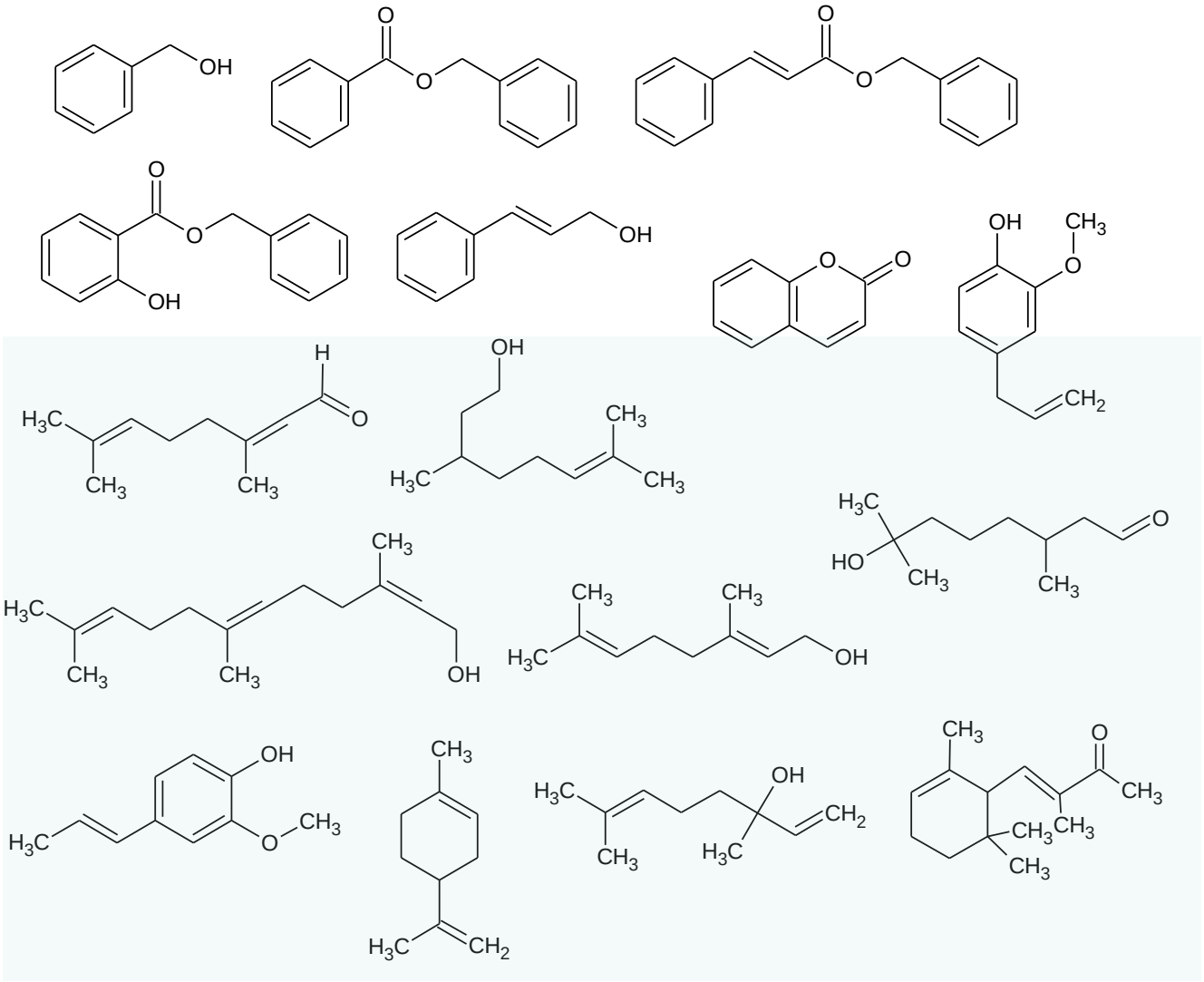
geraniol



pinokamphon



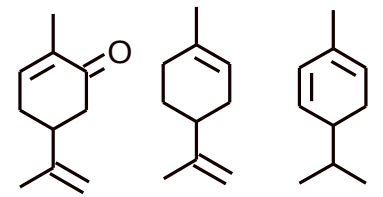
Chanel No5



Alcohol
Aqua
Benzyl alcohol
Benzyl benzoate
Benzyl cinnamate
Benzyl salicylate
Cinnamyl alcohol
Citral
Citronellol
Coumarin
Eugenol
Farnesol
Geraniol
Hydroxycitronellal
Isoeugenol
Limonene
Linalool
Alpha-isomethyl ionone
Yelow 5
Red 4
Yelow 6
Blue 1

Apiaceae

- *Carum carvi* Caraway
- *Pimpinella anisum* Anis
- *Foeniculum vulgare* Fennel
-
- *Petroselium hortense* Parsley
- *Apium graveolens* Celery
- *Coriander sativum* Coriander
- *Anethum graveolens* Dill



carvon limonen α-p

Asteraceae

- *Chamomilla recutita* Matricaria

- *Artemisia absinthum* Wormwood
- *Artemisia vulgaris* Mugwort

Lamiaceae

- *Ocimum basilicum* Sweet basil
- *Hysoppus officinalis* Hyssop
- *Majorana hortensis* Sweet majoram
- *Origanum vulgare* Oregano
- *Melissa officinalis* Balm
- *Lavandula spp.* Lavender
- *Salvia officinalis* Sage
- *Mentha x piperita* Peppermint
- *Metha spicata* Spearmint
- *Mentha arvensis* Cornmint

Lauraceae

- *Laurus nobilis* Laurel
- *Cinnamomum camphora* Camphor
- *Cinnamomum verum* Cinnamon

Myrtaceae

- *Eucalyptus globulus* Eucalyptus
- *Syzigium aromaticum* Clove
- *Melaleuca alternifolia* Tea tree
- *Myrtus communis* Myrtle

Rutaceae

- *Citrus aurantium* Bitter orange (Sevilla orange)
- *Citrus sinensis* Orange
- *Citrus limon* Lemon
- *Citrus paradisi* Grapefruit
-
-

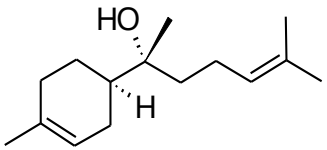
MYRISTICACEAE

- *Myristica fragrans* Nutmeg

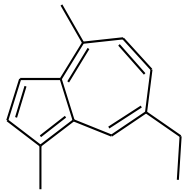
ARACEAE

- *Acorus calamus* Calamus

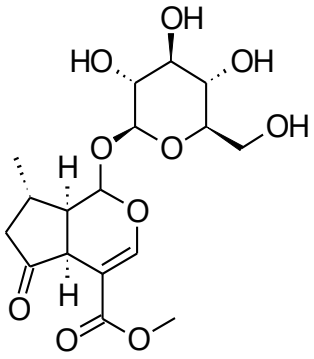
Sesquiterpenes - examples



bisabolol

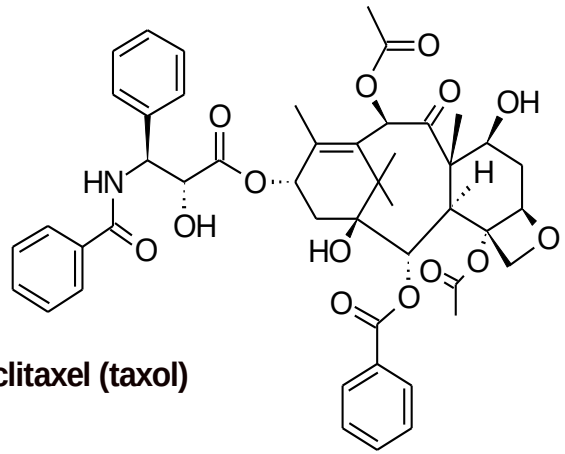


chamazulen

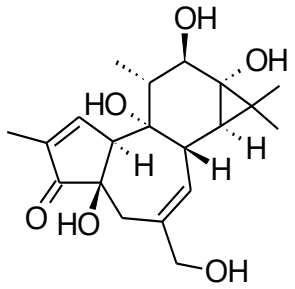


verbenalin

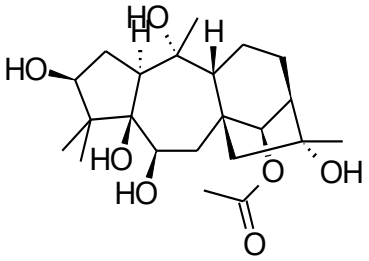
Diterpenes - examples



paclitaxel (taxol)



phorbol



grayanotoxin

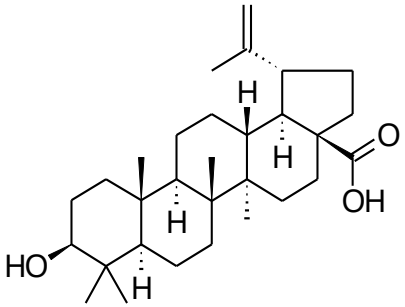
Paclitaxel

- Toxicity of yew (*Taxus* sp.) has been known for centuries

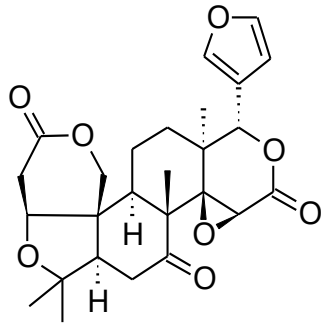
- Traditional use – criminal and war poison, abortifaciens

- Discovery of anticancer activity in yew extracts 1967
- Paclitaxel (originaly named Taxol) isolated 1969
- Structure determined 1971
- Molecular mode of action elucidated 1979
- Clinical Phase I studies initiated 1983
- Proved effective against ovarian cancer 1989, against breast cancer 1991
- Approved by US FDA for ovarian cancer (1992) and breast cancer (1994)
- Total synthesis 1994 (Nicolau and Holton, independently)

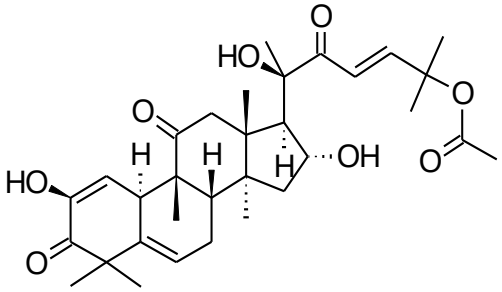
Triterpenes - examples



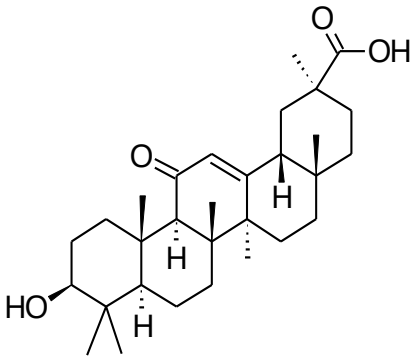
kyselina betulinová



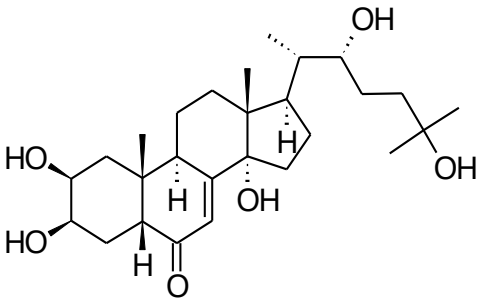
limonoic acid



cucurbitacin E

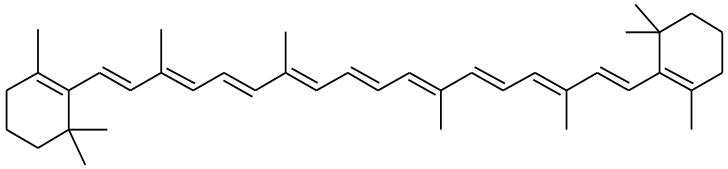


kyselina glycyrrhetová

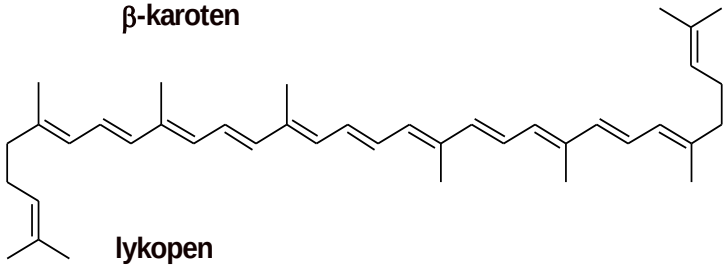


ekdyson

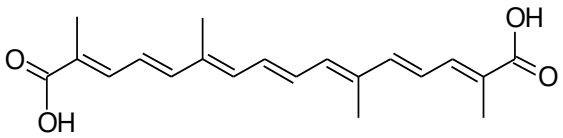
Carotenoids - examples



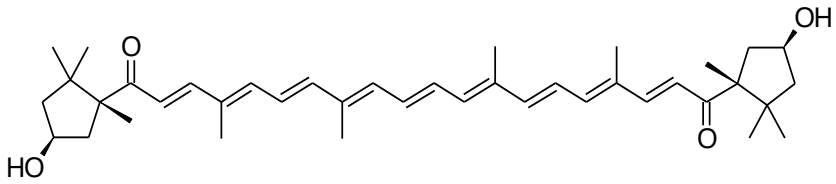
β-karoten



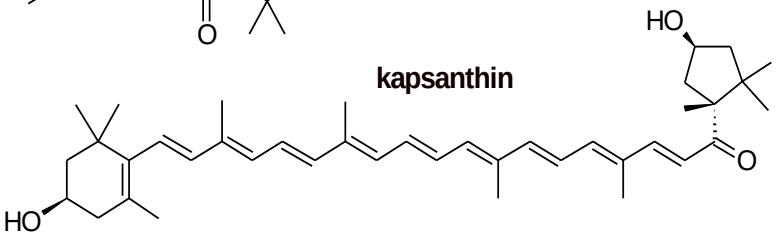
lykopen



krocetin

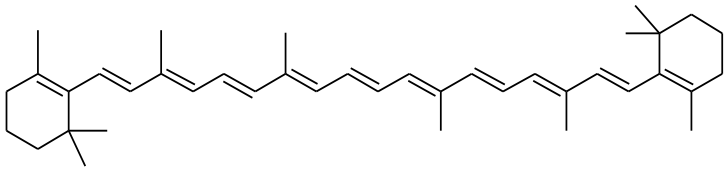


kapsorubin

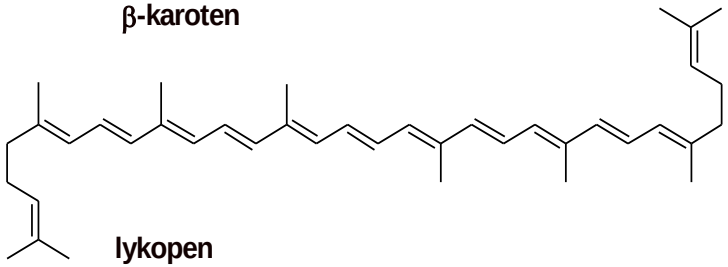


kapsanthin

Carotenoids - examples



β-karoten



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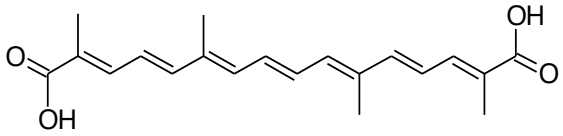
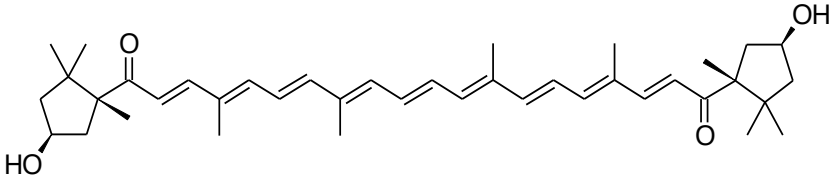


krocetin



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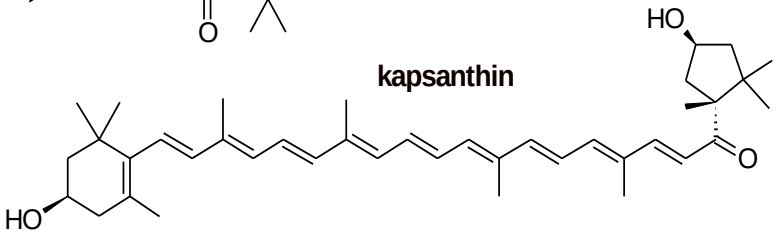
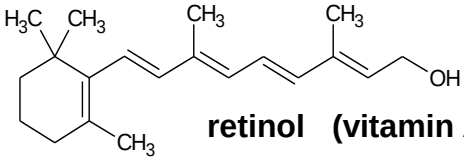
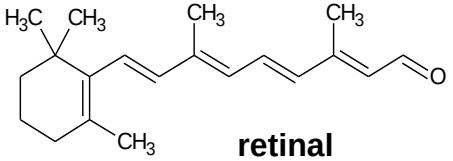


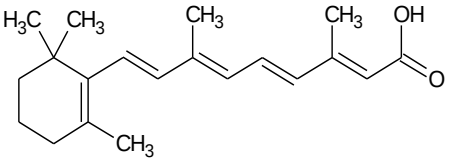
kapsanthin



retinol (vitamin A)



retinal



retinoic acid

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

12. Vitamin C

Lecturer: Oldřich Lapčík

VITAMIN C – HISTORICAL CONTEXT

- 1754 James Lind -In his book ***A Treatise on Scurvy*** proposed using lemons as prevention of scurvy

- 1761 Lemons and oranges reccommended for use on ships of East-Indian Society.

- 1795 Preventive use of lemon juice on ships of British Royal Navy.
  - "A lemon a day keeps scurvy away"

- 1907 A. Holst and T. Fröhlich recognised that guinea pig is prone to scurvy (only humans and apes had been known to be prone to scurvy till that time).

VITAMIN C – HISTORICAL CONTEXT

- 1911 Kazimir Funk introduced the term "VITAMIN".

  The first diseases assumed to be caused by avitaminosis were xerophtalmia (night blindness), beri-beri and scurvy.

  The hypothetical vitamins were designated A, B and C.

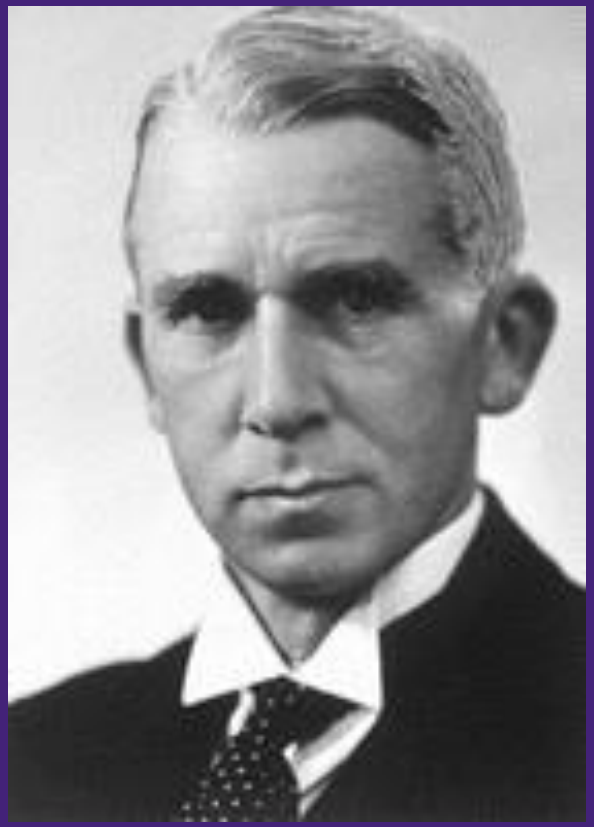
- 1928 Albert von Szent-Györgyi isolated ascorbic acid from paprika.

  (Nobel prize 1937)

- 1933 Tadeus Reichstein and Walter Norman Haworth

  independently invented chemical synthesis of L-ascorbic acid

Albert von Szent-Gyorgyi Nagyrapolt
Nobel Prize 1937



Walter Norman Haworth

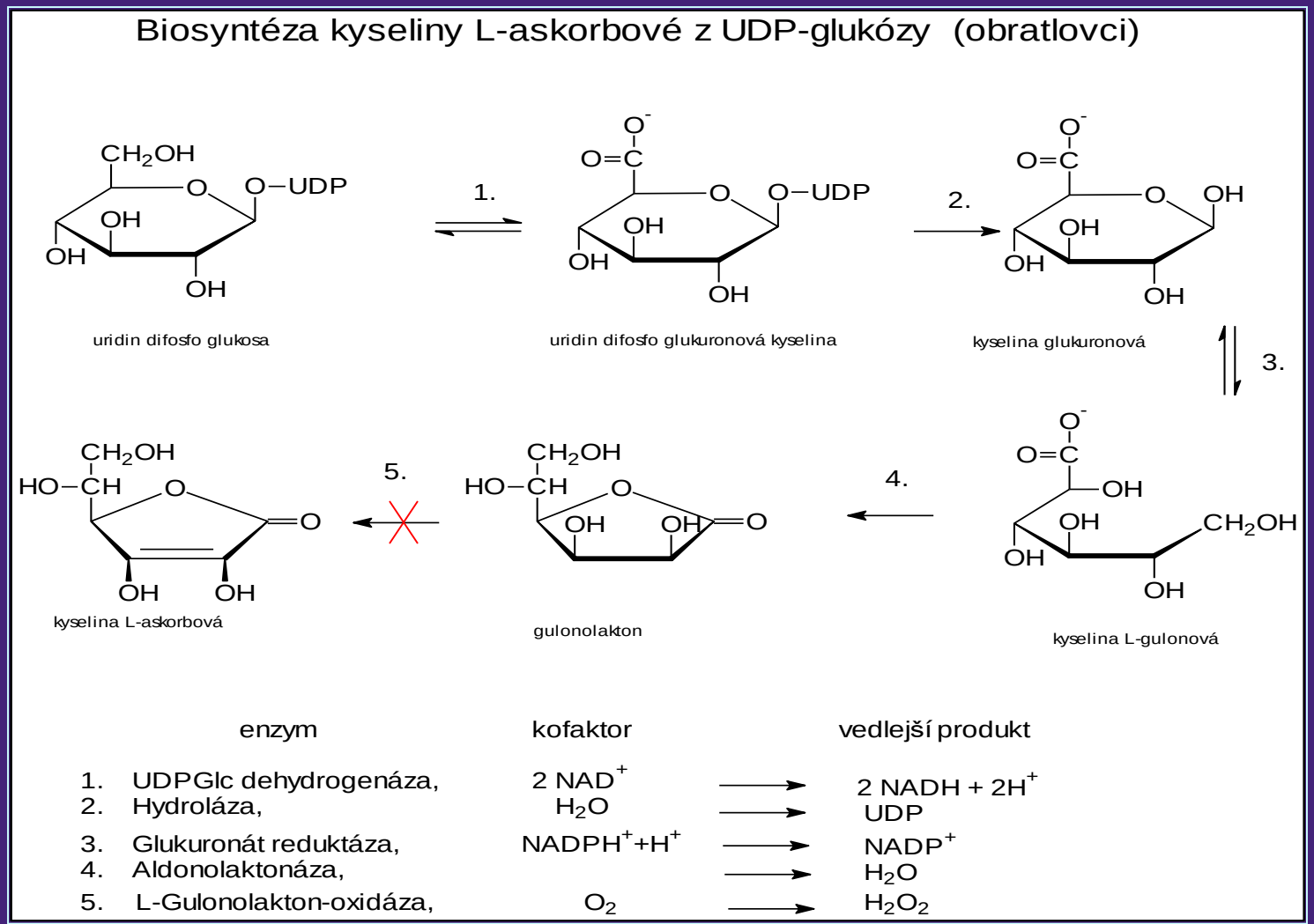
Nobel Prize 1937

Tadeusz Reichstein

Nobel Prize 1950

VITAMIN C – HISTORICAL CONTEXT

- 1957 J. J. Burns clarified the biochemical reasons why humans, monkeys and guinea pigs are not able to synthetize vitamin C.



VITAMIN C –HISTORICAL CONTEXT

- 60.-70-ies . I. B. Chatterjee and B. C. Guha demonstrated GULONOLACTONE-OXIDASE activity in livers and klidneys of majority of terrestrial vertebrates.

- 1980 Morimitsu Nishikimi showed imunochemical crossreactivity between frog, hen and rabbit GLO.
-
- 90 -ies. M. Nishikimi sequenced GLO genes in rat, human and several other primates and in guinea pig.
- 1997 A. Nandi and I. B. Chaterjee hypothesized that the onset of vitamin C biosynthesis in amphibians was an adaptation to terrestrian way of life.
- 1997 R. Moreau and K. Dabrowski discovered GLO in sturgeon and paddlefish (*Chondrostei)* and two years later also in lamprey (*Petromyzon spp.*)

- 1998 G. Wheeler described biosynthesis of ascorbic acid in plants

GLO Gene

Rat: 12 exons , 11 introns.

Human: On the chromosome 8p21.1 – a sequence homologous to rat GLO.
The sequence corresponds to the exons VII, IX, X, and XII.
The equivalents of exons VIII and XI are missing.
Numerous mutations, deletions and insertions, two stop-codons.
Instead of the XI codon there are two retroviral *Alu* sequences.
Pseudogene – no expression.

Anthropoid primates: GLO pseudogenes homologous to human GLO found in chimpanzee, orangutan, macaque. They differ only in point mutations.
The gene ceased to function 45-70 million years ago.

Guinea pig: Different remnants of GLO gene.
Missing the exons I and V, 84 basepairs from exon VI, numerous point mutations.
The gene ceased to function approximately 20 million years ago.

Gulonolactone-oxidase is missing in:

- Anthropoid primates and Tarsiers
- Guinea pigs
- Bats
- Certain songbirds (swallows, butcher-birds)
- Osseous fishes

- Vitamin C is not synthesized also by certain insects (*Diatrea grandiosella*)

Antioxidative potential of ascorbic acid

Radical scavenging:

- O_2^{-}, $HO_2^{\cdot}$
- soluble peroxy radicals ($RO_2^{\cdot}$)
- thiyl and sulfenyl radicals
- nitroxide radicals

Scavenging of O_3 a $NO_2^{\cdot}$

Reduction of carcinogenous nitrosamines to inactive products

Reduction of hypochloric acid (HOCl)

Regeneration of α-tocopheryl radicals in cell membrane lipoproteins

Ascorbic acid and biosynthesis of connective tissue

Ascorbic acid is an indispensable cofactor for proline hydroxylation in collagen and elastin.

Ascorbic acid protects collagen from oxidative degradation in the absence of Fe^{3+} ions

In presence of Fe^{3+} ions ascorbic acid accelerates collagen degradation.

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUROPEAN UNION
European Structural and Investing Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Bioactive Natural Compounds

13. Non-sugar sweet natural compounds

Lecturer: Oldřich Lapčík

Non-sugar sweet natural compounds

Oldřich Lapčík
Department of Chemistry of Natural Compounds
UCT Prague

What is the sweetest item in the world?

a) a barell of mature bee honey
b) good sleep

Sweet World

- Sweet – milý, miláček, brouček, zlato
- Sweetheart – milenka
- Sweetie – drahoušek
- Sweet seventeen – - děvče v rozpuku

- Sweet lipped – úlisný
- Sweet spoken - lichotivý

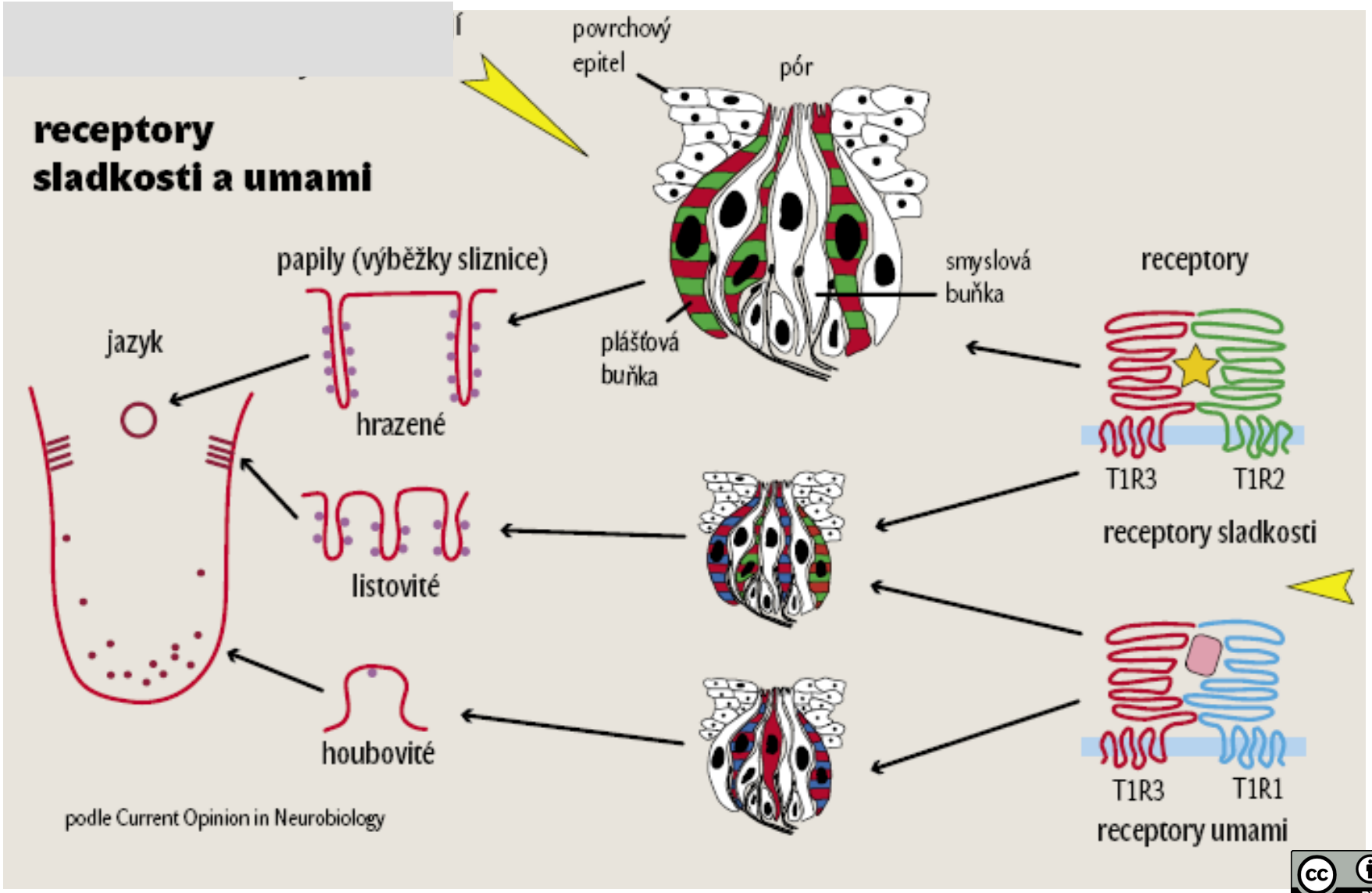
Sweet world

- Honey – miláček, zlatíčko
- Honeymoon – líbánky
- Honey cooler – lichotník

- Sugar – miláček, zlatíčko
- Sugar daddy – kořen
- Sugarer – ulejvák
- Sugary – lichotivý, lákavý

- High life – sladký život

Sweet taste perception

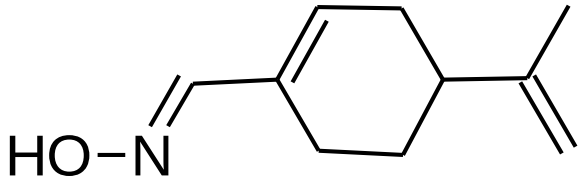


Non-sugar sweet natural compounds

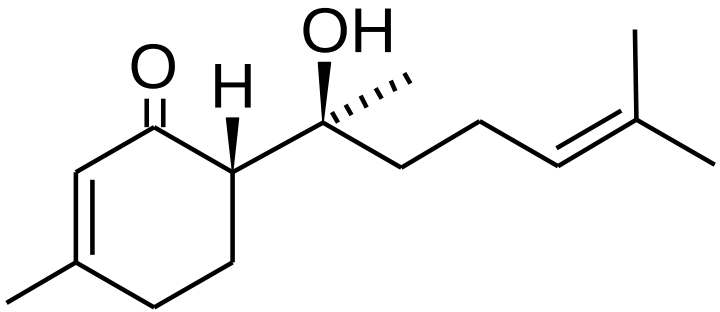
- **Terpenoids**
- Steroids
- Flavonoids, chalcones and coumarins
- Aminoacid derivatives
- Proteins

Monoterpenes and sesquiterpenes

Perilla frutescens (L.) Britton (Lamiaceae)
Perillartin is 350 × sweeter than sucrose.
Use: perfumery, tobacco flavoring (Japan)



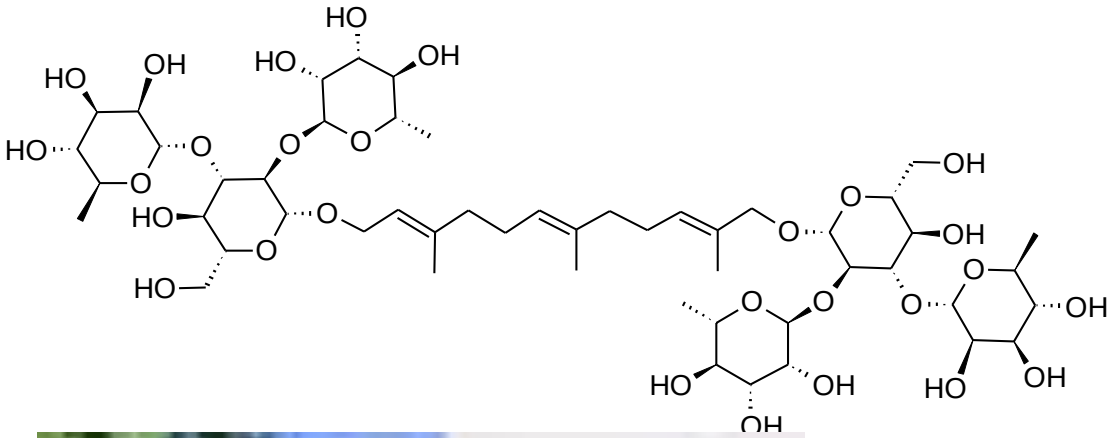
Lippia dulcis Trevir. (Verbenaceae)
Hernandulcin, 1000 × sweeter than sucrose



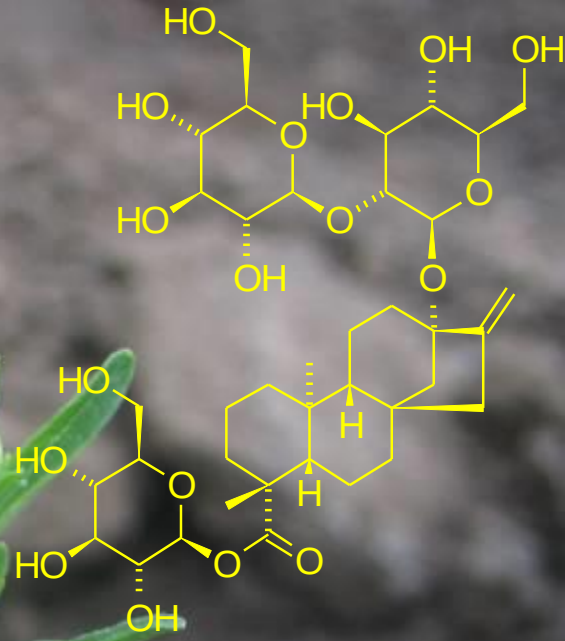
Monoterpenes and sesquiterpenes

Sapindus rarak (Sapindaceae)

mukurosioside IIb – as sweet as sucrose



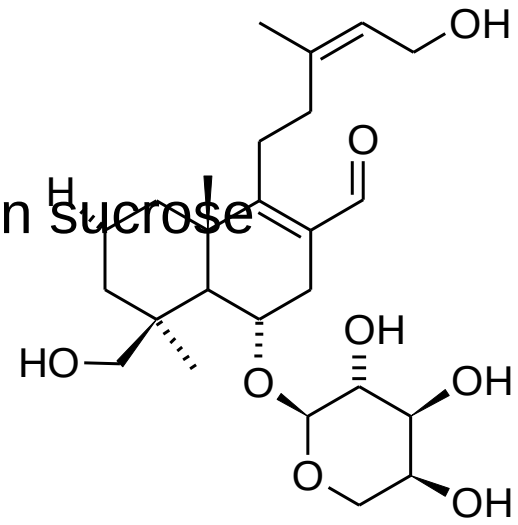
Diterpenes

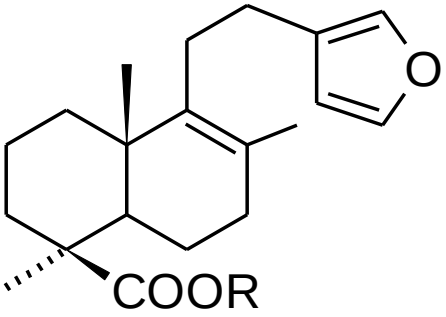


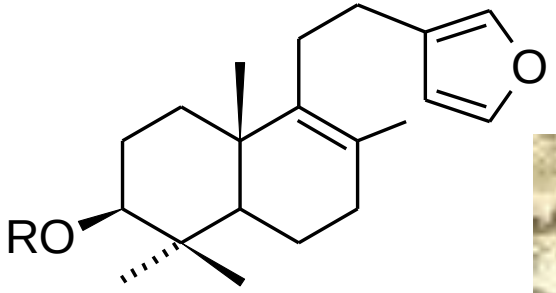
Stevia rebaudiana (Compositae)
Stevioside – 300 x sweeter than sucrose
Dulcoside A – C
Rebaudioside A - D

Diterpenes

Baccharis gaudichaudiana (Compositae)
Gaudichaudioside A – 55 x times sweeter than sucrose
Gaudichaudiosides B – D, bitter sweet







Phlomis younghusbandii (Labitae)
baiyunosidnde and flomisoside I

R = Glc - Xyl

Triterpenes

Licorice, *Glycyrrhiza glabra*

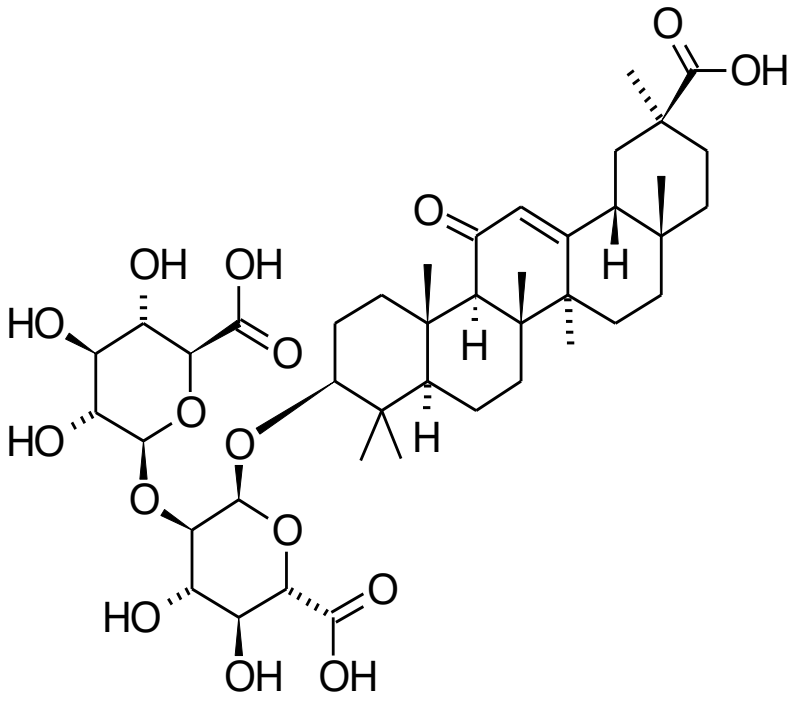
Licorice, *Glycyrrhiza glabra*

Root ... the taste is somewhat bitter,
but sweet and pleasant ...

We dig it and collect it while it fits
7 stars ... called chickens in Czech.

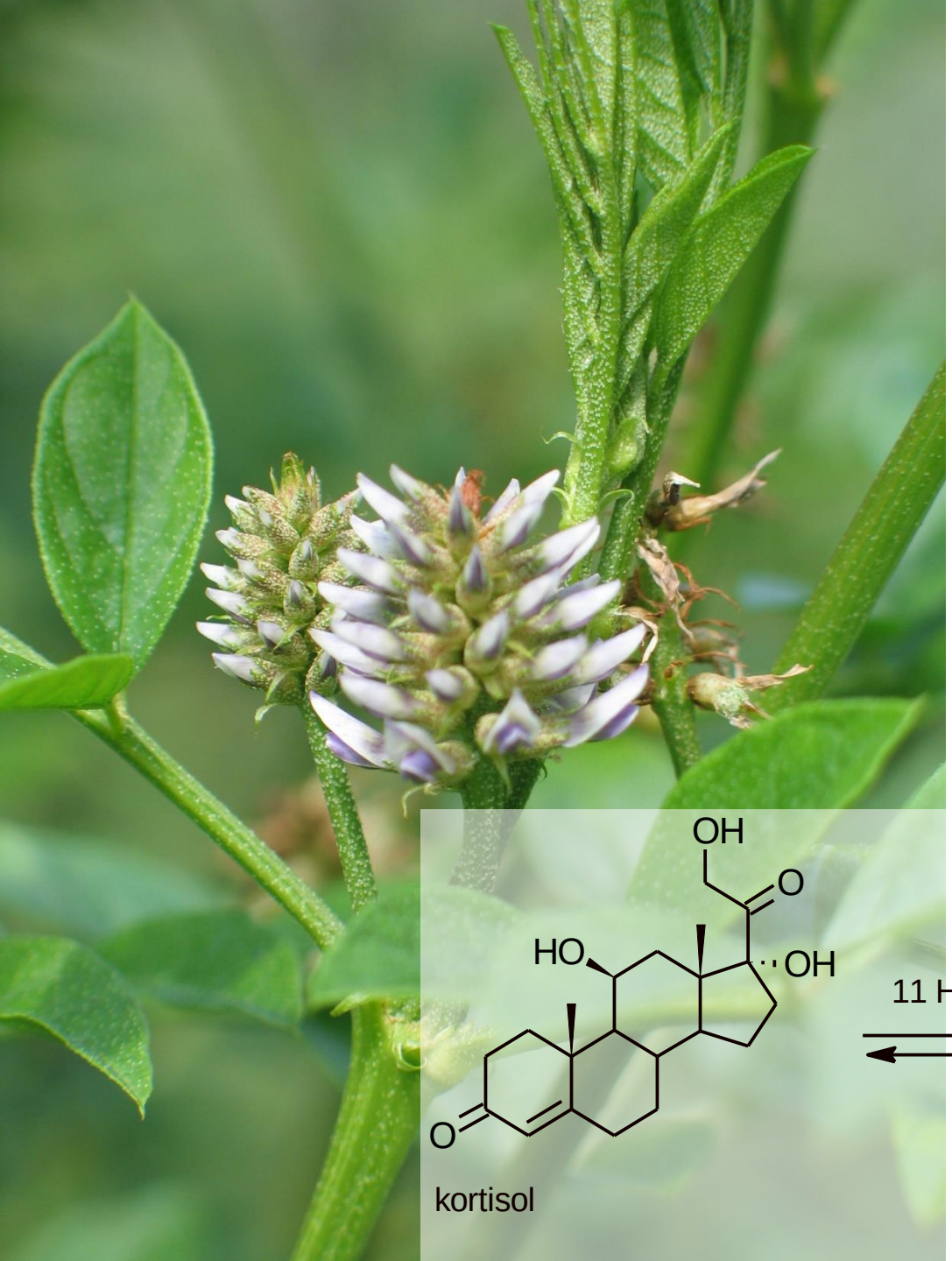
Chewing the root to thirst, hunger,
It is good for liver, hot stomach, removes heartburn, cleanses breasts and lungs, helps free coughing,
it relieves the dryness and roughness of the throat and the bronchus or the abdomen, softens swelling and ulcers. Therefore, it is beneficial against wheezing, dry cough, heavy breathing, constipation, ribbed tubercle and swollen hips.

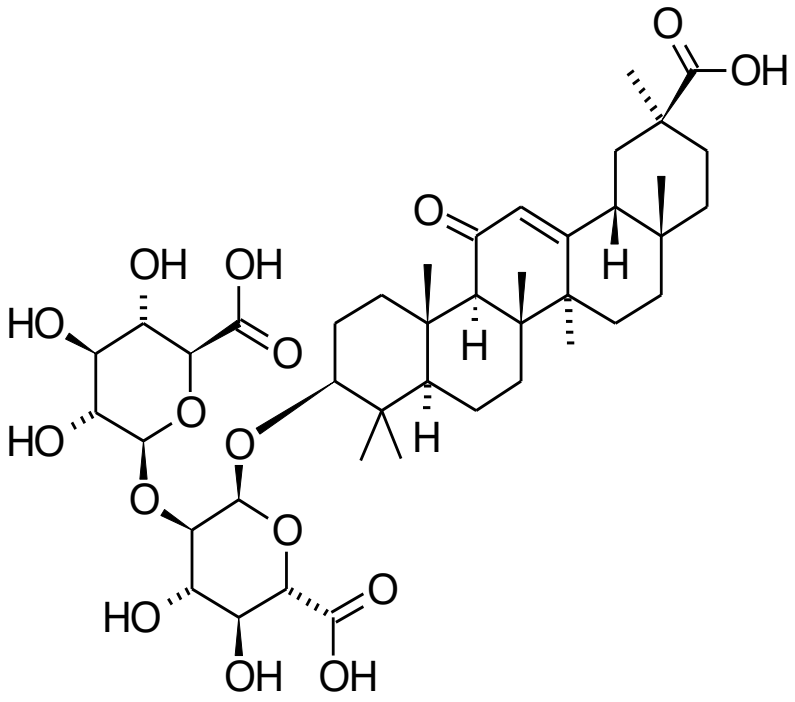
Dr. Petr Ondřej Mathioli,
Herbář neboli bylinář
Dobra & Fontána 1999, str 494.



Glycyrrhizic acid

Licorice, *Glycyrrhiza glabra*
Glycyrrhizic acid is 100-200 times sweeter than sucrose
a noticeable liquorice flavor

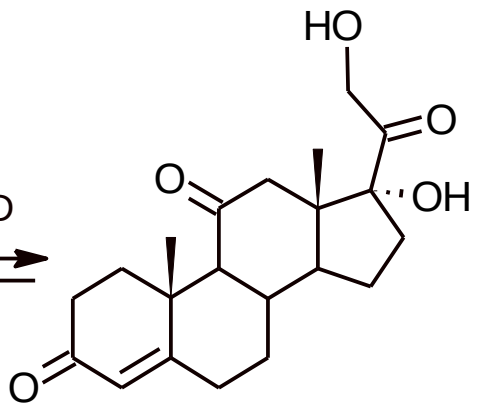




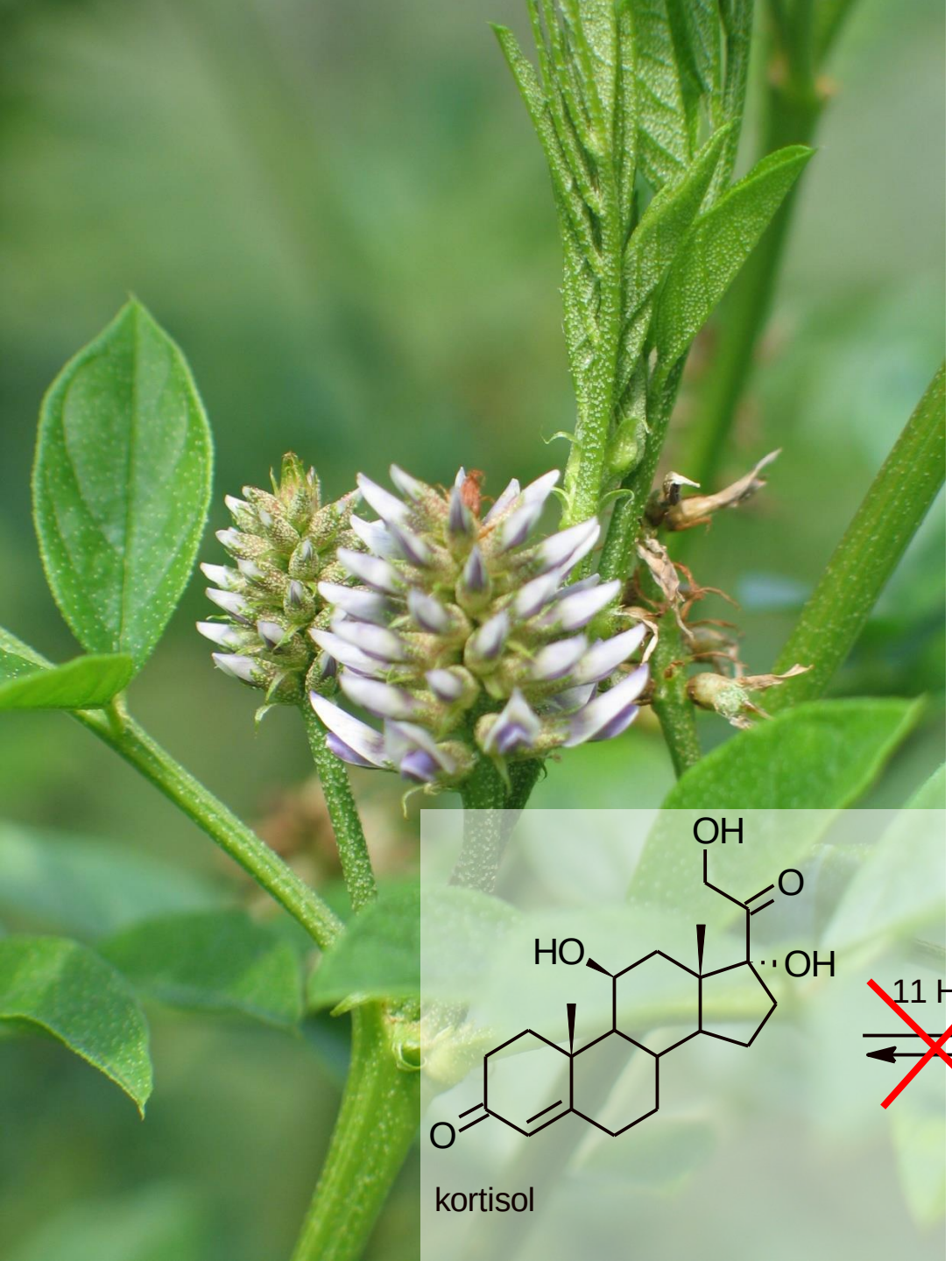
Glycyrrhizic acid

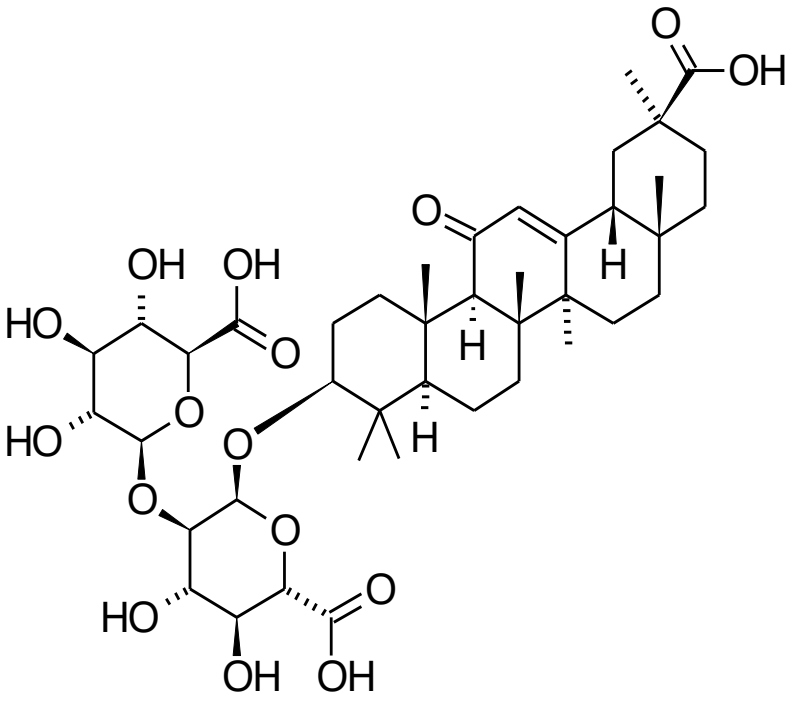
kortisol

11 HSD



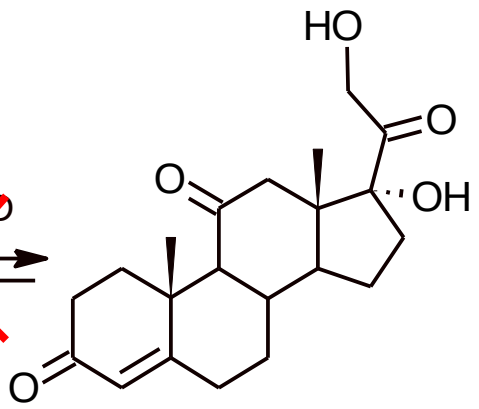
kortison



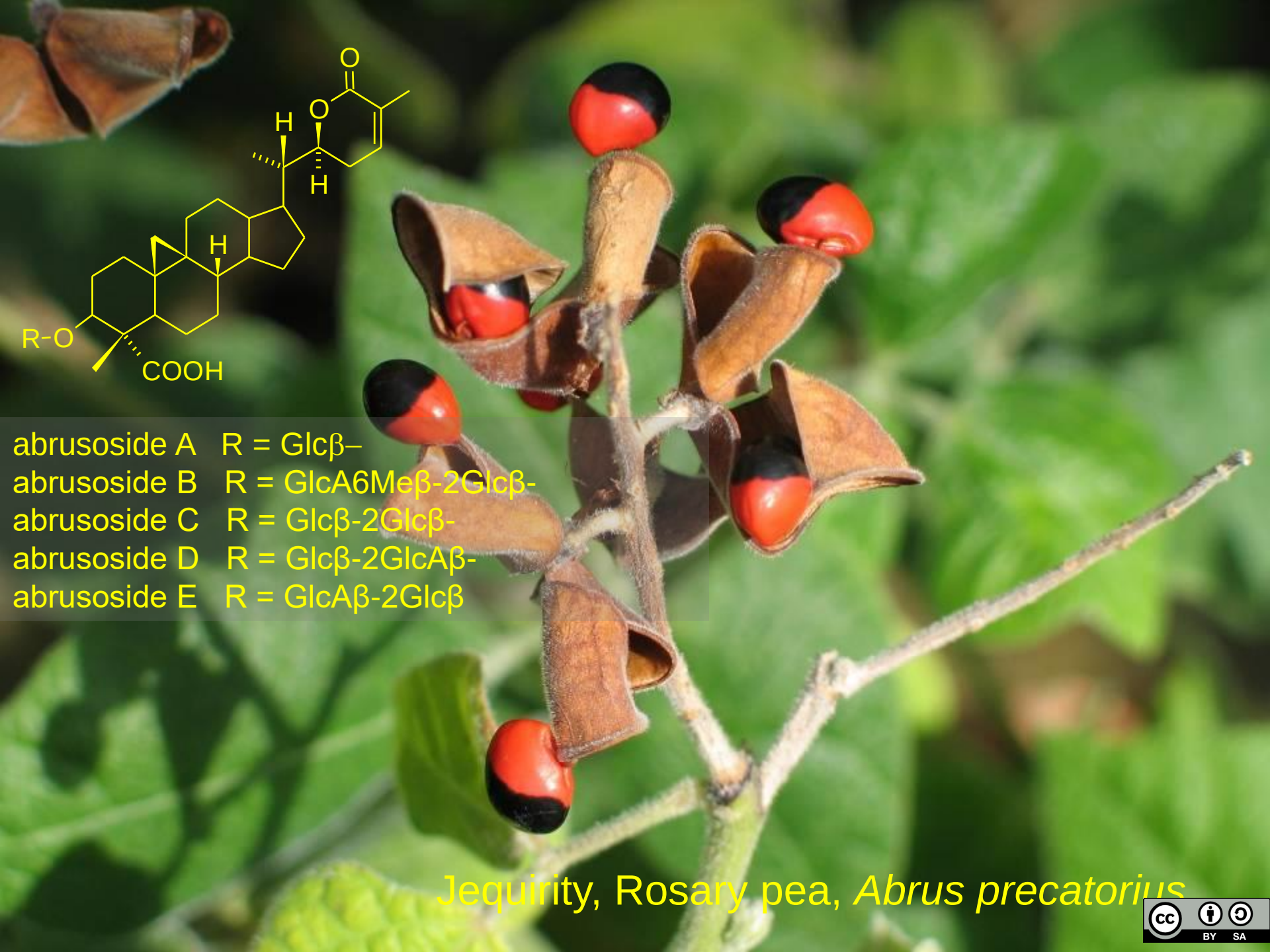


Glycyrrhizic acid

kortisol



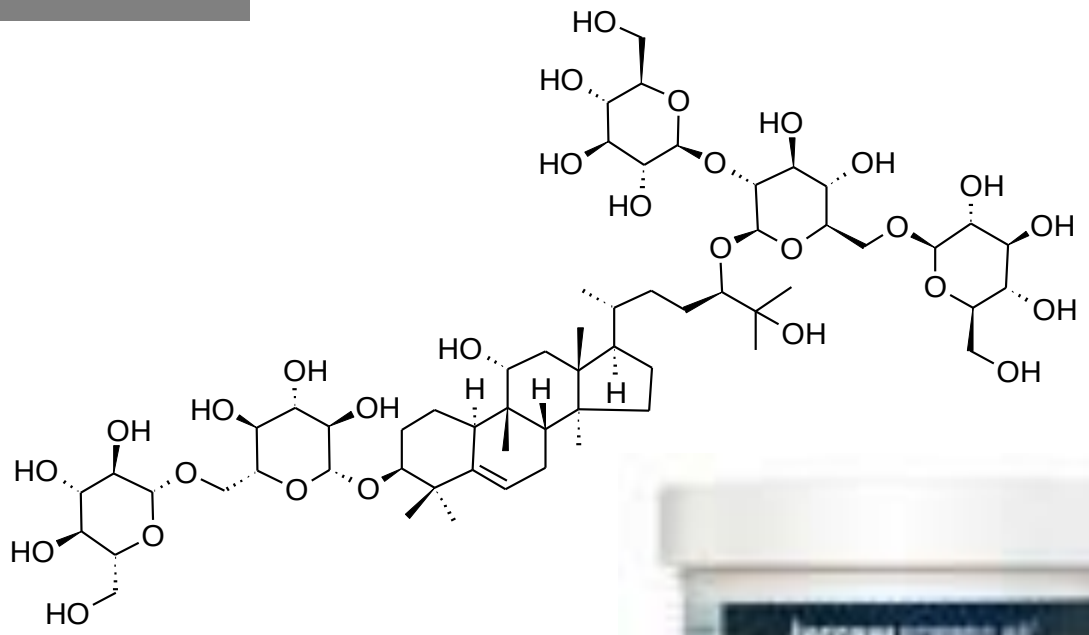
kortison



abrusoside A R = Glcβ–
abrusoside B R = GlcA6Meβ-2Glcβ-
abrusoside C R = Glcβ-2Glcβ-
abrusoside D R = Glcβ-2GlcAβ-
abrusoside E R = GlcAβ-2Glcβ

Jequirity, Rosary pea, *Abrus precatorius*

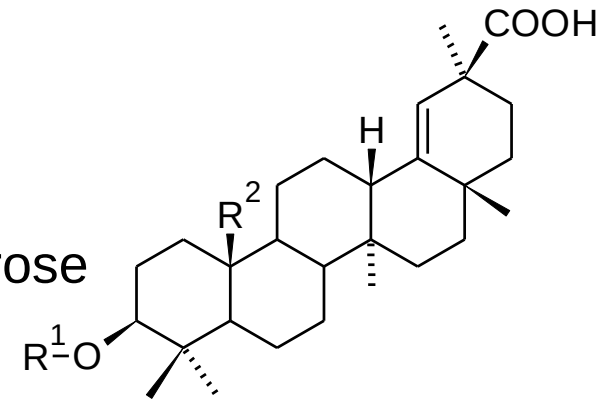
Triterpenes



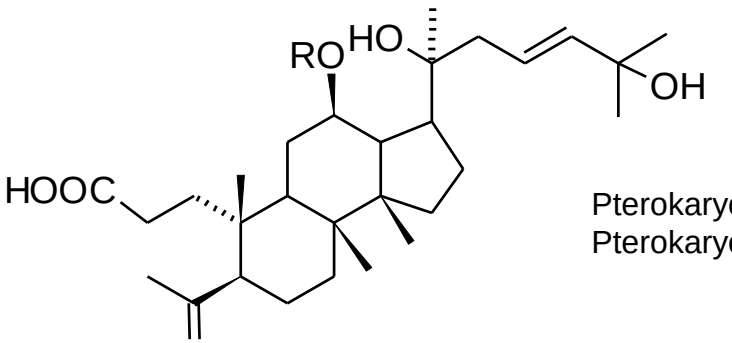
Momordica grosvenori Swingle (Cucurbitaceae)
mogroside V, 400 × sweeter than sucrose
siamenoside, 560 x sweeter than sucrose

Triterpeny

Periandra dulcis (Fabaceae)
periandrin I – V, 100-200× sweeter than sucrose



periandrin I R^1 = Glcβ-2GlcAβ- R^2 = CHO
periandrin II R^1 = Xylβ-2GlcAβ- R^2 = CHO



Pterokaryosid A R = Quiβ-
Pterokaryosid B R = L-Araα-

Pterocarya paliurus (Juglandaceae)
pterokaryoside A a B, 50-100× sweeter than sucros
bitter aftertaste

Non-sugar sweet natural compounds

- Terpenoids
- **Steroids**
- Flavonoids, chalcones and coumarins
- Aminoacid derivatives
- Proteins


Osladič obecný, *Polypodium vulgare*

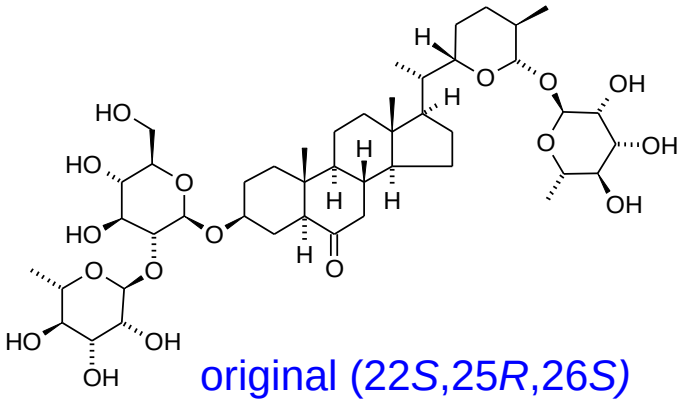
Kořen netkví hluboko
a rozkládá se v zemi do šířky;
jest křivý, uvnitř pak barvy zelené,
chuti sladké se skrovnou nebo
tajnou trpkostí.

Když se ho mnoho žvýká, vzbuzuje
v žaludku nelibost a nechutenství.
Nemá květu ani lodyh.

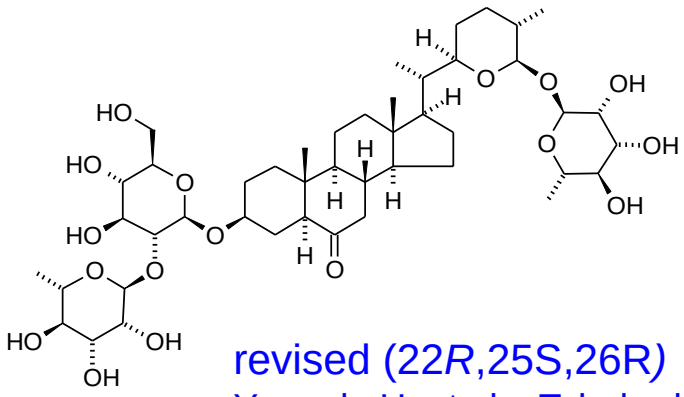
Trhati se má při ubývání měsíce.

Osladič je kořen počišťující;
pití teplého odvaru osladiče se slepicí,
nebo manholtem, nebo slezem na
lačný život, vyhání stolicemi
všelikou choleru i šlemy.

Dr. Petr Ondřej Mathioli, Herbář neboli bylinář.
Dobra & Fontána 1999, str. 1040-1041



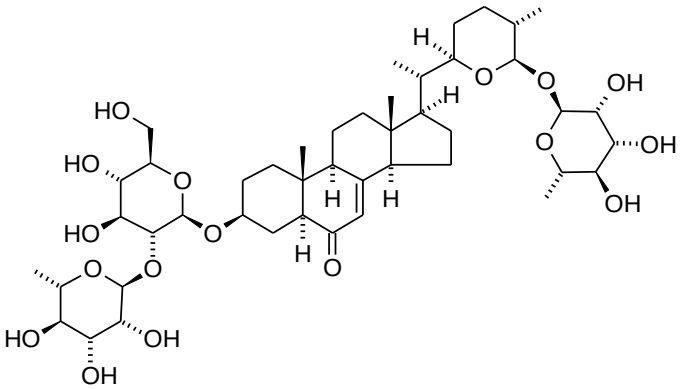
original (22*S*,25*R*,26*S*)

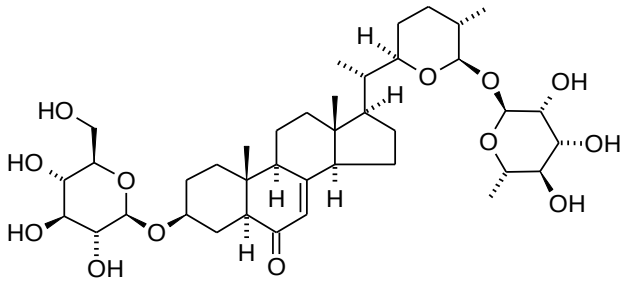


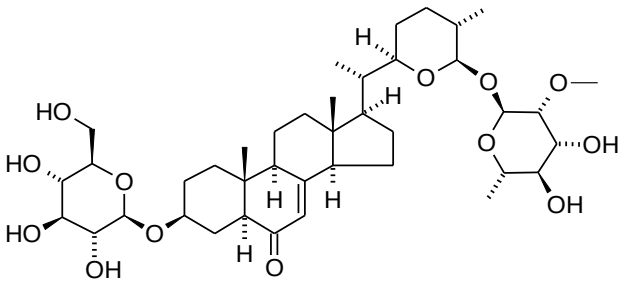
revised (22*R*,25S,26R)

Yamada H. et al.: Tehahedron Lett. *33*, 4009 (1992).

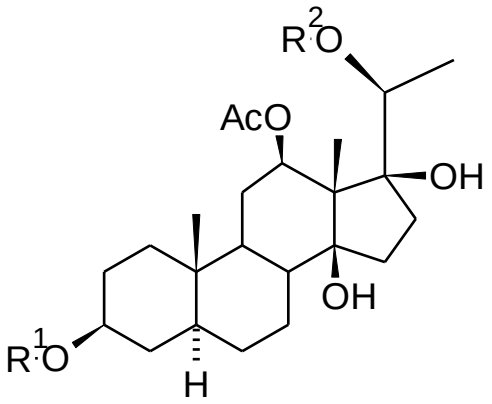
Poplypodium vulgare (Polypodiaceae)
osladine, 500× sweeter than sucrose







Poplypodium glycyrrhiza (Polypodiaceae)
polypodoside A - C, 600× sweeter than sucrose



Telosma procumbens (Asclepiadaceae)
telosmoside A15, 1000× sweeter than sucrose

Telosmosides A_1-A_{18}.

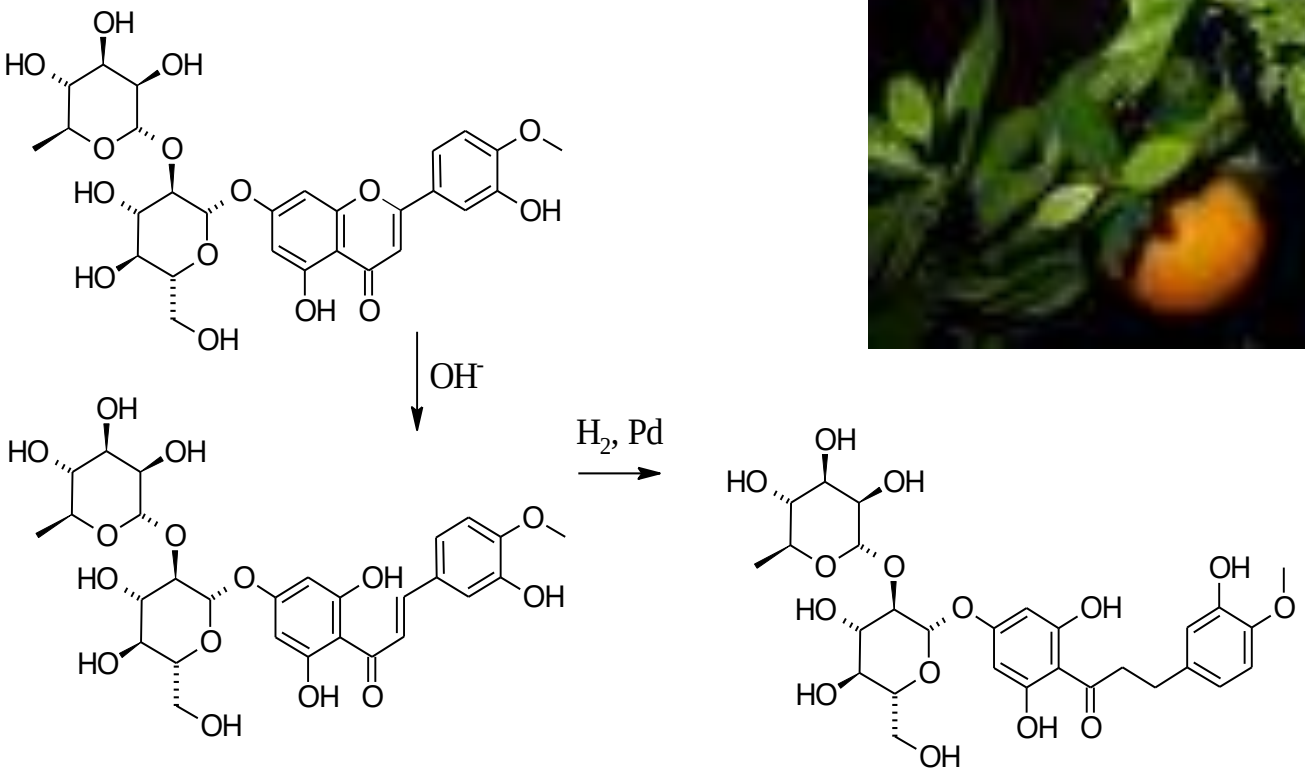
Glykosides derived from the same aglycone of polyoxypregnane type
Eleven sweet compounds, one bitter (A_2), six tasteless.

Non-sugar sweet natural compounds

- Terpenoids
- Steroids
- **Flavonoids, chalcones and coumarins**
- Aminoacid derivatives
- Proteins

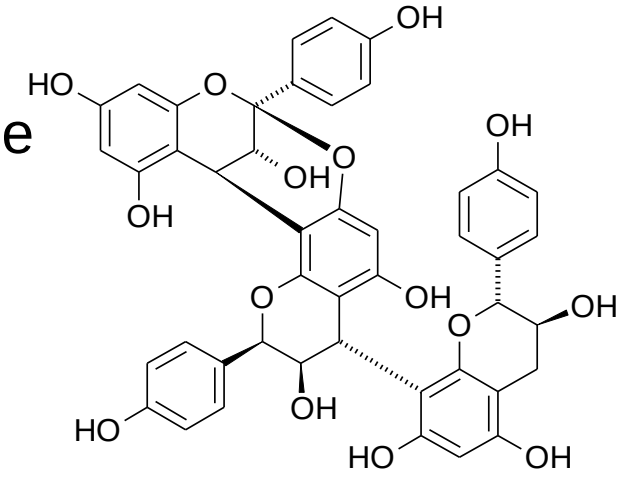
Flavonoids and chalcons

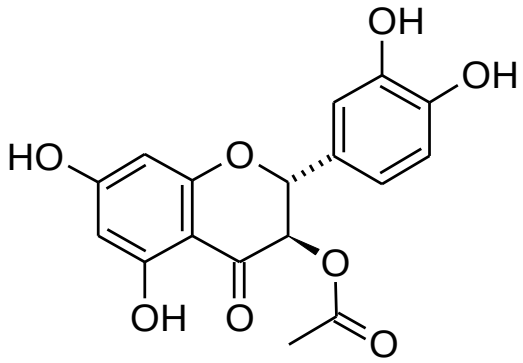
Citrus aurantium (Rutaceae)
neohesperidin, bitter
neohesperidin DHC, 1000x swee
E_{959} (EU), GRAS (USA)



Flavonoids and chalcons

Polypodium feii (Polypodiaceae)
selligueain, 32x sweeter than sucrose





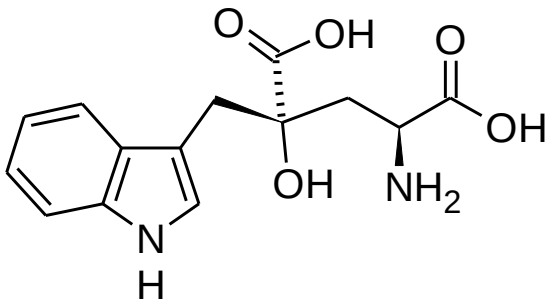
Hymenoxys turneri, Tessaria dodoneifolia, Bachcharis varlans, Inula viscosa (Compositae)
(2*R*,3*R*)-dihydroquercetin-3-acetát, 80x sweeter than sucrose

Non-sugar sweet natural compounds

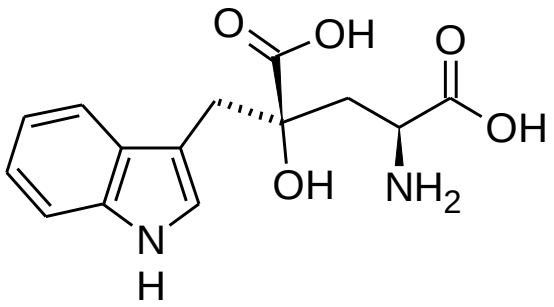
- Terpenoids
- Steroids
- Flavonoids, chalcones and coumarins
- **Aminoacid derivatives**
- Proteins

Aminoacid derivatives

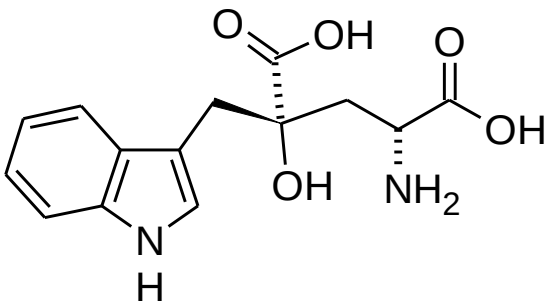
Sclerochiton ilicifolius (Acanthaceae)
monatin, 1200 – 1400 x sweeter than sucrose



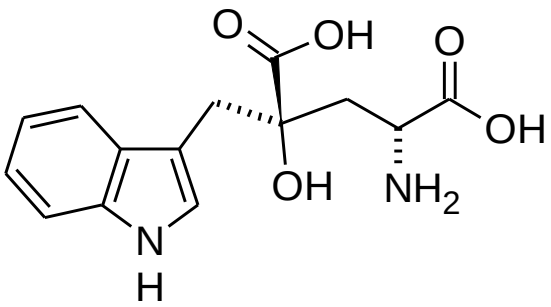
(2*S*,4*S*)-monatin



(2*S*,4*R*)-monatin



(2*R*,4*S*)-monatin



(2*R*,4*R*)-monatin

Non-sugar sweet natural compounds

- Terpenoids
- Steroids
- Flavonoids, chalcones and coumarins
- Aminoacid derivatives
- **Proteins**

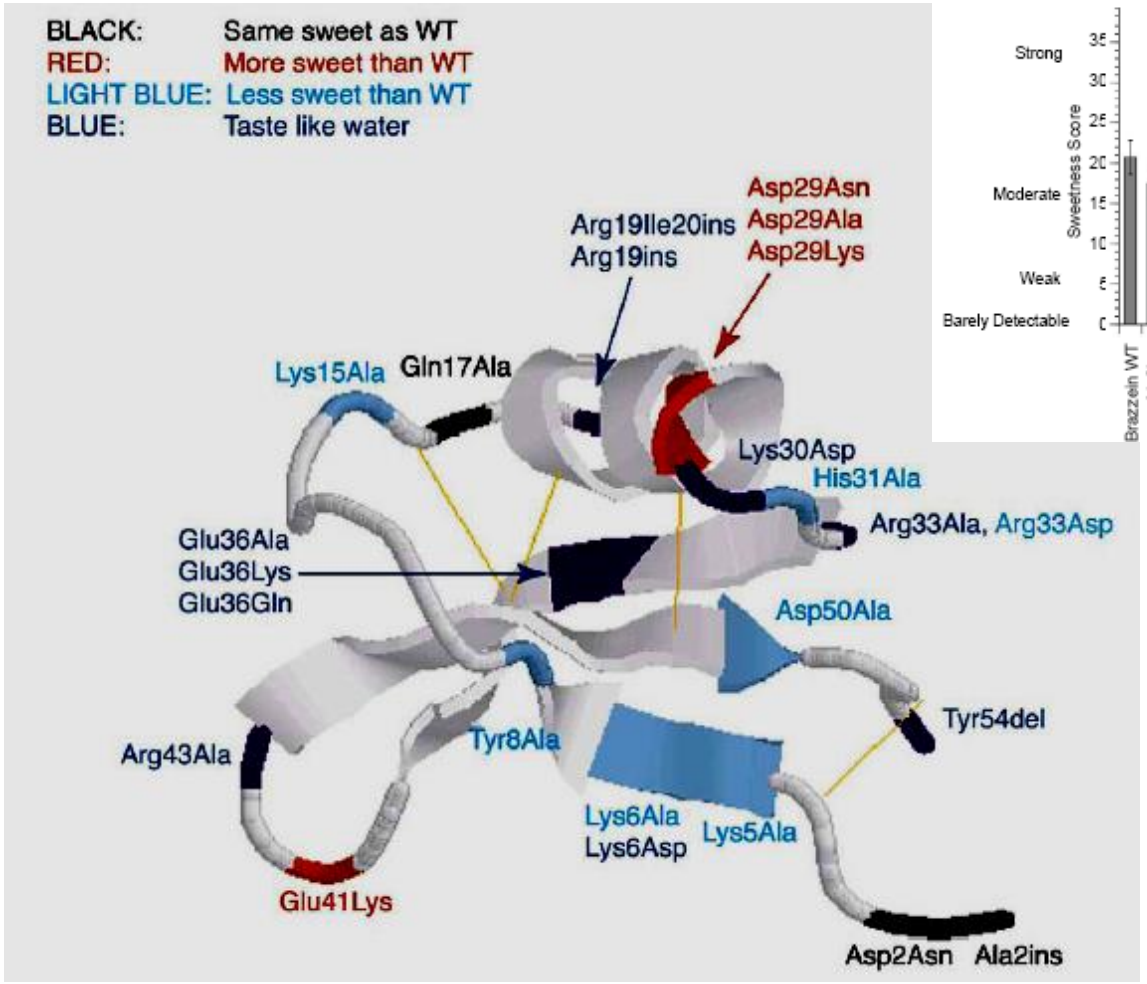
Proteins

Thaumatococcus daniellii Benth (Marantaceae)
thaumatin, 3000 – 15000x sweeter than sucrose
E_{957} (EU), GRAS (USA)

Dioscoreophyllum cumminsii (Menispermaceae)
monellin, 3000x sweeter than sucrose

Pentadiplandra brazzeana (Capparaceae)
brazzein, 1200x sweeter than sucrose

Brazzein mutants



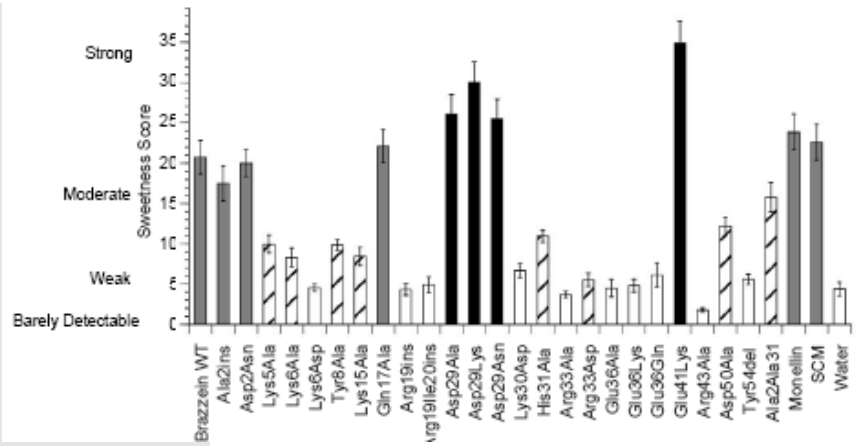


Fig. 3. Diagram showing the three-dimensional backbone of brazzein [10] with the position of mutations studied. The residues are color-coded to indicate the taste properties of mutants at these positions relative to those of WT brazzein: red, increased sweetness; black, the same sweetness; light blue, decreased sweetness; dark blue, taste equivalent to water. Intramolecular disulfide bonds are indicated as yellow lines.

Z. Jin et al./FEBS Letters 544 (2003) 33–37

Proteins

Capparis masaikai (Capparaceae)
mabinlin I-IV, 1000x sweeter than sucrose

Curculigo latifolia, (Liliaceae)
curculin and neoculin, 500x sweeter than sucr

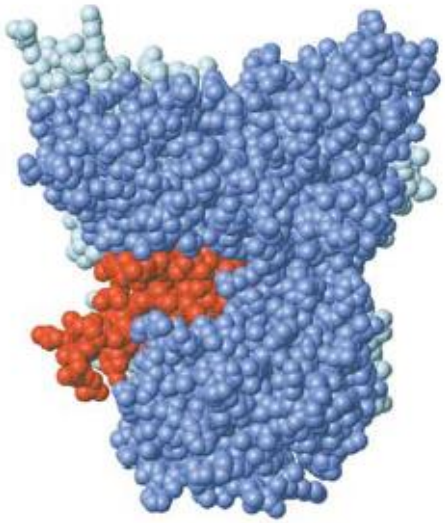
Synsepalum dulcificum syn. *Richardella dulcifica*, Sapotaceae
miraculin: conversion of sour taste to sweetness

Sweet proteins – do they have common features?

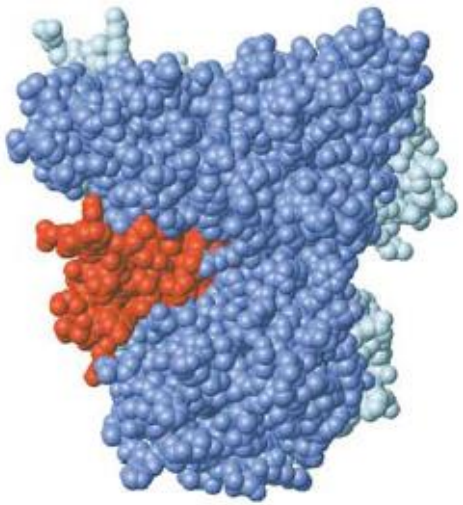
Protein	Sub-units	Aminoacids	m.w. (kDa)	SS bridges	Sweetness (sucrose = 1)	Note.
Thaumatin I	1	207	22,2	8	3000–15 000	E_{957}
Thaumatin II	1	207	22,2	8		
Monellin	2	44 + 50	10,7	0	3000	
Brazzein	1	54	6,5	4	1200	
Mabinlin I	2	32 + 72	12,3	4		
Mabinlin II	2	33 + 72	12,4	4	1000	
Mabinlin III	2	32 + 72	12,3	4		
Mabinlin IV	2	28 + 72	12,1	4		
Curculin	2	114 + 114	23	4	500	
Neoculin	2	114 + 113	23-24	4		
Miraculin	4	191	24,6		Tasteless	
Lysozyme (hen)	1	211	14,4	4		

Proteins and sweet taste receptor

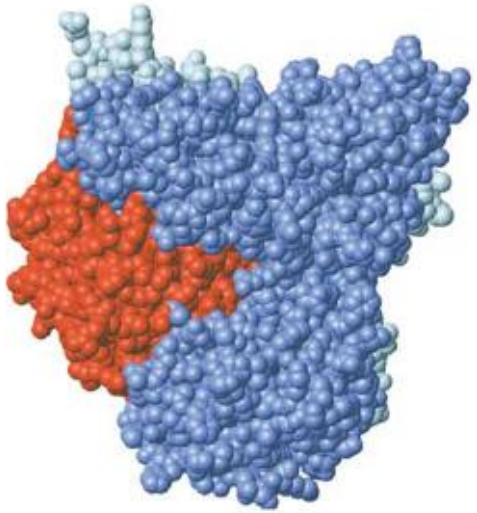
P.A. Temussi/FEBS Letters 526 (2002) 1–4



brazzein



monellin

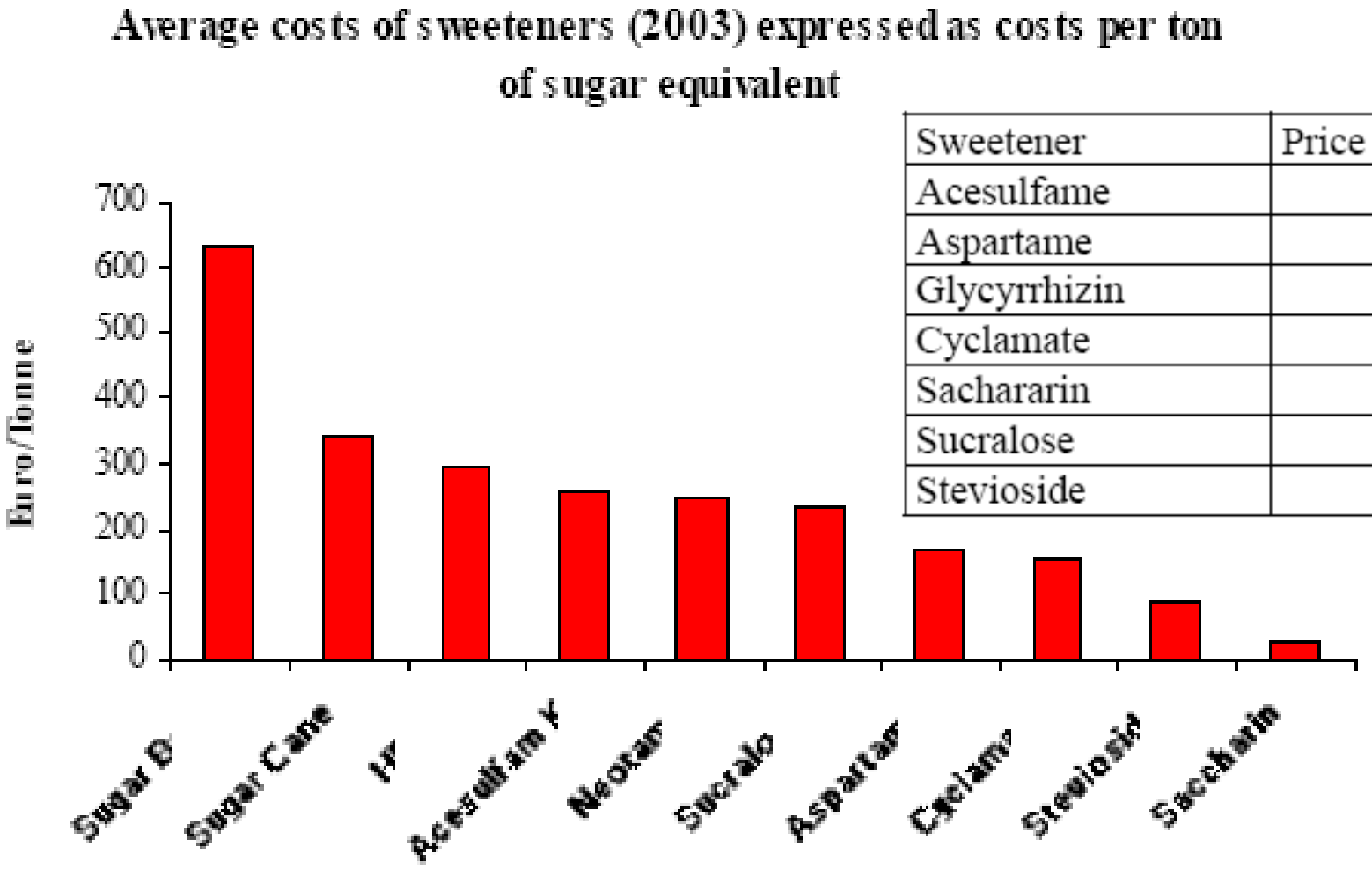


thaumatin

Non-sugar sweeteners market

- Thaumatin - E_{957} (EU), GRAS (USA)
- Brazzein – GM maize (USA)
- Stevioside – in EU since 2012, in USA since 2007
- Glycyrrhizic acid – not in EU nor in USA
- Cucurbitans (Lo-Han Guo) – Asia
- Neohesperidine DHC - E_{959} (EU), GRAS (USA)

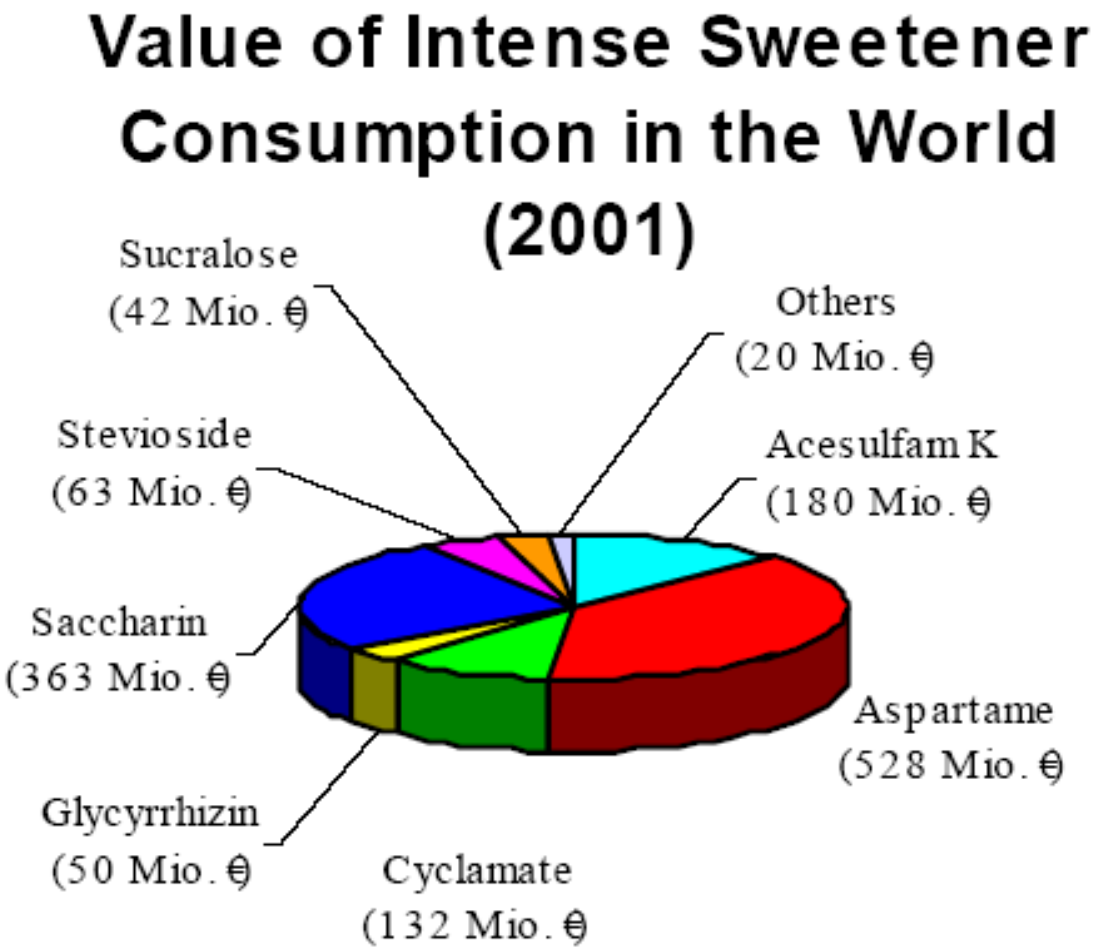
Non-sugar sweeteners market



Sweetener	Price per kg
Acesulfame	80 Euro
Aspartame	40 Euro
Glycyrrhizin	50 Euro
Cyclamate	4.5 Euro
Sachararin	6.7 Euro
Sucralose	139 Euro
Stevioside	50 Euro

Bahndorf D., Kienle U.: http://www.uni-hohenheim.de/~www440/VTP/stevia/B0/B5 (15.2.

Non-sugar sweeteners market



Bahndorf D., Kienle U.: http://www.uni-hohenheim.de/~www440/VTP/stevia/B0/B5 (15.2.

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•Lapčík O., Čopíková J., Uher M., Moravcová J., Drašar P. (2007) Necukerné přírodní látky sladké chuti. *Chem listy* **101**, 44-54.

Bioactive Natural Compounds

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2018

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1.

Introduction

The knowledge of a scale of natural remedies, spices, perfumes and also of psychotropic substances is inherent to every civilization. The way that individual species are used depends on culture. A spice, a remedy, a poison – the difference may be only a matter of dosage and the way of application. Numerous foods contain also substances displaying biological effects which are fully effective only after a long intake, a monotonous diet or after inappropriate preparation of food.

Therefore, in the “Bioactive Natural Compounds” course we will focus together with the medicinal plants also on the important substances found in certain fruits and vegetables.

Effects of natural compounds were known and exploited since immemorial. Study of their molecular basis started about two hundred years ago - the important milestone was the crystallization of pure morphine from opium tincture by Friedrich Serturner in 1806.

The process of discovery of bioactive compounds has several logical phases, and each of them may have numerous pitfalls. The first phase consists of description of a particular bioactivity and identification of the active compound(s). Next steps consist of purification of the compound(s) of interest from natural source(s) followed by determination of the structure. New structures challenge the chemists – synthesists. Total synthesis was considered to be the final proof of correctness of structure determination for many decades prior to the advent of hyphenated NMR and MS-MS techniques. Together with the compound itself, the chemists try to prepare also its derivatives. Availability of a pure compound and its analogues is the indispensable prerequisite for the study of relations between the structure and the function. Compounds with more advantageous properties may appear amongst the synthetic analogues.

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Basic terms

PHARMACOGNOSY (from the Greek *pharmacon* –poison, *gnosis* – knowledge) – study of the starting natural materials and substances intended for therapeutics. Nowadays, the field of pharmacognosy is limited to plants and a limited number of fungi.

PHARMACOPOIEA – The law defining assortment of materials used for preparation of remedies.

DRUG – dried part of a medicinal plant or dried amorphous product used for preparation of remedies.

ALLOPATHY – "official medicine", the remedies are designed to act either against the cause of disease (causal treatment) or against the symptoms (symptomatic treatment).

HOMEOPATHY – one branch of "alternative medicine", founded by Samuel Hahnemann, (1755-1853). Homeopathy is based on the presupposition, that illness may be treated or prevented by highly diluted solutions of substances that cause symptoms similar to the respective illness in higher concentrations (*similia simile curantur*). Homeopathic remedies are highly diluted, sometimes so that any biochemical interaction may be excluded. Their observed effects (if any) may be partly explained by principles known to the "official medicine", e.g. the PLACEBO effect, manipulation of BIORYTHMS or usual molecular interactions in the case of less diluted solutions (up to 10^{-15}M).

PLACEBO – The psychological effect of treatment elicited independently on chemical composition of remedies, just due to the patient´s **positive expectations**

NOCEBO – The psychological effect of treatment elicited independently on chemical composition of remedies, just due to the patient´s **negative expectations**

SYNERGY – complementary action of two or more substances (the effect is higher than would correspond to a simple addition of individual effects).

ANTAGONISM – the opposite action of two substances.

LATIN TERMS FOR ANATOMICAL PARTS OF PLANTS:
-AERIAL-
Tops – *herba*, stalk – *caulis*, bud – *gemma*, leaf – *folium*, wood – *lignum*, bark, peel – *cortex*, flower – *flos*, fruit – *fructus*, seed – *semen*, spore – *sporae*.
-UNDERGROUND-
Root – *radix*, rhizome – *rhizoma*, tuber – *tuber*, bulb (onion) – *bulbus*.

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DRUG EFFECTS:

ANTISCLEROTICS – drugs used against arteriosclerosis
(*Alium sativum* – garlic, *Drosera rotundifolia* – sundew (drosera))

ANTIHYDROTICS – drugs that decrease sweating
(For local applications e.g.: *Quercus robur* – oak tree (bark), *Juglans regia* – wallnut tree (leaves), *Agrimonia eupatoria* – agrimony)

CARDIOTONICS – drugs influencing cardiac action. (Usually poisonous).
(*Adonis vernalis* – spring adonis, *Digitalis lanata* – Grecian foxglove)

DIAPHORETICS – drugs that cause sweating
(*Salix alba* – willow, *Tilia cordata* – linden, *Sambucus nigra* – European elder)

HYPOTENSIVES (ANTIHYPERTENSIVES) – blood pressure decreasing drugs
(*Valeriana officinalis* – valerian, *Humulus lupulus* – hops)

EXPECTORANTS – the drugs making easier to cough-out the mucus from bronchi

Mucus dissolving drugs
Glycyrrhiza glabra – licorice, *Primula veris* – primrose, *Saponaria officinalis* – soapwort

Irritation decreasing drugs (antitussives):
Bellis perennis – daisy, *Plantago lanceolata* – ribwort

Mucous:
Verbascum thapsiforme – mullein, *Tussilago farfara* – coltsfoot

Disinfectively acting:
Thymus serpyllum – thyme, *Pinus silvestris* – pine

STOMACHICS – drugs influencing stomach function. (Often drugs containing bitter substances).

CARMINATIVES – drugs against flatulence.
(*Carum carvi* – caraway (caraway seed), *Coriandrum sativum* – coriander, *Majorana hortensis* – marjoram, *Pimpinella anisum* – anise (aniseed)

OBSTIPANTS – anti-diarrheal drugs.
(*Daucus carota* – carrot, *Salvia officinalis* – sage, *Vaccinum myrtilus* – cranberry)

LAXATIVES (purgatives, aperients)

1. Bulk laxatives: materials that bind water and thus influence the volume and consistency of intestinal content. e.g. pectins, mucins, agar-agar.

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2. Substances influencing the motility of smooth muscles. Anthraquinone laxatives, e.g. *Frangula alnus* – buckthorn, *Cassia senna.*

3. Drastic purgatives. *Ricinus communis* – castor (castor oil).

DIURETICS – the drugs supporting the function of kidney

ANTIHELMINTICS – act against parasitic worms

ANTIPYRETICS – act against fever. Antipyretics usually have also ANTIINFLAMMATORY activity

ANTIVIROTICS –act against pathological viruses

LACTAGOGUES – substances supporting secretion (or rather ejection) of maternal milk. (Caraway seed, aniseed, fennel).

CYTOSTATICS – substances that block the cell cycle. Cytostatics are used against cancers. *Vinca rosea* – Madagascan periwinkle, *Podophyllum peltatum* – May apple, *Taxus sp*. – yew.

EXTERNA – drugs for external use (used for skin care or for mouthwashes). Antibacterial and balmy drugs.
Matricaria chamomilla – chamomile, *Symphytum officinale* – coltsfoot, *Agrimonia eupatoria* – agrimony.

PANACEA – a miraculous universal remedy.
Panacea (Panakeia) and Hygieia were daughters of Greek god Asclepios (Aesculapos). Panacea knew the remedies for every disease. Her name sounds in the Latin name of Zhen-Shen: *Panax ginseng*.

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3.

A few historical names

HIPPOCRATES 460-377 B.C. – an ancient Greek physician and a teacher of physicians. The oath dedicated to his name (Hippocrates oath) has been taken by physicians throughout the centuries as a basis of professional ethics. Despite the uncertainty of its true authorship, this ancient text documents the permanent importance of ethical issues. Together with other ties, candidates of medicine were committed to reject performing euthanasia and artificial abortions – the topics still controversial in 21st century.

ARISTOTELES 384-322 B.C. – Greek philosopher, founder of principles of science

THEOPHRASTOS 371-287 B.C. – Greek philosopher, founder of botany

DIOSCORIDES 40-90 A.D. Greek physician and botanist. During his service in Roman army, Dioscorides travelled around the Mediterranean area and studied local healing practices. The author of book "*De Materia Medica"*, where over 600 medicinal plants were described (e.g. mint, chamomile, sage, licorice, oak bark, etc.)

AULUS CORNELIUS CELSUS 30 B.C. – 53. A.D. Roman physician. His monography "De Re Medicina" includes first definition of inflammation and its symptoms (in Latin terms: tumor, rubor, calor, and dolor, i.e. swelling, redness, heat and pain, respectively)

GALÉNOS CLAUDIUS 129-211 A.D. Greek botanist from Pergamon. The author of about 500 works about medicinal plants. His books became a recognized model for over a thousand years.

AVICENNA 979-1037 A.D. – Arabian scientist Abu Ali Ibn Sina, an expert in ancient medicinal publications. The author of "Canon Medicinae" (Al quantum fi-at tibb).

PARACELSUS 1493-1541 A.D. – German physician Theophrastus Bombastus von Hohenheim. Paracelsus opposed non-critical reproducing of Galenos and other old classics.

PIETRO ANDREAS MATHIOLI (Petr Ondřej) - Italian botanist; his herbal (first issued in Nurnberg) was translated into Czech by Tadeáš Hájek z Hájku (1562).

CARL von LINNÉ 1707-1778 – Swedish botanist and physician. He introduced the systematic nomenclature into biological sciences.

EDWARD JENNER 1749-1823 British physician. Edward Jenner discovered in 1796 that intended infection of humans with low-pathogenic cowpox (*Vaccinia* in Latin) could prevent against smallpox (*Variola vera*), for centuries one of the most serious infection diseases. The concept of protective immunization was named vaccination (according to Latin *vacca* – cow).

LOUIS PASTEUR 1822-1895 French chemist and physician. First studies of chirality. Study of fermentation, detection of microbial origin of fermentation. Discovery of sterilization (pasteurization). Improving hygiene practices.

Pasteur has expanded the use of active immunization (vaccination) to protect humans and domestic animals against a number of pathogens (e.g. rabies, anthrax, chicken cholera, swine fever) and developed the theoretical basis for the concept of this technique.

FRIDRICH SERTURNER 1783 - 1841, German pharmacist, pioneer of alkaloid chemistry. He first isolated morphine as a pure compound by crystallization from opium in 1804.

CHARLES FÉDERIC GERHARDT 1816-1855 has prepared acetylsalicylic acid for the first time by acetylation of salicylic acid isolated from a natural source.
HERMAN KOLBE 1818 – 1894 developed total synthesis of salicylic acid (1860) and made it available in large quantities. The Bayer Group introduced acetylsalicylic acid under the trademark ASPIRIN in 1899. Aspirin was the first commercially available natural compound analogue with significantly improved pharmacological activity (antipyretic, anti-inflammatory and analgesic).

CASIMIR FUNK introduced the term VITAMIN in 1911

Casimir Funk

ALBERT von SZENT GYORGYI isolated vitamin C from pepper in 1928. Synthetic vitamin C is available since 1932 thanks to the inventions of Tadeusz Reichstein and Norman Haworth.

ALEXANDER FLEMING discovered penicillin in 1929

4.

Metabolic origin of biologically active compounds

Primary and secondary metabolites

Despite the fantastic variability of Nature, all living beings share remarkably high number of biochemical features. Certain metabolic processes are common to all organisms from bacteria to multicellular organisms of all types (i.e. animals, plants and fungi) and their chemical principles are amino acids and proteins, nucleotides and nucleic acids, lipids and carbohydrates. This includes the building and maintaining body structures, genetic information, and energetic metabolism. These processes are called PRIMARY METABOLISM.

In the second half of nineteenth century, scientists realized the existence of another branch of metabolism, assigned as SECONDARY METABOLISM by Albrecht Kosseln in 1891. Its definition was formulated by Julius Sachs in 1873:

„*One can designate as by-products of metabolism such compounds, which are formed during metabolism but which are no longer used in the formation of new cells. ……. Any importance of these compounds for the inner economy of the plant is so far unknown*“

Primary metabolism is characterized by a network of reversible (at least partly) pathways and cycles, which enable the interconversion of metabolites leading to their optimal utilization. Secondary metabolism gathers substances and energy from primary metabolism, however, there is virtually no reverse current (Fig 1).

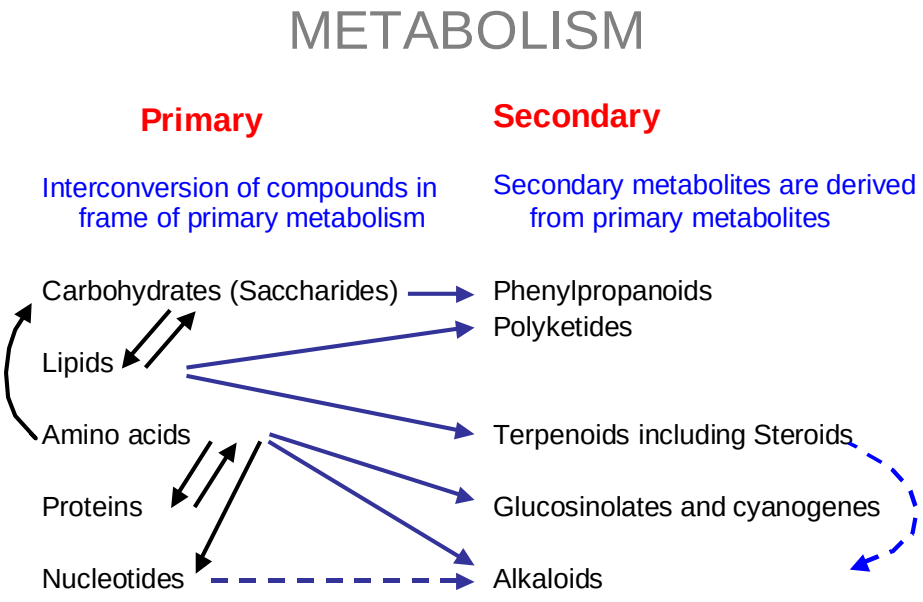

Fig. 1 Matter and energy fluxes in primary and secondary metabolisms

While the first note concerning the secondary metabolism was accompanied with the statement about its unclear physiological function(s), following research through the whole 20th century up to now has elucidated its numerous physiological and ecological roles. Plants and fungi differ from animals in many aspects. Living in the same place whole lifespan, they have to compete for space and defend themselves from attacks of numerous organisms – competitors (other plants or fungi), parasites, predators etc. Their physiology and reproduction depends on collaboration with other organisms, e.g. with symbiotic bacteria, pollinators or with animals who transport their seeds to other locations. Plants and fungi also do not have typical excretion organs capable to remove non-volatile catabolizes. Secondary metabolites were found to play important roles in all these processes (Table 1) as defense molecules, phytoalexins, attractants, metabolic end products etc. In contrary to the primary metabolism, the secondary metabolism is highly variable. The occurrence of some structural types may be limited to closely related taxa or may be even species specific (e.g. the occurrence of morphine, limited to *Papaver somniferum*). As secondary metabolites act in numerous interspecies ecological interactions, it is not surprising that many of them have interesting pharmacological activities.

Table1. Biological functions of secondary metabolites

Functions of secondary metabolites

Defense and competion	**Attraction and stimulation**
Herbivores	Pollination
-Arthropods	Seed dispersal
-Vertebrates	Food-plant recognition
-Invertebrates	Oviposition
Pathogens	Sequestration
-Viruses	Symbiosis
-Bacteria	-Bacteria
-Fungi	-Fungi
Plants	

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Products of primary metabolism

5.1.

Saccharides (Carbohydrates)

Saccharides are universal constituents of living organisms. They are polyhydroxylated organic compounds with a carbonyl (aldehyde or ketone) function. The saccharide group contain also oxidized or reduced derivatives (uronic acids, polyalcohols), their esters, ethers and amine derivatives.

They are found as

- support elements, fibers (cellulose and other parietal polysaccharides)
- energy reserves; polymers of glucose (starch), fructose (inulin) etc.
- constituents of various metabolites, nucleic acids, coenzymes, glycosides
- precursors of other metabolites – first formed from carbon dioxide and water, they are the basis of all organic compounds of the living world

The estimated production of saccharides by all photosynthetically active organisms is around 10^{14} metric tons per year.

Saccharides may be divided into

- **monosaccharides**, of the general formula $C_n(H_2O)_n$, characterized by the presence of an aldehyde (aldoses) or ketone (ketoses) carbonyl function and (n-1) hydroxyl functions. The number of carbon atoms is most often five or six (pentose, hexose) and ranges from three to nine.

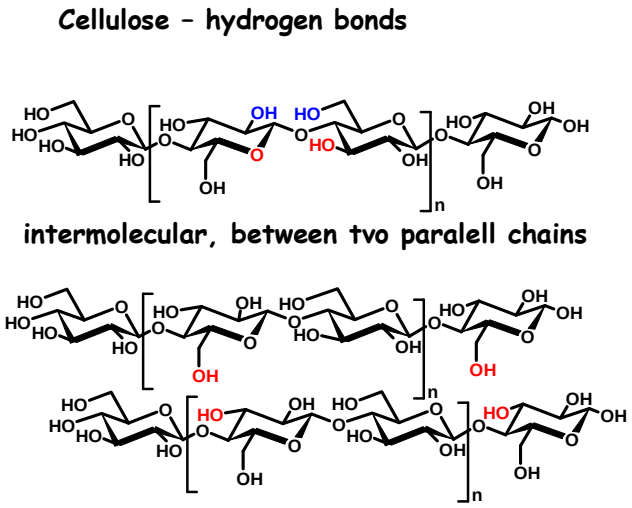
- **oligosaccharides** and **polysaccharides**, resulting from the combination through a glycoside bond, of several saccharide molecules (less than 10 units for oligo-; up to from 10 for polysaccharides).

- **glycosides** or **conjugate saccharides**; they result from the establishment of a bond between a saccharide moiety (mono- or oligosaccharide) and a non-sugar molecule. Saccharides are present as building units of many complex biomolecules, including nucleosides and nucleic acids, glycoproteins and proteoglycans, and also in a wide variety of glycosylated secondary metabolites (e.g. phenolics, terpenoids, cyanogic glycosides, glucosinolates and many others)

Monosaccharides are easily digestible, therefore they are important namely as nutrients.
On the other hand, numerous polysaccharides are used in many pharmaceutical and industrial applications. We will mention some of them.

Cellulose

Together with starch and chitosan, cellulose is one of the most common polysaccharides in the nature. Cellulose is a linear polymer of D-glucopyranose (polyβ-(1→4)-D-Glcp), usually very long (2000-14000 glucose units per molecule). It is water insoluble. Individual polymeric chains interact by multiple hydrogen bonds, forming a mechanically resistant supramolecular structure which is main component of wood and of plant fibers (e.g. cotton). Due to limited occurrence of β-glycosidases, cellulose is non-digestible. Those animals which use cellulose as a source of energy take the advantage of using enzyme apparatus of their symbiotic bacteria to cleave β-glycoside bond and digest thus cellulose to glucose (e.g. ruminants).



Despite its non-digestibility, cellulose plays an important role in human nutrition and physiology as the main component of **dietary fiber**. This term describes the non-digestible part of food. Fiber is necessary for maintaining good rheological properties of the intestinal content; it also serves as scaffold for the growth of symbiotic intestinal bacteria and enhances the excretion of biliary metabolites (e.g. bilirubin) by speeding the intestinal passage.

Fig 2. Molecular structure of cellulose

Applications of cellulose:

Food and pharmaceutical industry:	emulsifiers, stabilizers, fillings.
Other use:	paper, filters, carriers, bandage materials

Gums and mucilages

Gums and mucilages are polysaccharides of higher plant origin which form colloidal solutions or gels with water. The term "**gum**" was originally coined for **polysaccharidic exudates** secreted by plants after injury (e.g. gum Arabic), however it is used also for some products which come from polysaccharidic endosperms of plant seeds which in fact should be called **mucilages**. Most gums dissolve in water to form viscous solutions, some are insoluble and form gels.

Gum Arabic
Acacia senegalensis. (Mimosaceae), known as *verek* (in western Africa) or *hashab* (in Sudan) is a small tree with curved thorns. The gum is obtained from long transverse, ribbon-like, incisions made without injury of the cambium. The gum (1-2kg/tree/year) is collected and sorted to differential commercial categories.
Gum arabic consist of yellowish-white or pale amber mass, it is glassy and transparent. The gum is odorless, tasteless and adheres to the tongue. In the excess of water (2 or more) it forms viscous, adhesive, weakly acidic colloidal solutions. Optically active (levorotatory).
It may contain tannins (especially colored gums) but no starch.
Basic structure: 1→3 galactan substituted by arabinose units and by complex oligosaccharides comprising D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid.
Use: Stabilizer and emulsifier (food and pharmaceutical industry), apertures, glue (textile and paper industry). Authorized food additive (E_{414}).

Tragacanth gum
Astragalus gummifer (Fabaceae) is a bushy and thorny subshrub (0,5-1 m) indigenous in the mountain deserts of western Asia (Iran, Kurdistan, Armenia, Syria, Iraq, Afghanistan). *A. gummifer* has pinnately compound leaves and pale yellow flowers. The fruit is a small 1-seeded pod.
Tragacanth gum is collected from deep incisions made on the basis of the main stem.
Tragacanth gum is odorless and tasteless. It contains certain amount of round shaped starch granules (positive staining with Lugol solution). The raw gum may be separated to two fractions: (1) tragacanthin, a neutral arabinogalactan (1→3, 1→6) soluble in water and water-alcohol mixtures, and (2) bassorin, a partially methylated glycanogalactouronan.
Use: Bulk laxative, stabilizer of suspensions and emulsions. Authorized food additive (E_{413}).

Carob (carob gum, locust gum)
Ceratonia siliqua (Cesalpiniaceae) is a large evergreen tree of the Mediterranean rim. It has compound pinnate leaves and small reddish flowers. The fruit is thick and though pod containing 12-16 seeds separated by pulpous septa. The constant weight of the seed lead to its ancient use as a weight standard (1 carat = 200 mg).
The albumen of the seeds is called “carob”, “carob gum” or “locust gum”.
Carob is composed of an almost pure D-galacto-D-mannan. The backbone of the polymer is made of 1→4 linked β-D-mannose with lateral branches of only one α-(1→6) linked α-D-galactose units.
Carob is partially soluble in cold water, well soluble in hot water (80 C). It acts synergistically with carrageenans to form elastic gels.
Use: Symptomatic treatment of diarrheas, low caloric diets (food thickener), food technology (milk and bakery product stabilizer, EU code: E_{410}). Pharmaceutical and cosmetic industry.

Guar
Cyamopsis tetragonolobus (Fabaceae) is an annual herb cultivated in India and Pakistan, in the United States and in Central America. The endosperm of the seeds is a D-galacto-D-mannan. Guar is a creamy white powder, insoluble in organic solvents, gives viscous water solutions.
Use: Reduction diets, appetite suppressant. Emulsifier and gelifier (E_{412}).

Konjac
Amorphophallus conjac (Araceae) is originnally from Asia. It is characterized with a voluminous tuber, a large leaf and a solitary foul-smelling flower.

The tuber is rich in 1→4 glucomannan.
The konjac flour or conjac glucomannan are used in food preparation (filling in low caloric diets (bakery), jellies, capsules.

Psyllium (**Ispaghula**)
Psyllium ovata (*Plantago ovata*, *Plantago ispaghula*), Plantaginaceae, is an annual herb with lanceolate linear leaves. The flowers are white and grouped into cylindrical spikes.
The drug consists of the seed or the seed tegument.
The seed contains proteins, lipids, sterols, triterpenes and about 30% of mucilage. The swelling index of the seed is higher than 9 (or higher than 40 for tegument).
The polymer backbone is a xylan with 1→3 and 1→4 linkages. The monosaccharides in this main chain are substituted on C-2 or C-3 by L-arabinose, D-xylose, and a-D-galacturonyl-(1→2)-L-rhamnose.
Use: reduction diets, bulk laxative.

Flax
Flax is an annual herb with simple leaves that are alternate and lanceolate and the pentamerous blue flowers. The fruit is a capsule with 1-seeded locules. "Fiber" flax varieties have been cultivated in Europe since ancient times. The "seed" varieties are shorter and have higher yields of seeds.
Flaxseed (or linseed) is elongated, ovoid, flattened and rounded at the end (4-6x2-3.1,5-2 mm) its tegument is dark brown, smooth and shiny. The external walls of the epidermis are mucilaginous.
Linseed contains oil (35-45%), proteins (20-25%) and mucilage (6-10%); of note is the occurrence of cyanogenic glycosides (e.g. linustatin) and of lignans (secolariciresinol).
The mucilage can be fractionated into a neutral fraction – an arabinoglycan composed of D-xylose, L-arabinose, D-glucose and D-galactose – and an acidic fraction composed mainly of L-rhamnose and D-galactose.
The swelling index of linseed is over 4-5.
Linseed is used as a bulk laxative.
Another application of linseed is its use in bakery for decoration of dark type breads.

Pectins
Pectins are polymers built of 1→4 linked α-galacturonic residues associated with arabinan and galactan units. They are polyanions with gelating and metal ion-chelating activity.
Pectins are chiefly localized in the middle lamella of the vegetable cell wall. They are particularly abundant in unripe fruits. Pectins from individual sources differ in structure and in the degree of esterification of galacturonic units by methanol.
Main sources of pectins for industrial production are citrus waste and apple pulps.
Hydrolysis of highly methylated pectins found in some fruits represents the source of methanol contamination of alcoholic beverages prepared from these sources.
Use: Gelifiers, stabilizers (E_{440a} – pectins, E_{440b}- amidated pectins) in jams, candies, frozen desserts and sauces.

Polysaccharides from seaweeds, algae and microorganisms

Agar

Agar is a complex polygalactan obtained from several species belonging to the genera *Gelidium*, *Gelidella* and *Pterocladia*. It may be fractionated to agarose and agaropectin.
Agarose is a barely sulfated linear polymer of D-galactose of the $(AB)_n$ type with alternate 1→3 and 1→4 bonds, where the A units are partly methylated D-galactoses and the B units are L enantiomers of galactose, often of the 3,6-anhydro-L-galactose type.
Agaropectin is more heterogeneous, with highly sulfated and/or branched molecules.

Agar is colorless to pale yellow. Soluble in hot water forms gels after cooling to 30-40°C, which liquefy only after heating above 70-80°C.
Use: Over a century, agar is used as a superior carrier of solid cultivating media in microbiology. Agarose gels are used as carriers for electrophoretic separation of biomacromolecules (e.g. DNA sequencing) and for immunochemical precipitation methods. Agar is an authorized food additive (E_{406}), usable e.g. in confectionery.

Alginates

Alginic acid is a copolymer composed of poly-mannuronic and poly guluronic blocks. It is obtained from several genera of the pelagic or benthic algae belonging to the Pheophycae.
Alginic acid is insoluble in water, but its salts with monovalent cations (Na^+, K^+) are water soluble giving viscous colloidal solutions. With divalent cations (e.g. Ca^{2+}) they tend to form supramolecular structures – elastic gels with relatively large volumes of the pores.
Use: Alginates are used in gastroenterology as antacids (together with sodium bicarbonate), in obesitology (dietary additives). They are used also preparation of hemostatic wools, gauzes or powders.
Food additives: E_{400} (alginic acid), E_{401}, E_{402}, E_{403}, E_{404} (sodium, potassium, ammonium and calcium salts, respectively) and E_{405} (propylene glycol alginate).

Carrageenans

Carragenans are polysaccharides obtained from different species of the Rhodophycae (e.g. the Irish moss, *Chondrus crispus*, Giartiniaceae).
Carrageenans are polymers of D-galactose, highly sulfated, m.w. from 10^5 to 10^6. All carrageenans have linear structure of the $(AB)_n$ type with altering 1→3 and 1→4 bonds, where A and B are galactopyranosyl residues. According to the substitution pattern, seven types of carrageenans are distinguished (τ,κ,λ,μ,ν,θ,ξ).
Use: Reduction diets, bulk laxatives. Gelifiers for the production of creams, emulsions and pastes (pharmaceutic industry). Stabilization of milk products (creams, ice-creams). Carriers in the hygienic and cosmetic formulations (gels, emulsions, lotions, ointments etc.).

Dextrans

Dextrans are glucose polymers made of a-D-glucopyranosyl units linked 1→6. Natural dextrans are more or less branched, of high molecular weight (up to $50x10^6$). They are synthesized by extracellular enzymes of various bacteria of the genera *Leuconostoc*, *Lactobacillus* and *Streptococcus*. Pharmaceutically important are the dextranes produced by specific strains of *Leuconostoc mesenteroides*. After a partial cleavage they are fractionated.
Use: The fraction with m.w. around 60 kDa has been used as a substitute of blood plasma in emergency cases of serious bleeding. Cross-linked high molecular dextrans have been used as chromatographic and electrophoretic carriers (e.g. Sephadex).

5.2.

Specifically active proteins

5.2.1.

Enzymes

Carica papaya (Caricaceae) and several other *Carica* species.
C. papaya is a perennial tropical herb up to 10 m of height with a habit of a palm. The papaya fruits can reach from several hundred grams to 5 kilograms of weight. Ripe papayas are yellow, with orange-yellow flesh and black seeds in the central part. Unripe papaya secretes a rapidly coagulating white latex. When dried in temperatures below 50°C it gives white or brown amorphous material. Papaya latex is rich in proteolytic enzymes - papain, chymopapain and papaya proteinase Ω. The enzymes may be isolated by conventional protein separation techniques (precipitations, dissolutions and chromatography techniques).
Papain is a protein of 212 aminoacids, m.w. around 23,000 daltons. It is a cysteine endopeptidase, relatively thermostable, with optimal pH between 5-7. Papain is activated by thiols and reducing moieties (cysteine, dithiothreitol, glutathione) and inactivated by metal ions and oxidants. Chymopapain is a protein of 218 amino acids; its structure and properties resemble those of papain.
Use: Purified chymopapain can be injected into the place of an intervertebral disc prolapse to cleave the proteoglycans and thus initiate its resorption. Mixtures of papain, chymopapain, bromelain and trypsin are promoted as tools improving local availability of antibiotics and other therapeutics in low accessible sites of the body (e.g. in dentistry for treatment if gingival infections). Papain is promoted as therapy of digestive disorders. Crude papain/chymopapain preparations are used in marinades (together with spices) to achieve a partial cleavage of long protein chains and to improve palatability and digestibility of meat.

Pineapple
Ananas comosus (Bromeliaceae)
Pineapple is a herbaceous plant native to Central America, widely cultivated in all of the tropic regions of the world.
Pineapple is rich in soluble mono- and disaccharides, in organic acids and in vitamins. Its yellow color is due to carotenoids. The ripe fruit a stem contain a mixture of proteolytic enzymes called bromelain. Bromelains are glycoproteins, m.w. 18-28 thousand Daltons, with similar properties as papain and chymopapain.

5.2.2.

Lectins

Latin *lego, legere, legi, lectum* means to read, to choose, to select. Lectins are proteins able to bind saccharide residues on cell membranes, in a specific and reversible fashion, without displaying enzymatic activity. This binding is the principle of numerous cell-cell interactions in multicellular organisms as well as the recognition of target cells by certain pathogens and parasites. Certain amounts of lectins are thus present in each multicellular organism and in each of its tissues.
Numerous plants produce extra amounts of lectins to be stored in specific organs, mostly in seeds. They usually form during ripening and disappear during germination. Many lectins are

able to agglutinate red blood cells – they are referred to as phytohemagglutinins. Some lectins are able to cause hemolysis, some are mitogenic and some are highly toxic when applied parenterally. While lectins are usually inactivated by heating, some of them resist in the conditions of gastrointestinal tract. Therefore they may cause the toxicity of some raw seeds (e.g. green beans) which are perfectly edible when cooked.

Several lectis are used as model mitogens in immunology and cell biology (e.g. the Pokeweed mitogen from *Phytolacca americana,* Phytohemagglutinin from *Phaseolus vulgaris*, and Concanavalin A from *Canavalia ensiformis*). Some others are used for cell typing (e.g. lectins from *Dolicho bifloris* and *Ulex europeus*).

Lectin targeted toxins

A specific example of toxic proteins is lectin-targeted RNAse toxins from castor beans and jequirity beans – i.e. ricin and abrins, respectively. These intracellularly active toxins are composed of two subunits. The A subunit is a galactosyl specific lectin, the B subunit is an rRNA specific RNAse. Binding of the lectin subunit to a galactosyl residue on the cell membrane provokes a sequence of intracellular events resulting in internalization of the toxin. Inside the cell, the RNAse subunit destroys 28S subunits of ribosomes by cleaving the ribosomal RNA, which results in turning off the proteosynthesis.
Ricin as well as abrins belong to the most toxic proteins when applied parenterally, however, they are toxic also per orally. The lethal doses found in rats after intravenous applications were 0.4 μg/kg body weight for ricin and 0.1-0.3 μg/kg b.w. for abrins. The intoxications result in nausea, headaches, bloody diarrhea, ECG modifications, necrosis of liver and in damage to almost all organs (as found in autopsy).

Castor
Ricinus communis, Euphorbiaceae.
From ancient times, castor seeds are a source of valuable oil used in many technological applications (e.g. lamp oil for lighting, lubricant, more recently also biodiesel). Due to the content of irritating/toxic diterpenes it cannot be used for food production, however it is well known as a drastic laxative. Spent grounds (the cake) after pressing the oil from seeds are rich in proteins, and amongst them toxic ricin is relatively abundant. Ricin can be inactivated by heating, and so the spent grounds may be fed to certain animals after proper treatment.
Ricin was studied as a model compound for specifically targeted antitumor agents (lectin subunit should be replaced by a ¨tumor-specific antibody" in this approach).
There are also records about the abuse of ricin by criminals, terrorists and secret services (e.g. the case of Bulgarian dissident Georgi Markov, assassinated by a ricin intoxicated bullet).

Jequirity
Abrus precatorius, Fabaceae.
Also known as Indian licorice, this legume is known for its decorative red and black beans. The seeds have long been used to make necklaces and bracelets – from this they are also called rosary peas.
Jequirity peas contain four toxic proteins of the lectin-RNAse type (abrins A-D) and several toxic lectins. They have been used in biomedical studies.

The leaves of "Indian licorice" are non-toxic and are sweet due to the content of five sweet terpenoid glycosides (abrussoside A-E).

Fig.: *Abrus precatorius* – Jequirity bean.

5.2.3.

Protein sweeteners

Proteins are important nutrients. However, their taste usually is not much intensive. Sensoric quality of protein-rich foods is related rather to the interactions with the gustatory receptors of smaller peptides and even single amino acids. Namely the *umami* receptor appears to be important for perception of proteins, for which glutamate is a typical ligand.

A small number of proteins display strong interactions with the receptors of sweet taste. Some of them are used in food and pharmaceutical industry as non-caloric sweeteners and taste modifiers, other are interesting from theoretical point of view as tools for study of taste perception.

Thaumatin
Thaumatococcus danielli (Marantaceae) is abundant in Ghana, the Ivory Coast, Togo and Sierra Leone. Its fruits contain two almost identical proteins, both with 207 amino acids, named thaumatin I and thaumatin II, which can be isolated from the fruits as a mixture by water extraction and ultrafiltration. Thaumatin is soluble in water and in diluted alcohols, stable at pH from 2.7 and is not inactivated by short heat treatment (pasteurization). According to the test conditions, thaumatin is 3000-15000 times sweeter than sucrose.
Use: Non caloric sweetener. Approved food ingredient in EU (E_{957}), in the USA categorized as GRAS (**G**enerally **R**ecognized **A**s **S**afe). The gene for thaumatin II has been expressed in

several microorganisms and fungi (e.g. *Bacillus subtilis*, *Aspergillus niger*), nowadays also recombinant thaumatin is available and approved in the USA.

Monellin
Monellin is obtained from fruits of *Dioscoreophyllum cuminsii*, Menispermaceae, a tropical forest vine indigenous in western Africa. English names: wild red berry, guinea potato or serendipity berry. Preparation: Aqueous extraction of frozen fruits, membrane filtration of the extract.
Monellin is soluble in water and in diluted alcohols, unstable at low pH and thermally unstable. Lysine residues play role in the sweet taste. Limited applications.

Brazzein
Obtained from fruits of *Pentadiplandra brazzeana* (Capparaceae), brazzein is the smallest sweet protein with 54 aminoacids and four disulphidic bridges. It is 1200 times sweeter than sucrose, thermostable .and pH-stable. Brazzein gene has been expressed in several recombinant organisms including maize. Mutants of brazzein with increased or decreased sweetness have been also prepared.

Mabinlin
Four highly homologous sweet proteins have been identified in the seeds of a South-Asian tree *Capparis masaikai* used as traditional sweetener in folk cuisine. The most abundant of them, mabinlin II is composed of two subunits (33 and 72 amino acids) linked with two disulphidic bridges. Another two bridges stabilize the longer B-chain. Mabinlin II is exceptionally thermostable – keeping its properties even after 48 hours boiling. Mabinlins III and IV are relatively thermostable as well.

Miraculin
is a 473 amino-acids glycoprotein from fruits of *Synsepalum dulcificum* (alternative name *Richardella dulcifica*), Sapotaceae, a shrub indigenous in western Africa. Miraculin is virtually tasteless on its own, but it transforms acidic taste into a sweet taste and modifies perception of numerous flavors. The ability to modify taste persists for up to two hours. Preparation: Aqueous extraction of frozen fruits, membrane filtration of the extract.
Use: Recently miraculin started to be popular as an alternative sweetener in some countries, the miracle berries being imported e.g. to Japan. Transgenic lettuce and tomatoes expressing the miraculin gene have been also reported.

Curculin
from malaysian plant *Curculigo latifolia* (Liliaceae) is composed of two identical 114 AA polypeptides linked with two disulphidic bridges. On its own, curculin is 500 times sweeter than sucrose, and moreover it turns sour taste to sweet. Neoculin is a curculin homologue, composed of one curculin subunit and of a peptide sharing 77% AA identity with it.

Lysozyme
Lysozymes are hydrolytic enzymes (EC 3.2.1.17) playing important roles in non-specific defense against bacteria. The taste of some lysozymes is perceived as sweet (e.g. lysozymes from hen, goose, turkey and turtle eggs), some other are tasteless (e.g. human lysozyme).

Table 2. Structural features of sweet proteins: SubU: subunits; AA: amino acids; SS: disulfide bridges, Sweetness: compared to sucrose (w/w).

Protein	SubU.	AA	m.w. (kDa)	SS	Sweetness	note.
Thaumatin I	1	207	22,2	8	3000–15 000	E_{957}
Thaumatin II	1	207	22,2	8		
Monellin	2	44 + 50	10,7	0	3000	
Brazzein	1	54	6,5	4	1200	
Mabinlin I	2	32 + 72	12,3	4		
Mabinlin II	2	33 + 72	12,4	4	1000	
Mabinlin III	2	32 + 72	12,3	4		
Mabinlin IV	2	28 + 72	12,1	4		
Curculin	2	114 + 114	23	4	500	
Neoculin	2	114 + 113	23-24	4		
Miraculin	4	191	24,6		tasteless	
Lysozym (hen)	1	211	14,4	4		

6.
Products of secondary metabolism

6.1.
Alkaloids

The term alkaloid was introduced by Carl Fridrich Wilhelm Meissner at the beginning of 19th century to designate natural compounds reacting like bases (from the Arabic *al kaly* = soda and from the Greek *eidos* = appearance). There is no simple and precise definition of alkaloids and it is sometimes difficult to distinguish between alkaloids and other nitrogen containing compounds. When we use the term **alkaloid**, we **always** expect **the presence of nitrogen in the molecule** and a significant **pharmacological activity**.

True alkaloids are biosynthetically derived from amino-acids and nitrogen is a part of a heterocyclic system.

Protoalkaloids are biosynthetically derived from amino-acids but their nitrogen is not engaged a heterocycle. They are amines or amides. Some of them are intermediates on the metabolic pathways leading to the synthesis of true alkaloids in the particular plant species.

Pseudoalkaloids are products of some other branches of metabolism. While the carbon skeleton is synthesized in the particular biosynthetic pathway (most often they are terpenoids), nitrogen atom is built-in into the structure during late steps of the biosynthesis. Amino acids serve only as nitrogen donors.

Purine alkaloids are biosynthetically derived from purine bases.

Occurrence, distribution and localization of alkaloids

Alkaloids occur exceptionally in bacteria (e.g. pyocyanine from *Pseudomonas aeruginosa*) and are not frequent in fungi (e.g. ergolines from *Claviceps purpurea*, psilocin and other bioactive tryptamines from the Strophariaceae and Hymenogastraceae families, e.g. the *Psilocybe* sp.). Their occurrence is rather limited also in lower plants and Gymnosperms. The most frequent sources of alkaloids are Angiosperms – it is estimated that that 10-15% of them synthesize these products. Certain families have a marked tendency to elaborate alkaloids – e.g. Amaryllidaceae and Liliaceae in Monocotyledons and Annonaceae, Apocynaceae, Fumaricaceae, Lauraceae, Magnoliaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae, Solanaceae in Dicotyledons.

Majority of alkaloids occur only in a limited number of taxonomically related species (e.g. the occurrence of morphinans is characteristic for the genus *Papaver*, morphine has been found solely in *Papaver somniferum*), some are found in quite distant taxa (e.g. caffeine). Alkaloid concentrations in plants have a wide range of variation – from a few ppm (e.g. cytostatic alkaloids vinblastine and vincristine in *Cataranthus roseus*) to more than ten

percent of dry weight (e.g. morphinanes in opium). Also the distribution of a particular alkaloid in individual anatomical parts may differ by several orders of magnitude (e.g. morphinanes are almost absent in poppy seeds, chinin is found in bark but not in leaves of *Cinchona* sp., all parts of potato plant *Solanum tuberosum* except the tubers contain toxic levels of steroidal pseudoalkaloids). Different alkaloids may be present in different anatomical parts of the same plant, but in such a case they usually are of the same metabolic origin.

Physico-chemical properties of alkaloids

Alkaloids are low molecular compounds, with molecular weight ranging approximately from 100 to 1000 Daltons. Many of them are relatively stable in wide range of pH and temperatures (note that one way of application of alkaloid containing drugs is smoking).

Oxygen-containing alkaloids are usually colorless (except the protoberberine alkaloids), optically active, crystalizable solids. Pure compounds have sharp melting points, without decomposition. Oxygen-free alkaloids use to be liquids at room temperature, some of them are relatively volatile and may extracted from plant material by steam distillation.

Alkaloids are soluble in polar organic solvents (e.g. halogenated hydrocarbons, ethyl acetate, alcohols) and in acidified water. Their solubility is rather limited in non-polar organic solvents (hexane, cyclohexane), water and in alkaline solutions.

The pH dependence of the solubility in water of alkaloids is exploited in general approaches to their isolation from natural sources. Their separation from other types of biomolecules is achieved by several partitions between water and organic phases in properly set pH conditions.

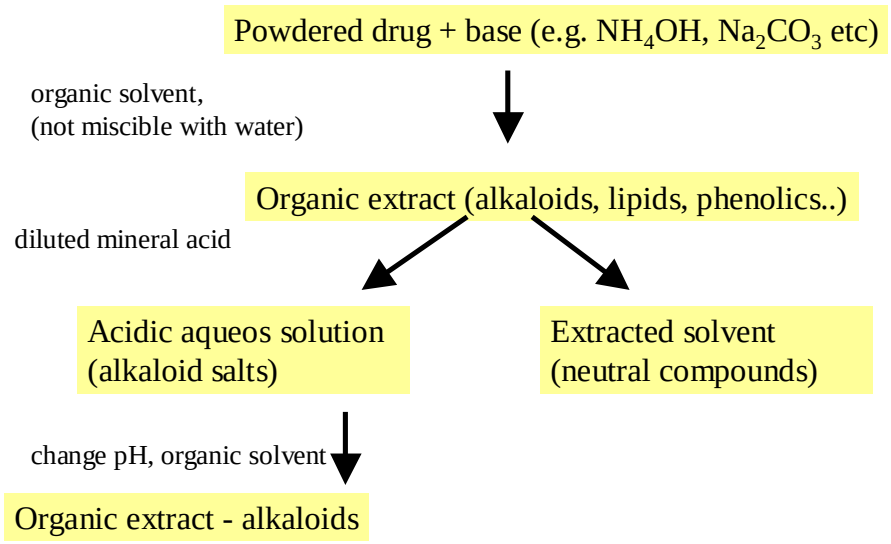


Fig. 3. A scheme of alkaloid extraction in an alkaline medium.

Pharmacological activities of alkaloids, their uses and abuses

Alkaloids are particularly important substances because of their multiple pharmacological activities which logically result in multiple aplications.
Among the alcaloids we can find:

- CNS depressants (e.g. morphine, scopolamine) and CNS stimulants (e.g. strychnin, caffeine)
- Sympathomimetics (e.g. Ephedrine)
- Sympatholytics (e.g. Yohimbine)
- Parasympathomimetics (e.g. Pilocarpine)
- Anticholinergics (e.g. Atropine)
- Local anesthetics (e.g. Cocaine)
- Antibacterials (e.g. Sanguinarine)
- Antimalarials (e.g. Quinine)
- Amoebicides (e.g. Emetine)
- Cytostatics (e.g. Vinblastine, Colchicine)

Or, from other point of view:

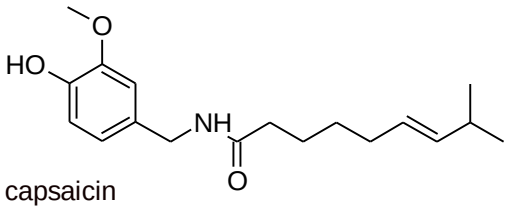
- Therapeutics
- Psychotropic illicit drugs
- Poisons – criminal, hunting, war and ordeal poisons
- Criminal abortives (e.g. acridone alkaloids from rue plant - *Ruta* spp.)
- Pesticides (e.g. Strychnine, Aconitine)
- Food contaminants (e.g. Solanine, ergot alkaloids)
- Culturally accepted addictions (e.g. Caffeine, Nicotine, Arecoline)

Important alkaloids and their natural sources

6.1.1.

Protoalkaloids

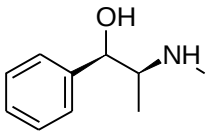
Capsaicin from *Capsicum* sp.
This simple amide and its analogues (capsaicinoids) are pungent (hot) taste principles of



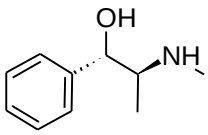
capsaicin

paprika and chilli peppers. Their activity is mediated via the activation of a Ca^{2+} ion channel type receptor called TRPV1 or vanilloid receptor.

Ephedrine and pseudoephedrine from *Ephedra* species.
Sympathomimetics, stimulating drugs, natural analogs of amphetamines.
Original source of ephedrine and pseudoephedrine is *Ephedra sinica* (Ephedraceae) and several other *Ephedra* species. Recently these alkaloids are obtained from a yeast based biotechnology.

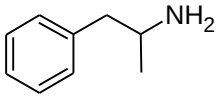


ephedrine

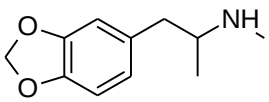


pseudoephedrine

Pseudoephedrine is widely used in combination with other active substances for symptomatic treatment of common cold, influenza etc. (e.g. Modafen, Nurofen, Paralen plus).
For their stimulating activity, ephedrine and pseudoephedrine have been on the doping list for a long time.



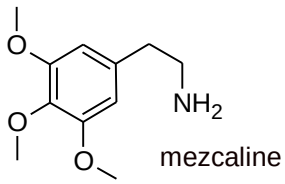
amphetamine



ecstasy

(synthetic, illicit addictive substances)

Hallucinogenic alkaloids from Peyote
Peyote – *Lophophora williamsi*, Cactaceae, is a globular cactus growing in in North Mexico and in Texas, reaching up to 20 cm in height, 5-10 cm in diameter. The aerial part contains about 50 alkaloids, phenethylamines and tetrahydroisoquinolines (up to 6% dry weight). The main active principle is mescaline, a potent hallucinogen. Peyote has been considered a divine plant by local tribes, being used for ritual purposes.



mezcaline

6.1.2

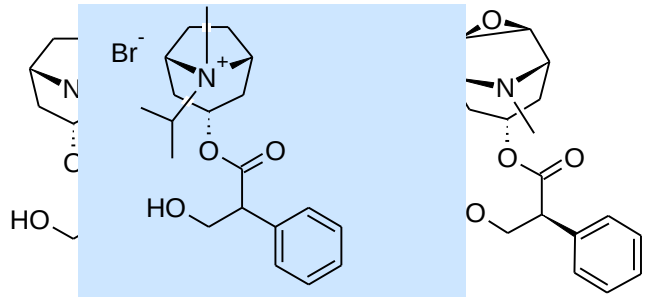
True alkaloids

6.1.2.1.

Tropane alkaloids

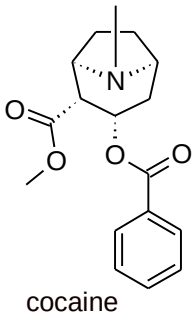
Tropane alkaloids are esters of tropanols, a group of heterocyclic nitrogen containing alcohols, with different organic acids. Biosynthesis of tropanols in the Solanaceae (e.g. *Atropa*, *Hyoscyamus* and *Datura* spp) originates from ornithine via putrescine, the same precursors as for biosynthesis of nicotinoids in *Nicotiana* sp. from this family.

Atropine is obtained from herbs of Deadly nightshade (*Atropa belladona*)**,** Black henbane (*Hyoscyamus niger*) and Thornapple (*Datura stramonium*). Acting as a reversible acetylcholine receptor blocator, atropine is efficient myorelaxant with numerous medicinal applications. E.g.: Anesthesiology (preanesthesia), symptomatic treatment of GIT, biliary tract, antispasmodic for ureteral colic, specific antidote to treat acetylcholinesterase poisoning (e.g. organophosphates), eye drops, treatment of Parkinson disease. Several decades ago, *Stramonium* containing cigarettes were used to achieve relaxation of bronchi in acute asthmatic attacks – later replaced with inhalation formulations of micronized atropine analogues (e.g. Atrovent).



atropin atrovent (Boehringer, Ing.) scopolamine

Cocaine and its analogues occur in *Erythroxylon coca* (Erythroxylaceae) and several other *Erythroxylon* sp. referred as coca. Coca grows naturally in Bolivia, Peru, Venezuela and Columbia. It is a shrub with ovoid leaves, small pentameric white flowers and small oval red fruits. Individual cultivars differ in the composition of essential

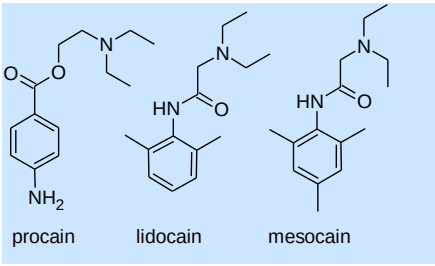


cocaine

oils and thus in sensoric evaluation by „*coqueros*“ (the coca users).

Coca leaves have been used for centuries in a traditional fashion with different dosage as a stimulant, an anesthetic, and to suppress hunger and thirst.

Isolation of pure cocaine opened the way to additional applications – unfortunately, the abuse soon prevailed.



In medicine, cocaine had been used as one of the first efficient local anesthetics. Later it was completely replaced by synthetic analogues with more advantageous properties, which are not active in CNS nor they are addictive (e.g. mesocain, procain, lidocain).
Recently, cocaine is just an illicit drug.

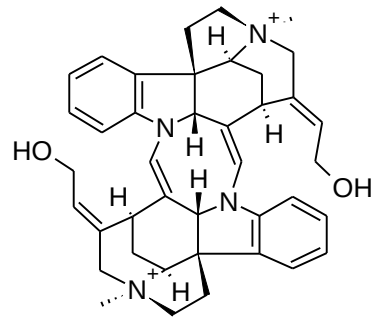
Symptoms of cocaine intoxication (abuse):
hyperthermia, mydriasis, vasoconstriction,
hypertension, tachycardia
effects on CNS: euphoria, hyperactivity, sensation of intellectual stimulation,
- psychic dependence develops rapidly (especially in intravenous users and smokers)

6.1.2.2.

Bisbenzyltetrahydroisoquinolines

This group of alkaloids is represented by over 400 known compounds found in about ten families (Menispermaceae, Ranunculaceae, Berberidaceae, Monimiaceae, Annonaceae, Lauraceae etc.) The most interesting of them are the efficient inhibitors of acetylcholine receptor. This group of compounds has been found the active principle of CURARE poisons.

CURARE is a term for a group of complex products, of variable botanic origin but with almost identical pharmacological properties. These poisons have been used for coating the tips of the blow darts or the arrows used for hunting by the natives of South America rain forest regions. Parenteral intoxication causes immediate muscle relaxation – the injured animal cannot flee, neither is able to stay attached to the tree. It falls down and is easily collected. The immediate cause of death after curare intoxication is suffocation from failure of breathing movements (relaxation of diaphragm). Peroral toxicity of curare is negligible – so the game may be safely consumed.



toxiferine I

In 1820, Charles Wallerton brought the evidence that the effect of curare is temporary – he succeed to save intoxicated animals by artificial ventilation. A century later, Frederic Prescott introduced the use of curare to anesthesiology to achieve relaxation of large muscles before surgery (1935). Currently, several curare-like compounds are being used in medicine, with different duration of the myorelaxing effect, e.g. tubocurarine or semisynthetic derivatives of toxiferine from *Strychnos toxifera*.

6.1.2.3.

Morphinans

Biosynthesis of morphinan alkaloids is typical for the *Papaver* genus (family Papaveraceae) – a large genus with more than 100 species. About 10 *Papaver* species produces thebain – a compound structurally close to morphine enough to serve as its precursor in a semisynthetic procedure. Only one species is known to produce morphine – the opium poppy.

Poppy

Poppy (*Papaver somniferum*) has been cultivated since ancient times in Europe, primarily for the production of its oily and nutritionally valuable seeds. The analgesic and sedative effects of opium and of poppy straw, has been also known for centuries. Morphine as a pure substance was crystalized from opium tincture by Friedrich Serturner already in 1806. Currently, the world leading producers of poppy for pharmaceutical industry are France, Turkey, India and Australia, the main producer of poppy seed for nutritional purposes is Czech Republic. In addition to these legal uses, large quantities of poppy are cultivated namely in Afghanistan, Pakistan and Burma to provide precursors for the market of illicit drugs.

Opium

Opium is a latex secreted after injury by unripe poppy capsules. Dry opium is white or brown amorphous material, it has a characteristic smell and bitter taste. Crystals, latex granules, call walls and starch grains may be found by microscopic examination. Opium is composed of saccharides (about 20%), organic acids (lactic, fumaric, meconic, oxaloacetic), water and up to 20% of alkaloids. Among alkaloids, morphine is the leading compound together with codeine (up to 10% and 2% of dry weight, respectively), accompanied by a dozen of other, structurally related compounds, e.g. thebaine, oripavine, codeinone and others.

Pharmacological activity

Morphinans influence the signaling pathways of endorphins, a class of peptidic hormones connected with regulation of reactions to stress and perception of pain.

The effects of **morphine**:

Analgesia. The effect vary depending on pre-existing pain. Morphine is efficient namely against a chronical (long lasting) pain.

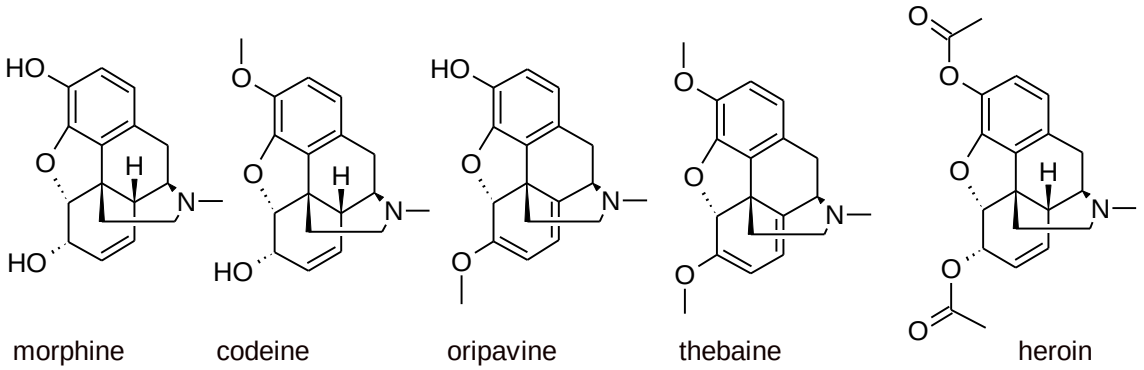
Respiratory effects. Morphine depresses the respiratory centers in the brain stem: the decrease of sensitivity of these centers to carbon dioxide and hypoxia causes bradypnea and rhythm irregularities with higher doses.

Depression of cough centers: Morphine is effective even in cases of cough not responding to other antitussives (e.g. emergency situations after massive chemical irritation of airways or embolia)

Decrease of pituitary function: Morphine decreases secretion of ACTH, LH and FSH, and by this mechanism influences also levels of steroid hormones from subsidiary endocrine glands, i.e. cortisol (a stress hormone) and sexual hormones.

Codeine: Codeine is a potent antitussive. Much less intensive is its analgesic activity. Its salts (e.g. phosphate) are used as antitussives in form of aerosols or in peroral formulations – often in combination with other active compounds (e.g. paracetamol).

Heroin: Heroin (diacetylmorphine) is a semisynthetic derivative of morphine. Originally intended for use as morphine replacement in disaccustoming treatment of addicts, finally it was find to be even more addictive. Currently heroin is not used in any medicinal application.

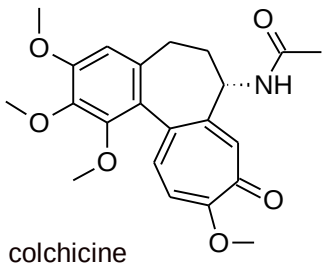


6.1.2.4.

Isoquinoline alkaloids

Autumn crocus *Colchicum autumnale* (Liliaceae)**.**
Autumn crocus, called also meadow saffron, is a herbaceous plant of wet meadows of Europe. It is characterized by an unusual life cycle. In October, purplish-pink flowers, single or in groups up to six of them, grow from underground bulbs. In springtime, leaves appear on the meadows together with the fruits – three-celled capsules, developing from the previous autumn flowers. The green parts cease and disappear at the beginning of summer.

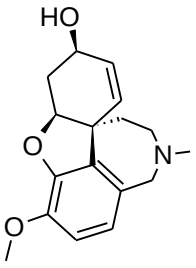
Autumn crocus was known already to ancient Greeks for its toxicity, since fifth century it was used to treat gout.
Colchicine, the main active principle of autumn crocus, was purified in 1884 by Laborde and Houdé and its structure was established by Dewar in 1945.



colchicine

Pharmacologic activity: The autumn crocus is highly toxic. The ingestion of all parts of the plant causes intoxication with swallowing difficulties, abdominal pains and diarrhea, muscle cramps, hypotension and respiratory difficulties. In serious cases, cardiovascular collapse or respiratory arrest may lead to death even several days after the intoxication.
Colchicine blocks mitosis at the metaphase stage by preventing the formation of mitotic spindle. This cytostatic activity is caused by binding of the alkaloid to tubulin. Colchicine was tested as a potential antitumor agent at seventies and eighties, but its high cellular toxicity avoided this use.

Galanthamine



galanthamine

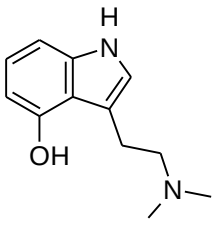
Galanthamine is a potent inhibitor of acetylcholine-esterase, and thus an antagonist of acetylcholine receptor blocking agents (e.g. curare, atropine). Pharmacologically favorable is that galanthamine crosses the blood-brain barrier and modulates the performance of cholinergic nerves in central nervous system. Several years ago it was approved for treatment of mild to moderate forms of Alzheimer disease.
Originally isolated from snowdrops (*Galanthus sp.,* Amaryllidaceae) and later found in several other genera of the same family (*Narcissus*, *Lycoris*), galanthamine is nowadays either isolated from *Narcissus* (daffodil) bulbs or prepared by chemical synthesis.

6.1.2.5.

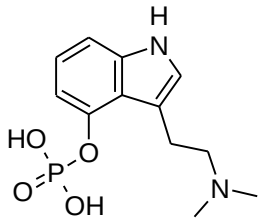
Alkaloids derived from tryptophan

Due to the presence of indol heterocycle, even the simplest tryptamines meet formal criteria for their categorization as alkaloids. More complex structures are derived from further cyclization and from different types of condensation of tryptamine.

Hallucinogenic tryptamines from mushrooms and other sources
Hallucinogenic mushrooms, belonging to the order Agaricales, Hymenogastraceae and Strophariaceae families, genera *Psilocybe*, *Conocybe*, *Panaeolus* and *Stropharia*, were used in some Central-American cultures from pre-Colombian era. Much more recently, an abuse of local *Psilocybe* species (namely of *P. semilanceolata*) appeared also in some social groups in European countries (including the Czech Republic, Austria, Poland, Finland, France and UK). About 10-20 carpophores are said to be enough for intoxication characterized by visual hallucinations with shape distortion, color intensification and changed perception of time and space. The active principles of these mushrooms are psilocin and psilocybin.



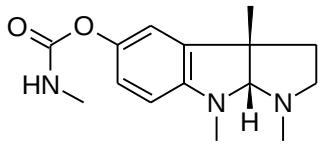
psilocine



psilocybin

Similar compounds and activities occur also in other fungi belonging to genera *Psilocybe, Conocybe, Gymnopilus, Inocybe, Panaelina, Panaeolus, Pholiotona, Pluteus* and *Stropharia*, in plants *Adenatera peregrina*, *Virola* spp. and even in a frog – toad *Bufo alvarius.*
Ayahuasca, psychoactive herbal preparation of South-American natives, includes extracts of tryptamine containing liana *Psychotria viridis* and monoamine oxidase (MAO) inhibitors (e.g. harmine and harmaline) containing *Banisteriopsis caapi*.

Calabar bean alkaloids
Physostigma venenosum (Fabaceae) is a climbing vine with trifoliate leaves and papilionaceous flowers. The fruit is a pod, the seed is a 2-3 cm long and 12-15 mm wide bean with brown tegument. The seeds contain 0.2-0.3% of alkaloids, chiefly represented by physostigmine (known also as eserine) accompanied by norphysostigmine, geneserine and several other substances. Physostigmine is relatively unstable and is oxidized when exposed to air and light.



physostigmine (eserine)

Pharmacological activity: Physostigmin is a reversible acetylcholine esterase inhibitor. Its affinity to the enzyme is four orders of magnitude higher than that of acetylcholine. It acts as a parasympathomimetic. Serious intoxications are characterized by myosis, sialorrhea, rhinorrhea, bradycardia, hypotension, bronchospasmus, nausea, vomiting, abdominal cramps and central effects. Physostigmin is a stronger antagonist of acetylcholine receptor blocking agents (e.g. curare, atropine) than galanthamine.

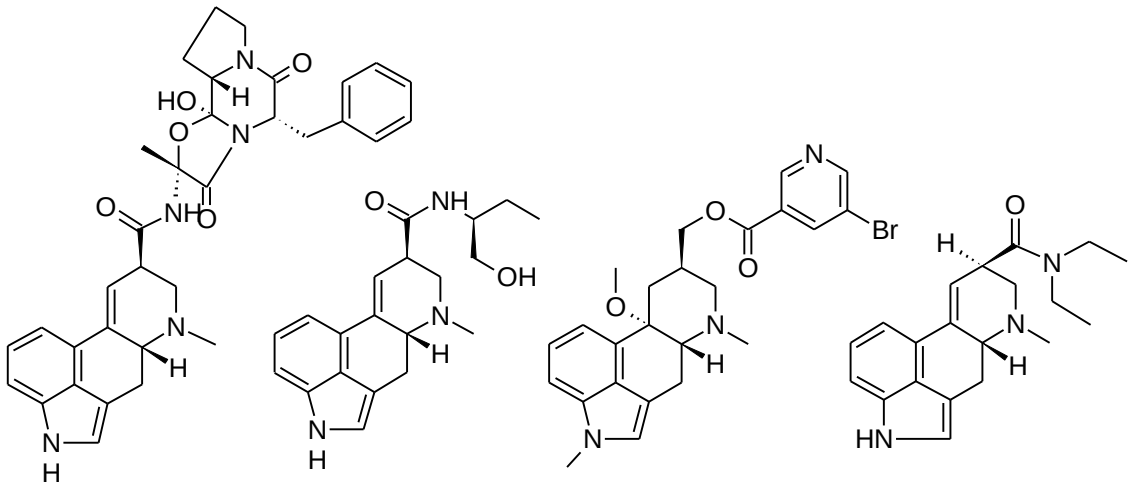
Ergoline alkaloids

Ergoline alkaloids are derived from a tetracyclic, indol containing, nucleus named ergoline. They are commonly classified to clavines (i.e. simple lysergic acid derivatives) and several groups of ergopeptines (i.e. compounds of lysergic acid with a proline containing oligopeptide). These alkaloids were initially characterized in the ergot of rye, *Claviceps purpurea*, and later found in other *Claviceps* species. Clavines are also synthesized by other fungi (genera *Aspergillus, Balansia, Penicillium, and Rhizopus*) and even in certain higher plants (e.g. the Convolvulaceae family genera *Argyreia, Ipomoea, Turbina* and *Stritocardia*).

Ergot of rye, *Claviceps purpurea*

Ergot of rye is a fungus parasiting on rye (*Secale cerale*) and triticale (a hybrid of rye and wheat, *Triticum aestivum*). It contains a rather complex mixture of alkaloids with different individual activities. Therefore also effects of a crude drug are highly variable, taking place namely in blood vessels and/or the central nervous system. Intoxications with infested rye has been recorded many times since the first description of „St. Anthony's Fire" in around 1000 A.D. The intoxication, now referred as "ergotism" has two main forms – the gangrenous form, characterized by painful inflammation of extremities (i.e. fingers etc.), sometimes ending with their spontaneous loss; and the convulsive form with mental agitation, delirium and sensory perturbations. The prevalence of ergotism decreased with the agricultural and social improvement up to its practical disappearance from Western and Central Europe during 19th Century.

For pharmaceutical purposes, ergot is cultivated either in field cultures using rye or triticale as feeding plants, or in fermentation cultures.



Pharmacological activities and uses

Ergot alkaloids interact with several classes of receptors for dopamine, noradrenalin and for serotonin. As these mediators regulate numerous physiological functions, including the function of blood vessels, immune system and nervous system, the effect of ergot alkaloids depend on a particular compound, its dosage and way of application.

Examples: Ergotamine is a potent vasoconstrictor. It is used to modulate the vascular tone, e.g. in treatment of migraines and related vascular headaches.

Methylergometrine maleate: Used in obstetric emergencies, post-partum, hemorrhage, after cesarean section, after abortion etc.

Dihydroergotamine: treatment of migraines, vascular headaches, the insufficiency of venous and lymphatic vessels, orthostatic hypotension.

Nicergoline and Cabergoline. Synthetic derivatives of lysergol. Used for treatment of the chronic arterial disease of lower limbs, treatment of senile cerebral insufficiency.

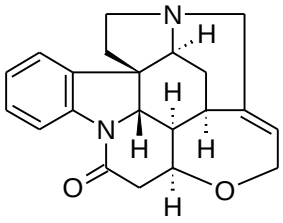
Bromocryptine. A synthetic derivative of lysergol. Used for basic treatment of Prolactin secreting adenomas, treatment of clinical consequences of hyperprolactinemia

Monoterpenoid indole alkaloids

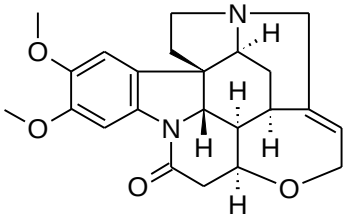
Nux vomica

Strychnos nux-vomica, Loganiaceae, is a South-Asian tree. The seed (nux vomica) contains 1-3% of alkaloids chiefly represented with strychnine and brucine.

Strychnine is a particularly toxic compound (lethal dose 0.2 mg/kg). The intoxication resembles tetanus; symptoms include increased sensitivity to light and noise, anxiety and convulsive attacks. Death occurs by suffocation following the spastic contraction of diaphragm. Nux vomica was introduced to Europe in 16th century as a pesticide. Brucine, a dimethoxylated strychnine derivative, is much less toxic and is intensively bitter. It had been used as a bitter taste standard, but it was been replaced by quinine and caffeine in this application.



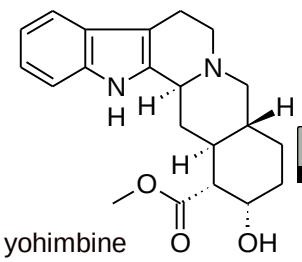
strychnine



brucine

Yohimbine

Found in bark of yohimbe (*Pausinistalia yohimbe*, Rubiaceae), a tree widespread in Cameroon, Gaboon and Congo, yohimbine is a selective inhibitor of the α-2-adrenergic receptors and is a sympatholytic. It has a biphasic effect to blood pressure, increasing it in low doses and acting as hypotensive in high doses. Before the introduction of sildenafil (i.e. Viagra), yohimbine was similarly reputable for its effect on the vasodilatation of the *corpora cavernosa*.



yohimbine

Madagascan periwinkle

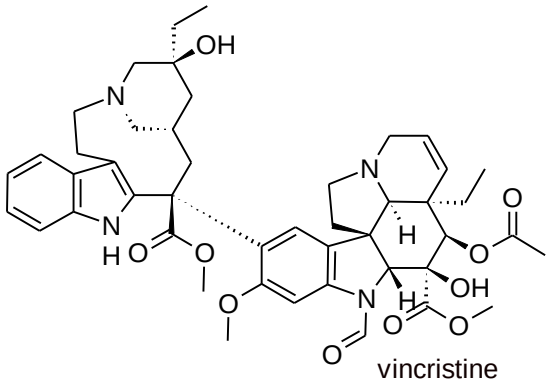
Cataranthus roseus, Apocynaceae, is a perennial subshrub with decorative flowers of pink or white flower. Madagascan periwinkle is widely planted as a decorative species and also to supply pharmaceutical industry.

The aerial parts contain from 0.2 to 1% of alkaloids. These form a complex mixture in which more than 95 constituents have been identified.

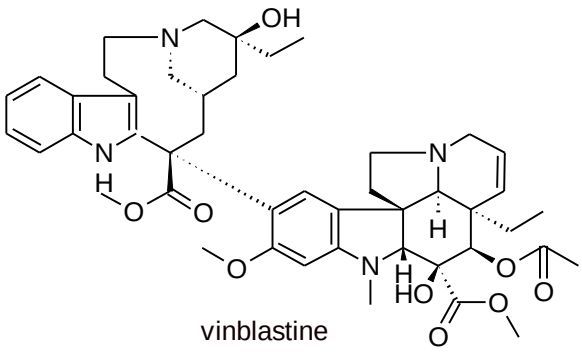
The compounds of pharmaceutical interest are the alkaloids formed by coupling of two units – an indole and a dihydroindole (referred as binary alkaloids).

Vincristine and vinblastine are highly efficient cytostatics approved for the therapy of several types of neoplasias. Their concentration in the drug is rather low (about 3 mg/kg for each), the efficacy of their production may be partly enhanced by isolation of structurally related compounds and their chemical conversion to one of the above mentioned substances.

Use: Vincristine sulphate – treatment of Hodgkin´s disease, non-Hodgkin´s lymphoma, breast cancer, uterine cancer, small cell bronchial cancer, various sarcomas.
Vinblastine sulphate – treatment of Hodgkin´s disease, non-Hodgkin´s lymphoma, testicular cancer, breast and ovary epithelioma, Kaposi´s sarcoma, choriocarcinomas.



vincristine



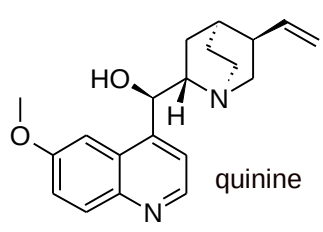
vinblastine

6.1.2.6.

Quinoline alkaloids

Cinchonas are a family of about forty species of a tree habitat. Cinchonas are indigenous in wet tropics of South America. Today they are planted in many other places worldwide with corresponding climatic conditions.

The drug is bark obtained from cultivated species namely *Cinchona officinalis*, *C. ledgeriana* and *C. succiruba*. The bark is rich in phenolics and contains up to 6% of alkaloids, mainly of a quinoline structure.



quinine

Quinine was the first efficient antimalarial drug (and practically the only one until the 40-ies of the 20-th Century). It is also a mild antipyretic and an analgesic. Quinine is used for treatment of flu-like states, aches and fevers, often in combination with vitamin C, caffeine, codeine, paracetamol and other active substances.

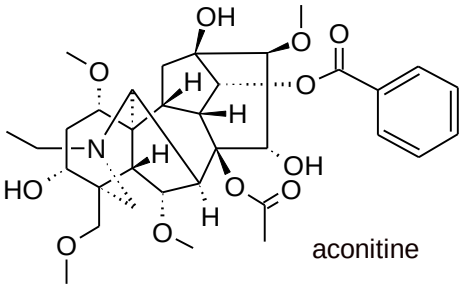
Quinine is used as an ingredient in bitter drinks (e.g. tonic waters), and also as a standard of bitter taste.

6.1.3.

Pseudoalkaloids

Aconite

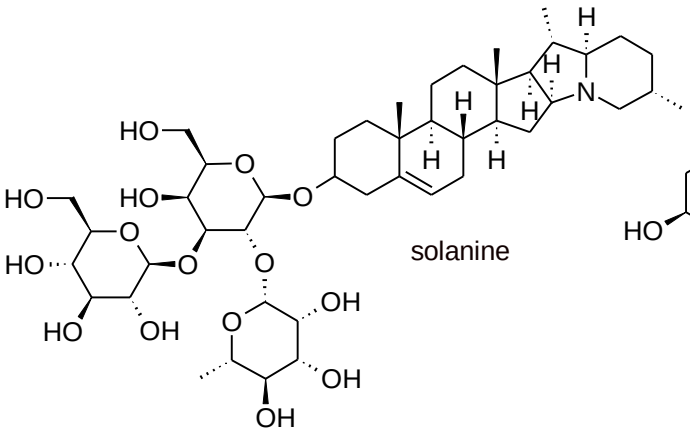
Aconitum napellus (Ranunculaceae) is a herb up to 1 m of height, with blue flowers and palmilobate leaves. Its tubers contain up to 1% of diterpenoid alkaloids with aconitane-type skeleton. Aconitine belongs to the most toxic alkaloids (LD 0.25 mg/kg.). The intoxication causes paralysis of the breathing center in the brain stem and cardiovascular collapse. During the history, aconite had been used as a pesticide (to control populations of big predators, i.e. bears, wolfs, foxes), a criminal and war poison, but also as an analgesic and antitussive.



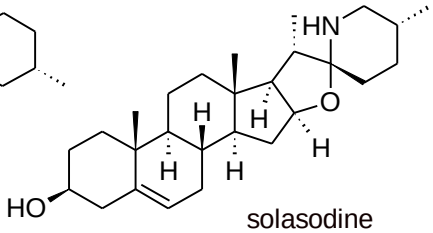
aconitine

Steroidal alkaloids

Nitrogen-containing steroids are synthesized by several families (e.g. Solanaceae, Buxaceae), acting as phytoalexins. The potato plant (*Solanum tuberosum*, Solanaceae) contains up to 500



solanine



solasodine

mg/kg of solanine, solasodine and several other steroidal alkaloids in all its parts except the tuber. After consumption of fruits or sprouting potatoes, intoxication may occur accompanied with gastrointestinal symptoms and tachycardia.

6.1.4.

Purine alkaloids

Purine alkaloids are derived from common purine bases. Their biosynthesis often consist in a limited number of simple steps (Fig 4). It is not much surprising that they occur in multiple non-related taxa. Caffeine is produced by *Camellia sinensis* (Theaceae), *Coffea arabica* and *C. canephora* (Rubiaceae), *Cola acumitata* and *Theobroma cacao* (Sterculiaceae), *Paulinia cupana* (Sapindaceae), *Illex paraguariensis* (Aquifoliaceae).

Pharmacological activity:

Caffeine acts on the CNS and on the cardiovascular system.

- CNS activity. A cortical stimulant, caffeine enhances alertness and decreases sensation of fatigue. High doses can induce nervousness, insomnia and tremors. It stimulates the respiratory center in the brain stem.
- CV activity. Caffeine causes tachycardia and increases cardiac output. It has also mild diuretic effect.

Theophylline is a non-specific bronchial smooth muscle relaxant, which counteracts the effects of various bronchoconstricting mediators. It stimulates the respiratory center in the brain stem.

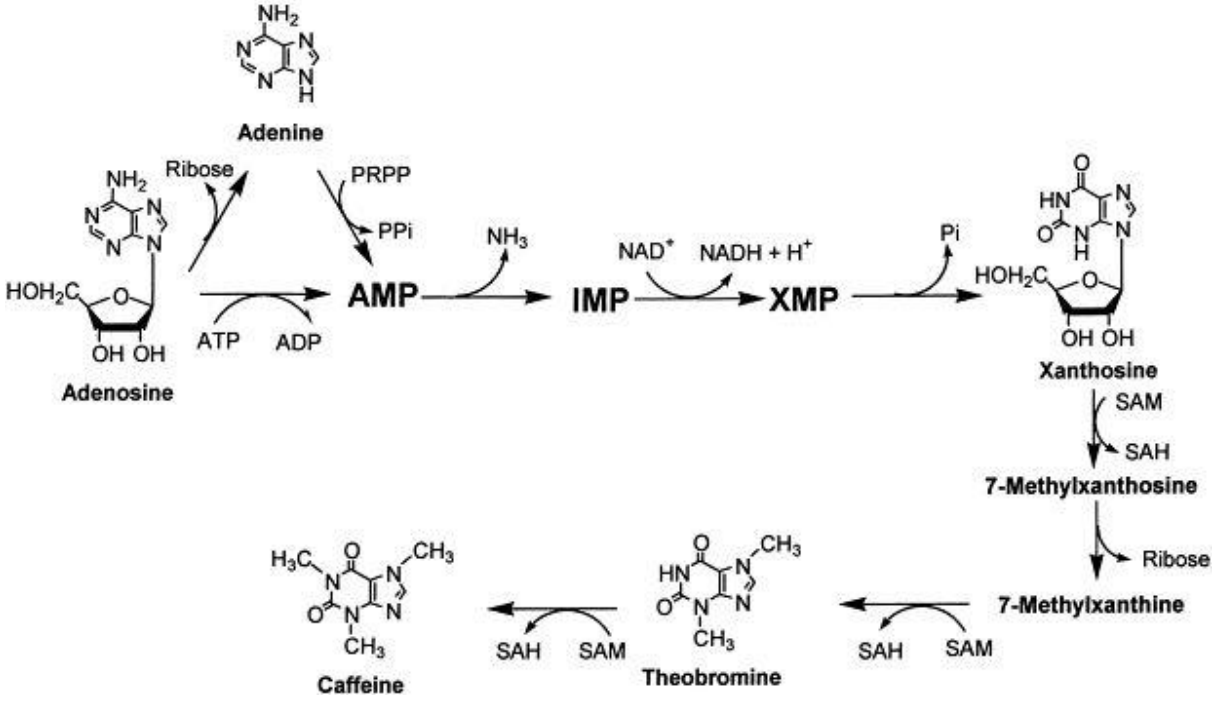


Fig. 4 Biosynthesis of caffeine.

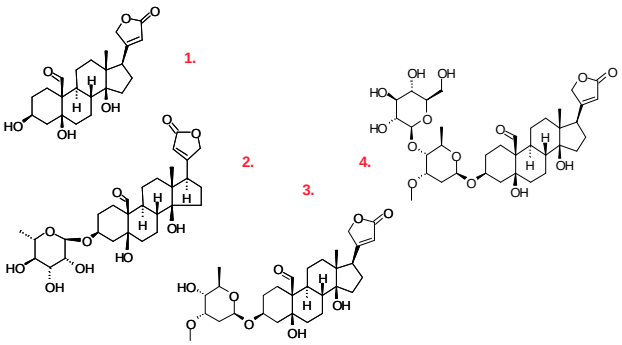
6.2.

Glycosides

Glycosylation is a way of derivatisation of compounds widely used by living organisms. It consist in the attachement of a saccharide through the glycosidic bond between the hemiacetal hydroxyl on the C1 and a hydroxyl, suphydryl or amino group on the particular aglycone. (Note, that saccharide hydroxyls at C2 - C6 may be esterified or etherified, but they do not form glycosidic bond)

Glycosides are composed of two distinct parts:
1. Saccharide part, called glycone
2.. Non-sugar part, called aglycone or genin

Different glycosides may be derived from the same aglycone – either due to the attachement of different sugars or due to the modification of different positions.
(example: glycosides of strophantidin – Fig.5)



1. Strophantidin
2. Strophantidin-α-L-rhamnoside
3. Strophantidin-D-cymaroside
4. Strophantidin-glucocymaroside

Fig.5 Glycosides derived from strophantidin

Biochemical origin of the glycosidic bond
Glycosidic bond is formed by the action of glycosyltransferases – the enzymes which use uridine diphosphate saccharides as the donors of glycone. Glycosyltransferases are highly specific with respect to the the glycone. (UDP-glucose is the substrate for glucosyltransferases, UDP-galactose for galactosyltransferases, UDP-rhamnose for rhamnosyltransferases etc.). However, they may be much less specific as regards the acceptor of the sugar moiety. Some glycosyltransferases are able to modify rather large groups of structurally related compounds. The most usual type of glycosides, so-called *O*-glycosides, are derived from hydroxylated aglycons (e.g. phenols, sterols etc.). *S*-glycosides are derived from thiols, *N*-glycosides are derivatives of amines. So called C-glycosides containing a carbon-carbon bond between the glycon and the aglycon are a less common type of glycosides and differ in their chemical properties.

Glycosidic bond in *O*-,*S*- and *N*- glycosides is energetically unfavourable and relatively unstable. It may be cleaved enzymatically, by corresponding glycosidases, or hydrolyzed in acidic conditions. Glycosidic bond is relatively stable in neutral and alcaline conditions.

When compared with parental aglycone, glycosides differ in many parameters.
The attachement of a polar (hydrophilic) sugar moiety leads to:

- increase of solubility in water
- decrease of solubility in organic solvents
- decrease of sensitivity to oxidation (sacharide as a protecting group)
- often also to decreased toxicity (masking the pharmacophore)

6.2.1.
Specific groups of biologically active glycosides

Cardiac glycosides

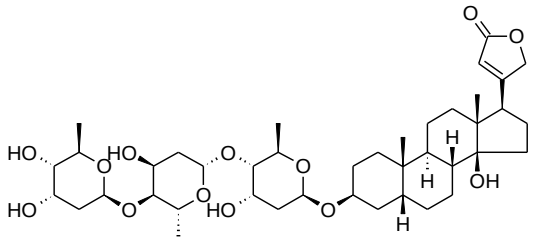
Cardiac glycosides are found in a limited number of genera belonging to about 15 families, e.g.: *Asclepiadeaceae, **Apocynaceae**, Brassicaceae, Celestraceae, Crassulaceae, Fabaceae, Iridaceae, **Liliaceae**, Moraceae, **Ranunculaceae**, **Scrophulariaceae**, Tiliaceae*
Aglycones of the bufadienolide type are produced also by some amphibians and stored in their skin, certain insect are able to accumulate cardiac glycosides from food.

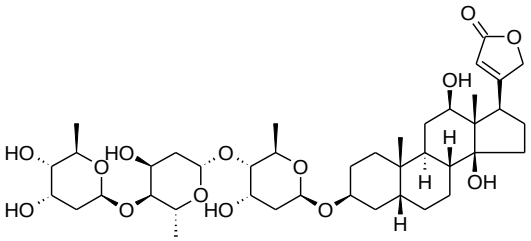
There are two main types of cardiac glycosides: cardenolides and bufadienolides. Their aglycones are steroids carrying a beta hydroxy-group on C3, glycosylated often by unusual sugars, and substituted at C17 with a lactone ring. The lactone is built of five atoms with one unsaturated bond for the **cardenolides** and of six atoms with two unsaturated bonds for the **bufadienolides**.

Principal activity of cardiac glycosides: Modulation of NaK ATPase

Cardiac glycosides act as inhibitors of Na^+K^+ ATPase - the enzyme responsible for maintaining the electrochemical gradient on the plasma membrane. Concentrations of sodium (main extracellular cation) and potassium (main intracellular cation) differ by two orders of magnitude on both sides of the plasma membrane. (Plasmatic Na^+ levels are about 135-150 mM, while that of K^+ are about 3.0-5.0 mM). This gradient is absolutely necessary for many vital functions of all living cells, including the excitability of neurons and muscle cells. Heart is the most sensitive organ, the increase of plasma potassium level above 7.0 mM leads to heart failure. When dosed properly, cardiac glycosides can stimulate the heart action – cardenolides obtained from digitalis and strophantus have been used as **cardiotonics** in official medicine, some other plants have been used for these purposes in folk medicine. Higher doses are toxic. Cardiac glycosides containing drugs have been used e.g. as pesticides, criminal poisons, war poisons.

Foxglove
Digitalis lanata and *Digitalis purpurea*, Scrophulariaceae.

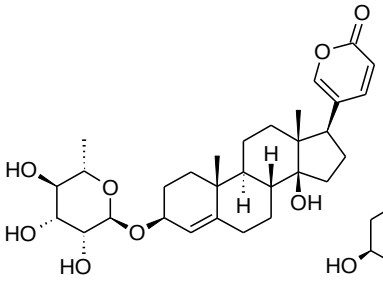

digitoxin

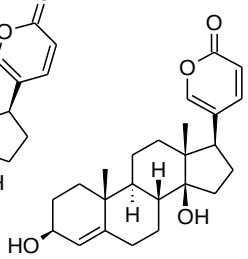

digoxin

Squill
Drimia maritima, Liliaceae

Voluminous bulbs up to 3 kg
contain bufadienolides (up to 4%, dry weight)

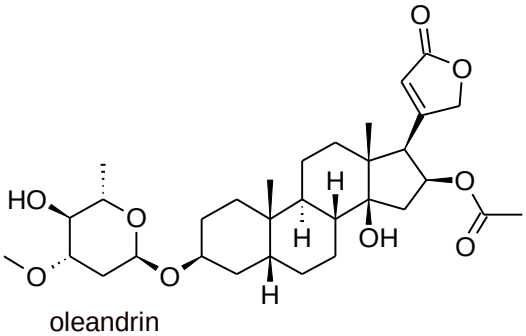
Squill extracts have been used as
- heart tonic
- rat poison
- expectorant


proscillaridin

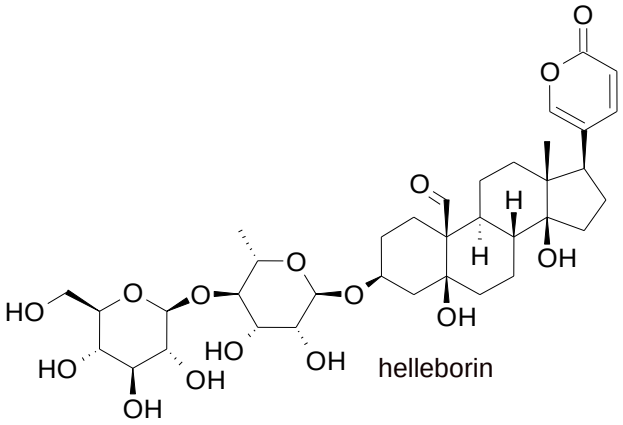

scillarenin

Oleander; Rose laurel
Nerium oleander, Apocynaceae

A shrub, indigenous in Mediterranean area, is often used as a decorative plant.
Leaves contain about 1.5% cardenolides, namely oleandrin and gitoxigenin.


oleandrin

Intoxication after the ingestion of leaves or seeds is accompanied with:
nausea, vomiting, bradycardia
hyperkalemia
ventricular fibrilation



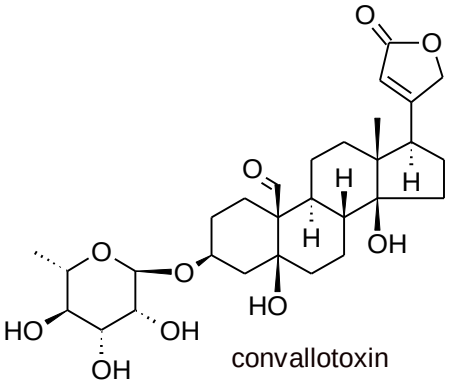
helleborin

Christmass rose
Helleborus spp., Ranunculaceae

Hellebores are often used as decorative plants. Intoxications occur relatively rarely (children, domestic animals)
Symptoms: Tingling in the throat, vomiting,
diarrhea, mydriasis.

Lilly of the valley
Convallaria majalis, Liliaceae.

Well known decorative plant, appreciated for its smell.
All parts contain convallotoxin – a cardenolide.
Intoxications occur occasionally, when children or pets taste lily of the valley bulbs or fruits.
Convallotoxin is poorly absorbed in the intestine
Intoxications usually are not serious – gastrointestinal symptoms. The use of *C. majalis* cardiotonic water extracts has been reported from folk medicine.



convallotoxin

Saponins

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. They are able to emulsify lipids. Most saponins have hemolytic properties and are toxic to fish and amphibians.
Saponins deserve attention because of their industrial applications – some of them are starting materials for the semisynthesis of steroidal drugs – and because of their pharmacological properties.

Structure of saponis
Structurally, saponins may be classified into two groups based on the nature of their aglycone.
Steroidal saponins are found mainly in monocotyledon angiosperms (Liliaceae: genera *Allium, Smilax, Asparagus;* Agavaceae: genera *Agave, Yucca;* Dioscoridaceae: *Dioscoridea*), some of them are found in dicots (Solanaceae, Fabaceae, Scrophulariaceae)

Triterpenoid saponins are found mainly in dicotyledons: Cucurbitaceae, Fabaceae, Primulaceae, Ranunculaceae, Rosaceae.

The aglycones of steroidal saponins possess a skeleton with 27 carbon atoms, which generally comprises six rings (two more in addition to the general steroid structure).
The aglycones of triterpenoid saponins are usually pentacyclic compounds categorized to the structural groups of oleananes, ursanes, lupanes and several others.

The glycon moiety is attached to the beta hydroxy group at C-3. The saccharides taking place in saponins formation are: D-glucose, D-galactose, L-arabinose, L-rhamnose, D-xylose, and D-glucuronic acid. The glycon usually consist of a linear or a branched oligosaccharide, a monosaccharide saponins (monosides) are less common.
Saponins glycosylated only on C-3 are monodesmosides.
Fairly often, the molecule includes a second sugar moiety, in addition to that on C-3, linked to the aglycone by an ester bond at the 28-position of the triterpenoid aglycone (or C-26 of steroidal aglycone): this is a bidesmoside.

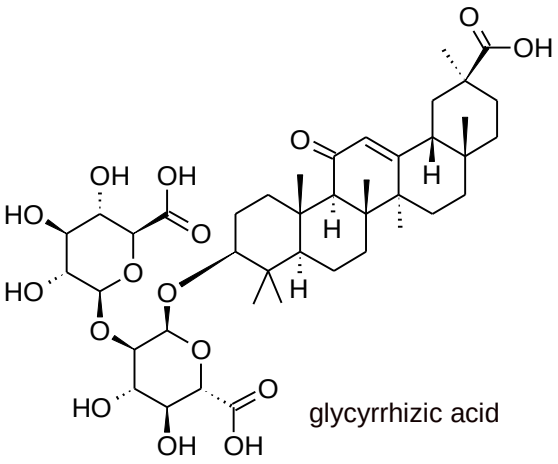
Extraction: Saponins are water soluble, therefore they can be extracted with water or with water-alcoholic media, generally at higher temperatures. Partition between water and butanol may be used for separation of saponins from other water-soluble compounds.

Pharmacological properties:
Saponins commonly have hemolytic properties that are attributed to the interaction with the components of plasma membrane (lipids, sterols). It is assumed that saponins ensure the defense of the plant against fungal and microbial attacks. The antifungal activity of saponins has been established also *in vitro*, toward phytopathogenic species as well as toward human pathogen *Candida albicans*. When taken orally by warmblooded species, saponins are most often only weakly toxic (i.e. they are non-toxic in low and moderate levels), probably because they are not absorbed much. Their effect is rather different when they are administered parenterally.
Many saponin-containing drugs are traditionally used for their antitussive and expectorant properties. Some saponins are known for their antiinflammatory and antiedema effects.

Licorice

Glycyrrhiza glabra **(Fabaceae)** is a perennial subshrub with erect and grooved stems (1-1.5 m), with alternate, compound, imparipinnate leaves. The inflorescences are erect racemes composed of lilac-colored flowers. The fruit is a small flattened pod. The drug is root of licorice.

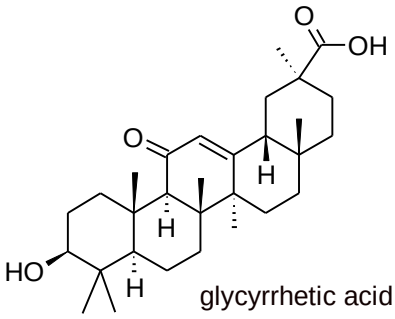
Licorice has been used in the Mediterranean area over two thousand years, and in China only a few centuries less. It was used against cough and to treat stomach ulcers. In addition to the use in traditional medicine, extract of licorice is widely used in many countries as a flavor and sweetener in pastry, candies, herb teas and other drinks including pastis, chewing gums, breath fresheners and even in chewing tobacco.



glycyrrhizic acid

Pharmacologically active substances: Along with 25-30% starch, 3-10% glucose and sucrose, coumarins, triterpenoids, sterols and other compounds, licorice contains flavonoids and saponins, to which the pharmacological activity is attributed.

Glycyrrhizic acid is an intensively sweet compound (2000 times more than sucrose). It is used as a non-caloric sweetener in certain countries. However, it is not approved as a sweetener in EU nor in the USA. Glycyrrhizic acid as well as its aglycone (i.e. glycyrrhetinic acid) are strong inhibitors of 11-hydroxysteroid dehydrogenase (11-HSD) – the enzyme responsible for the protection of mineralocorticoid sensitive tissues from mineralocorticoid activity of cortisol. Inhibition of 11-HSD leads to the **apparent mineralocorticoid excess** syndrome (AME), a misbalance in mineral metabolism resulting in sodium and water retention, losses of potassium, high blood pressure, alkalosis, risk of left ventricular hypertrophy and coronary artery disease. While the sensitivity to glycyrrhizic acid is relatively low in general population, serious clinical manifestations occur in predisposed individuals.

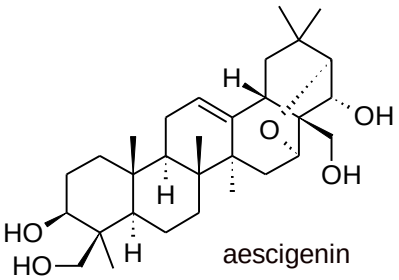


glycyrrhetic acid

Common chestnut (Horse chestnut)

Aesculus hippocastanum Hippocastanaceae

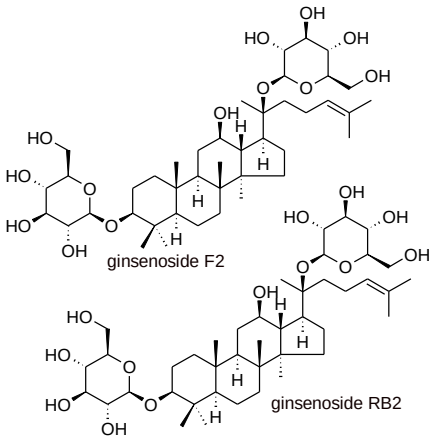
Drug: Chestnut seed contains 40-50% of starch and other sugars, 6-8% lipids, flavonol glycosides and triterpenoid saponins, called aescins, derived from the same aglycone. Aescins have anti-inflammatory, anti-edema, anti-exudative and veno-protective, being used in preparations for external application (lotions, gels, creams).



aescigenin

Primrose
Primula veris, Primulaceae
Drug: flos primulae,
radix primulae
Active substances: Flavonoids, primulic acid, saponins (2% - flower, 10% - root).
Bronchosecretolytic and expectorant. Primrose is mainly used in herbal tea mixtures intended for treatment of bronchial catarrh and mouthwashes.

Zhen-Shen
Panax ginseng Araliaceae
P. notoginseng

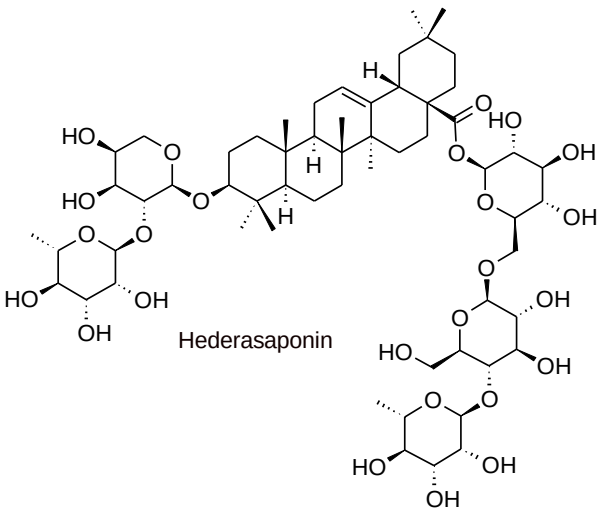


Plant: Small herbaceous plant, palmilobate leaves, white flowers, red berries
Drug: dried root.
Composition: Polysaccharides, glycopeptides, vitamins, sterols, essential oil – sesquiterpenes and polyalkines.
Saponins (about 20) – ginsenosides
Use: CNS stimulant, neuroprotective, "adaptogen", treatment of asthenia, modulation of immune response.

Marigold
Calendula officinalis, Asteraceae

Drug: flowers or capitulums
Composition: Flavonoids (isorhamnetin, quercetin), carotenes (lycopene), essential oil (sesquiterpenoids), triterpenoids (faradiol, arnidiol etc.), triterpenoid saponins.
Anti-bacterial and anti-inflammatory activity which results from the synergy of numerous marigold components may be partly attributed to saponins.
Use: topically used preparations – lotions, creams, soaps.

Common ivy
Hedera helix, Hederaceae



Drug: leaves, wood
Composition: Flavonoids, polyalkynes, saponins (5-8 %, bidesmosides and monodesmosides)
Activity: Expectorant, spasmolytic, fungistatic, anti-bacterial

Cyanogenic glycosides

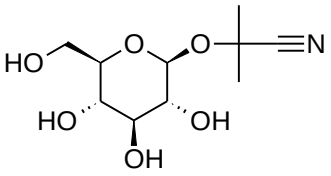
The aglycons of cyanogenic glycosides are 2-hydroxynitrils, which are metabolically derived from amino-acids. They are unstable, and after the enzymatic cleavage of the glycosidic bond hydrolyze spontaneously to several products, one of them being hydrogen cyanide.

Cyanogenesis was recorded in more than 2500 plant species belonging to numerous families, e.g. Rosaceae, Fabaceae, Poaceae, Araceae, Euphorbiaceae, Passifloraceae, etc.

The toxicity of the cyanide anion is based on complexing the heme iron resulting in inhibition of multiple heme containing enzymes and electron transporters. Acute intoxication with sub lethal doses of cyanide leads to asthenia, vomiting, hypotension and tachycardia. The effects of chronic intoxication are namely: a) inhibition of thyroid peroxidase, leading to thyroid gland disorders b) atrophy of optic nerves c) polyneuropathy.

Cassava

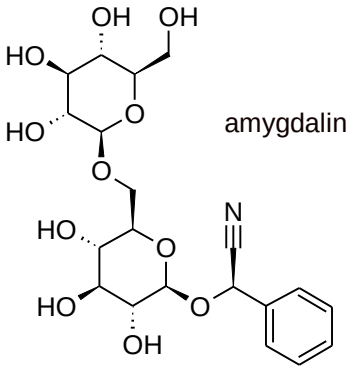
Manihot esculenta (Euphorbiaceae) is an important dietary staple for more than 500 million people in Asian and African countries in the warm-humid climatic zone. People eat 60% of the cassava produced and one third of the harvest feeds animals. All cassava cultivars contain the cyanogenic glucoside, linamarin, but in different concentrations. The roots of those cultivars with high cyanogenic content are processed to reduce the level of linamarin, because linamarin is hydrolysed in the intestinal tract of both men and animals by microbial flora and HCN is released. The technology includes peeling, grinding, fermentation and drying. The care given to this laborious procedure is decisive for the influence of cassava on human health in individual cultures. The recommended WHO limit of the cyanide content in cassava flour is 10 mg/kg. Legislation of individual states usually accepts several times higher levels; e.g. in Indonesia, the permitted value is 40 mg/kg. However, the real concentration of cyanide in cassava products often exceeds any acceptable levels, causing chronic poisoning to the consumers. Cyanide is detoxified to thiocyanate, which is a TPO inhibitor. Especially in conditions near to insufficient iodine intake, the consumption of cassava may be the crucial epidemiogenetic factor. The Bororos, a nomadic tribe in Central Africa, whose diet is based rather on milk products, have considerably low goiter prevalence when compared to local rurals, whose nutrition is based on cassava (17% vs. 76%, respectively).



linamarin

Rosaceae*: Prunus spp.*

The cyanogenic glycoside amygdalin is found in seeds of apricots, almonds, cherries, plums, peaches etc. Together with HCN it possess benzaldehyde after the hydrolysis – an important component of the bitter almond flavor.
Cyanogens are present also in apple seeds.



amygdalin

Glucosinolates

Glucosinolates are nitrogen-containing *S*-glycosides, metabolically derived from sulphur-containing amino-acids. Their aglycon is always glucose. An additional sulphur atom is present in the sulphate moiety bound to the nitrogen. Glucosinolates have been recorded in about twenty families, their most important producers are cruciferous plants (the Brassicaceae).

Brassicaceae include vegetables that are very important sources of vitamins, minerals, protein, oils and sugars – e.g. cabbage, kale, kohlrabi, cauliflower, broccoli, rape and mustard. They may contain up to 1% dry weight of glucosinolates. After a damage of plant tissues, glucose is cleaved and aglycones are liberated by the action of the myrosinase enzyme. Glucosinolates protect the plant from pathogens and pests; on the other hand, some insects use them as an oviposition signal. Amongst the others, these compounds are responsible for typical spicy tastes and aromas of cruciferous plants. Degradation products of glucosinolates include thiocyanates, isothiocyanates and cyanides (Fig 6) – all of them being TPO inhibitors. The cyanides are converted to thiocyanates again by the action of the sulphur-transferase enzyme.

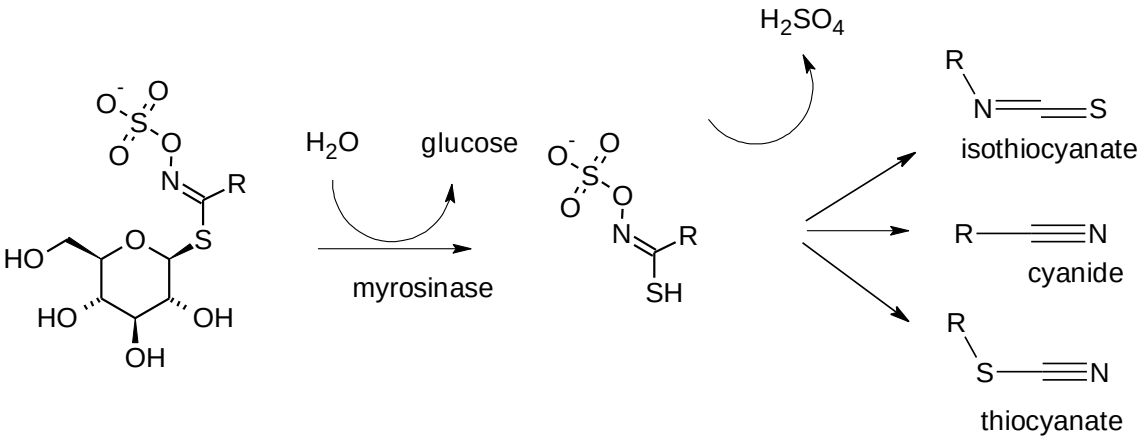
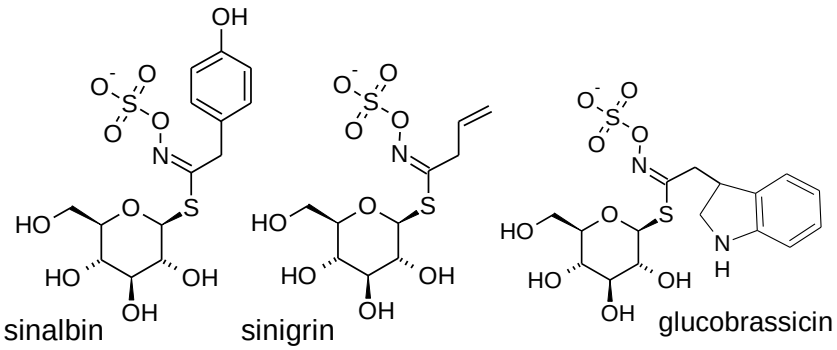


Fig 6. Degradation of glucosinolates

Toxicity of cruciferous plants to the thyroid gland was recognized already in the first quarter of the 20th century. The risk of adverse effects is high in individuals with a low iodine intake and a high consumption of the brassica vegetables. A striking evidence of this phenomenon comes from the veterinary field when sheep and cattle feed exclusively on the cruciferous plants. In Europe, the iodine deficient area includes namely the Alps and the Carpathian mountains, making thus an arc from Switzerland and Austria through the Czech Republic and Slovakia to Ukraine, Moldavia and Romania. For centuries, cabbage and kale have been an important part of traditional cuisine in the Carpathian area, due to their ease to grow and to be stored either fresh or fermented. Namely the lower social groups often suffered from poor and monotonous nutrition, lacking adequate amounts of basic nutrients. Resulting lower educability and working ability of these people formed a self-perpetuating circle of poverty Industrial development improved the social situation of large segments of the population in Europe during the second half of the 19th and the first half of the 20th centuries; iodine supplementation was introduced during the 20thcentury. Nowadays, glucosinolates are recognized to display a scale of beneficial effects, provided that their intake is moderate.



6.3.

Phenolics

Phenolics are compounds characterized by the presence of the aromatic ring substituted with at least one hydroxyl group, free or engaged in another function: ether, ester or glycoside. This structural feature is common to a virtually countless number of substances. It is worth to note that phenolics are produced by plats, fungi and microorganisms but not by animals. With one exception, which is the existence of phenolic steroids – i.e. estrogens, all other phenolics found in animal metabolome probably come from dietary intake of precursors already bearing the phenol group.

There are two main pathways leading to biosynthesis of phenolic substances in plants:

1) The shikimate pathway (assigned also the phenylpropanoid pathway), from saccharides, through several reduction steps to the phenylpropanoid skeleton. The phenolic amino acids, tyrosine and phenylalanine, are important products/intermediates of the shikimate/phenylpropanoid pathway.
2) The polyketide pathway, based on sequential building of the phenolic ring from two carbon units originating in decarboxylation of malonyl-coenzym A.

Some phenolics are pure products of one of these pathways (e.g. coumarines and lignans are pure phenylpropanoids), some phenolics are of a mixed origin (e.g. flavonoids).

Limited repertoire of phenolic originate in aromatization of six carbon rings in isoprenoids (e.g. thymol).

Main types of phenolics:

Phenylpropanoids

-Simple phenols and phenolic acids
-Coumarins
-Lignans
-Flavonoids: Flavonoids, Isoflavonoids, Anthocyanins
-Tannins

Polyketides

-Quinones and xanthones
-Orcinols and floroglucinols

Simple phenols and phenolic acids

Generalities

These low-molecular phenolics occur in biological material either free or bound in esters, glycosides and other more complex structures. In the free state they are

-Soluble in polar organic solvents

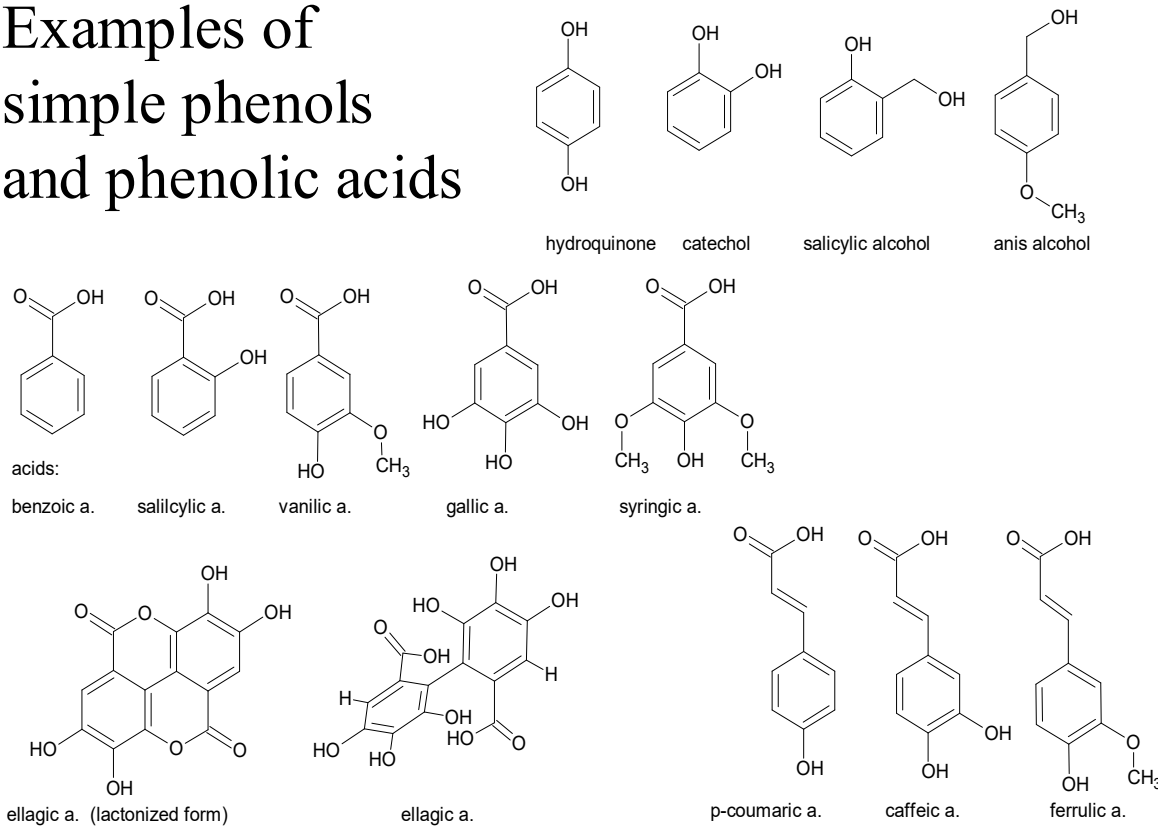
-Soluble in NaOH or Na_2HCO_3

-Unstable in alkaline conditions, prone to oxidation that leads to quinones

Extraction from fresh plant material should be preferred in order to prevent artifacts (acidified water-ethanol etc.)

Biological activities of simple phenolics are often based on their antibacterial and fungistatic activities (sometimes after oxidation to quinones), anti-inflammatory activities resulting from inhibition of cyclooxygenases and antioxidative activities.

Examples of simple phenols and phenolic acids



Botanical sources of simple phenolics

Bearberry
Arcostaphylos uva-ursi, Ericaceae

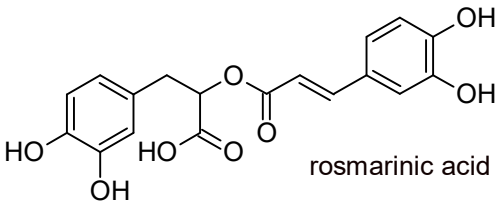
The leaves of bearberry contain:
- arbutin (6-10%)
- methylarbutin
- ursolic acid and other compounds.

The glycoside arbutin is hydrolyzed to hydroquinone which is subsequently oxidized to quinone – a bacteriostatic compound.
Bearberry leaves are used in urologic teas, considered as mild urinary disinfectants.

Similar repertoire of active compounds and related biological activities are attributed to several species of the genus *Vaccinium* (cranberry).

Rosemary
Rosmarinus officinalis, Lamiaceae

The drug: flowering tops
Composition:
-essential oil (10-25 g/kg): terpenoids: carnosol, borneol
- flavonoids (glycosides of luteolin)
-phenolic acids: rosmarinic acid, caffeic acid
Use: choleretic, diuretic, spasmolytic, anti-inflammatory.
Bacteriostatic. Supercritical rosemary extracts are used as food stabilizers.



rosmarinic acid

Myroxylon balsamum
Myroxylon balsamum (Fabaceae) is a tree indigenous in the Central and South Americas

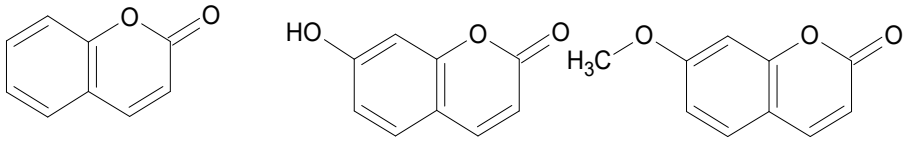
The exudate obtained from damaged bark of *Myroxylon* is called Peruvian balsam.
Peruvian balsam is composed of a mixture of
- benzoic acid esters, caffeic acid esters (50-60%)
- phenolic alcohols, free cinnamic and benzoic acids

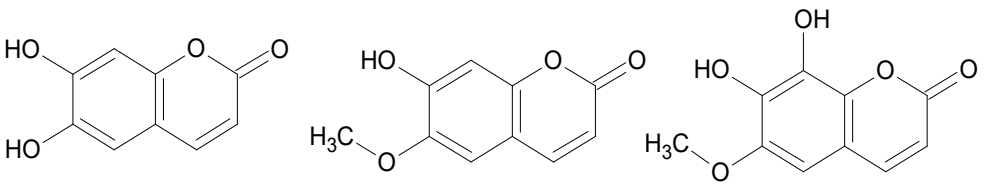
Individual components of Peruvian balsam have anti-inflammatory and bacteriostatic activities. Peruvian balsam has multiple applications in dermatology for its antiseptic and healing properties.

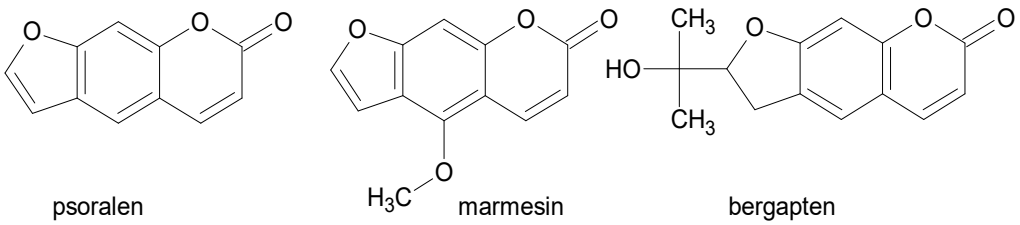
Use: topically, pure or more often in creams, ointments and lotions to treat burns, frostbites, cracks, erythema, pruritus, dermatitis.

Coumarins

Coumarins are a group of about 2000 known compounds derived from substitution and lactonization of 2-hydroxycinnamate (*o*-coumaric acid).
They are soluble in polar organic solvents; their solubility in water depends on the presence of polar substituents (hydroxyls, glycosides etc.). Coumarins have characteristic UV spectra; many of them are fluorescent compounds. The lactone ring is hydrolysable in alkaline conditions; this reaction sometimes may be reversible after the change of pH. The simplest coumarin is volatile and smells pleasantly creating a feeling of sweetness – the coumarin containing drugs are used as spices (e.g. sweet woodruff, *Asperula odorata;* sweet clover, *Melilotus officinalis*), ingredients of liqueurs and raw materials for perfumery.
Furanocoumarins found in some Rutaceae and Apiaceae are efficient photosensitizers. Serious burn-like dermatitis may develop due to the *in situ* generation of peroxyl radicals and other reactive oxygen species (ROS) after UV irradiation of the plant juice spoiled skin.







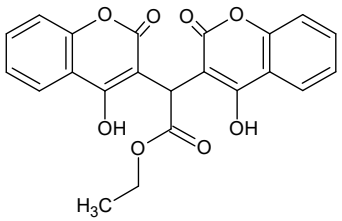
Botanical sources of coumarins

Sweet clover
Melilotus officinalis, Fabaceae.

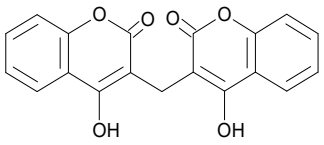
Sweet clover is a herbaceous plant, growing in Europe, reaching up to 1.5 m height.

Drug: Flowering tops
Composition: saponins, flavonoids, phenolic acids, coumarin, dicoumarol. Used as a spice, liquor ingredient or a component of mildly sedative herbal teas, sweet clover may be rather

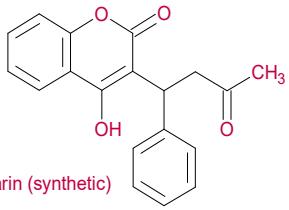
dangerous when overdosed. Dicoumarol, one of substances found in sweet clover, acts as a vitamin K antagonist and thus an inhibitor of hemocoagulation. Its intake may result in serious bleedings – the situation known more from the veterinary field.
Synthetic analogues of dicoumarol named warfarin and pelentan have been used as anticoagulants for therapeutic purposes, warfarin has been used also as a pesticide.



pelentan (synthetic)



dicoumarol (*from M. officinalis*)

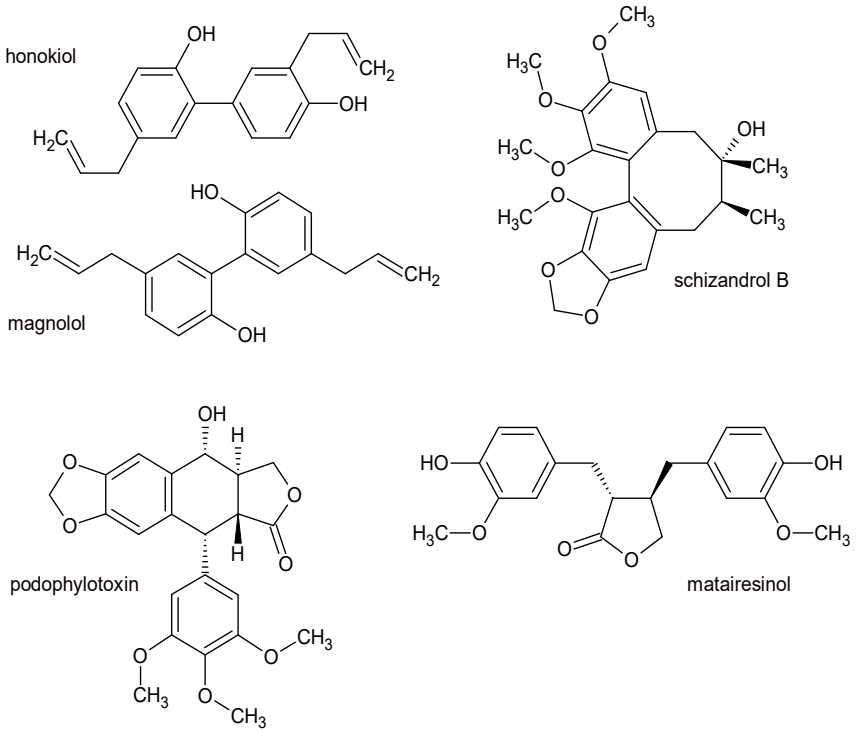


warfarin (synthetic)

Furanocoumarins have an additional five-atom ring derived from prenylation followed by cyclization. They occur especially in the Apiaceae and Rutaceae families.
Skin contact with plants rich in furanocoumarins may cause serious and painful dermatitis, which resembles burning (red skin, plasters) and its healing takes a long time. Namely the *Heracleum* and *Ruta* species are dangerous, particularly on sunny days.

Lignans

Lignans are derived from condensation of two phenylpropanoic units. Thus, they have 18 carbon skeleton with two aromatic rings and six non-aromatic carbons. Lignans are found ubiquitously in higher plants. While most of them do not display considerable pharmacological activities except the antioxidative activity, some lignans belong to pharmacologically important substances. Magnolol and honokiol from *Magnolia* species are mild anxiolytics. Lignans found in *Schizandra sinensis* are potent antioxidants and hepatoprotectives. Secolariciresinol and matairesinol found in linseed are precursors of estrogenically active enterolactone. Probably the most serious activities are attributed to toxic and cytostatic lignans from *Podophylum* sp.



Podophyllum peltatum (Berberidaceae)
English name: May apple

Rhizome extracts contain up to 20% of podophylotoxin
Cytostatic, toxic. Interaction with tubulin
Semisynthetic derivatives of podophylotoxin. are used in chemotherapy

Flavonoids

Flavonoids are a large group of compouds derived from condensation of a phenylpropanoic unit with three two-carbon fragmennt condensation carried out by a polyketide synthase type enzyme, resulting in formation of a fifteen carbon skeleton (Fig 7). Further functionalizations of the flavonoid skeleton lead to more than 8000 known compounds. The most frequent substituents are hydroxyl, methoxyl, glycosyl (O and C- glycosides) anf prenyl. The reaction between a prenyl and a hydroxyl may lead to formation of an additional ring. Flavonoids are ubiquitous in higher plants. Some of them are quite common, the occurence of other substitutional patterns is taxonomically limited.

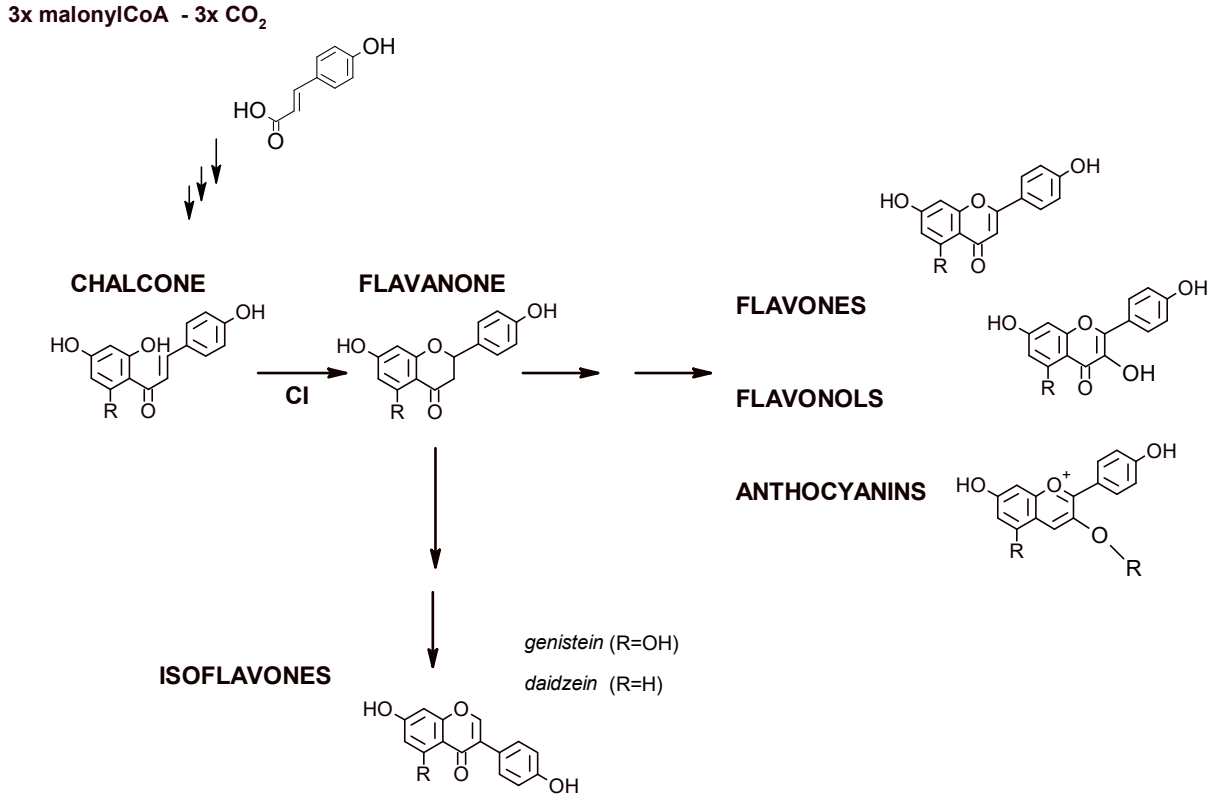
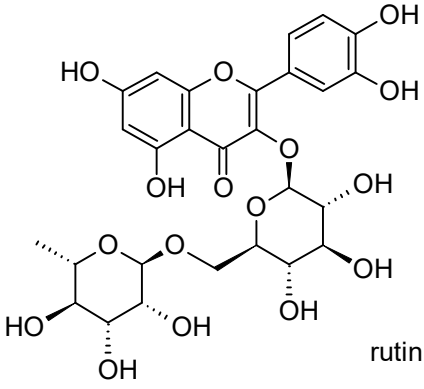


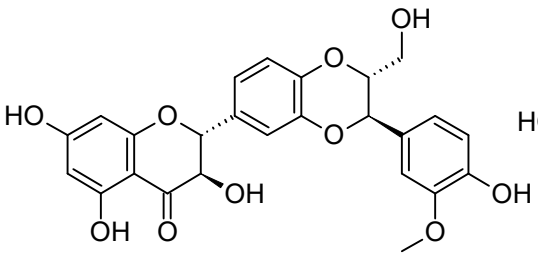
Fig. 7 Simplified scheme of biosynthesis of flavonoids and isoflavonoids

Among the pharmacological activities of flavonoids, their antioxidative activity is often mentioned. Blessed milk thistle (*Silybum marianum*, Compositae) is reputable since medieval times for its hepatoprotective activity, based on flavonolignans (e.g. silybin and silychristin). Venoprotective activity of buckwheat (*Fagopyrum esculentum*, Polygonaceae) and Japanese pagoda tree (*Sophora japonica*, Fabaceae) is attributed to the presence of rutin – a relatively common glycoside derived from quercetin. Naringin and neohesperidin are bitter principles of grapefruits and Sevilla oranges, respectively.

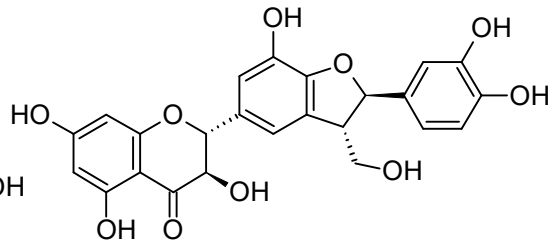


rutin

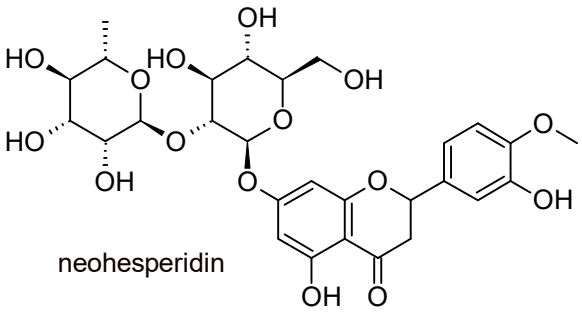
Simple isoflavonoids (namely daidzein and genistein) are known for their estrogenic activity.



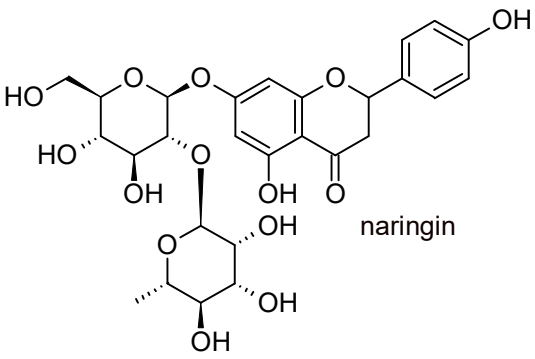
silybin



silychristin



neohesperidin



naringin

Phytoestrogens

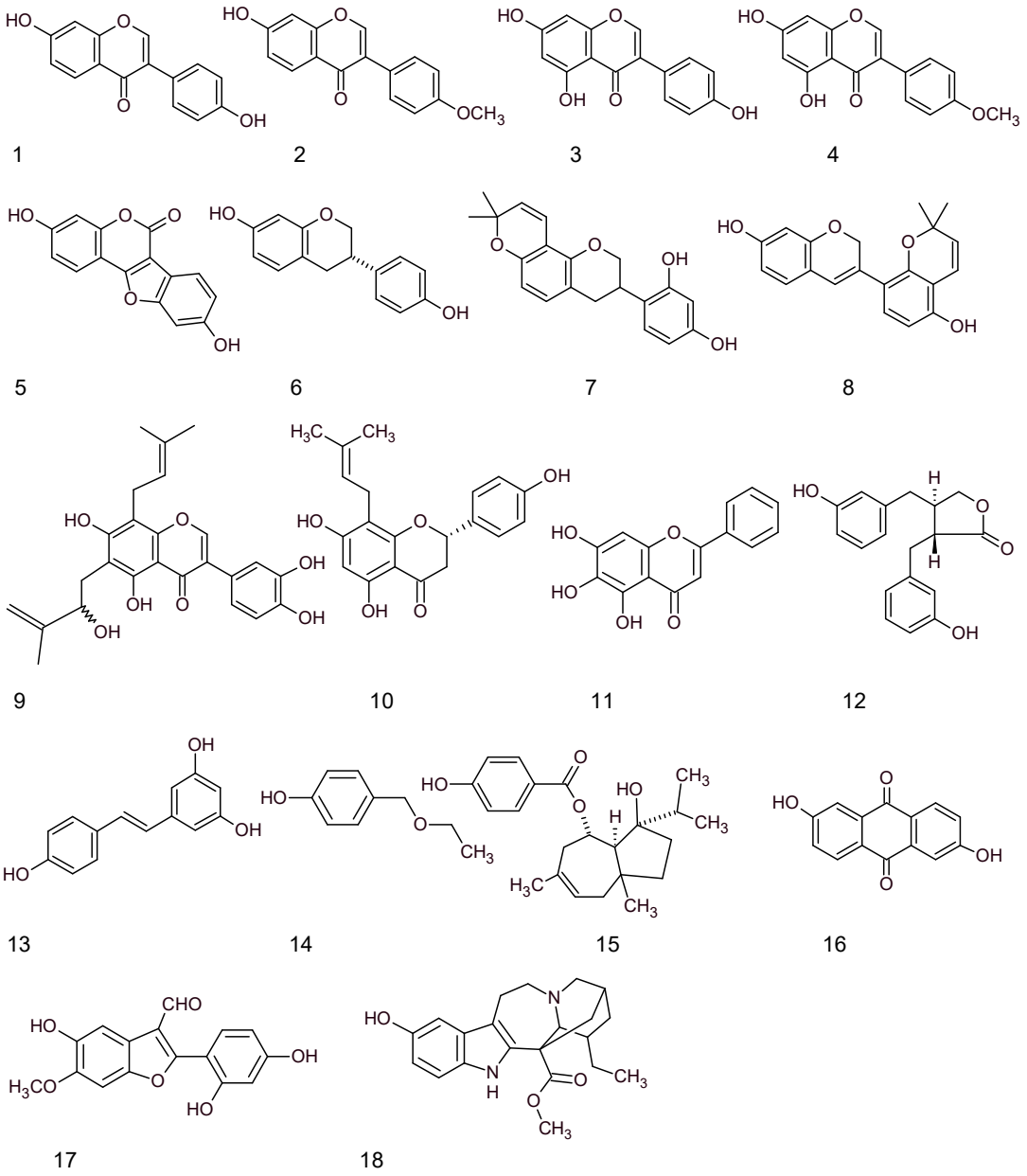
Estrogens were originally discovered as regulators of female fertility and the reproduction-related behaviour. Later, many other functions of estrogens were recognised that are important equivocally for both sexes, e.g. in bone development and homeostasis, lipid metabolism, cardiovascular system, cognitive functions etc. The estrogen signalling system is rather complex. The main estrogen-secreting gland is ovary in females; however, considerable amounts of estrogens are produced in testes in males, and in extra-gonadal places in both sexes, including breast, bone, adipose tissue, vascular smooth muscles and brain. There exist two types of estrogen receptors, with different tissue distribution and functions in gene regulation. Estrogen levels depend on age, sex and the physiological status of the individual. They are relatively low and stable in males, while in females during the fertile period of life estrogen levels are considerably higher and depend on the menstrual cycle. After menopause, estrogen levels drop down dramatically, which is accompanied by menopause related symptoms, e.g. osteoporosis, hot flushes, changes in vaginal histology, increased risk of cardiovascular diseases. In order to prevent menopause related complications, a considerable part of women uses estrogen substitutes as a hormone-replacing therapy (HRT). Nevertheless, HRT appears double-edged, as it increases the risk of breast cancer. Noticeable efforts have been developed to overcome this inconvenient feature of HRT or to find some non-risky alternatives. For the last two decades, many expectations have been connected with non-steroidal estrogenic mimics of plant origin – the phyto-estrogens.

Benets et al. (1946) postulated the estrogenic activity of plant origin in order to explain specific fertility problems of sheep grazing on subterranean clover. This hypothesis

was later verified. Formononetin, an isoflavonoid abundant in clover was found weakly estrogenic itself and recognised as a precursor of the potent estrogen equol. The common term "phyto-estrogen" was coined for estrogenically active compounds of plant origin including their active metabolites. Now we know several groups of phyto-estrogens of different structure and taxonomic origin. Unlike the authentic estrogens, phyto-estrogens are not steroids (Figure 5). Their common feature is the flatness of molecule and the presence of phenol moieties with appropriate distance of hydroxyl groups. Major phytestrogen groups represent individual branches of phenyl-propanoid metabolism in plants, i.e. isoflavonoids, coumestans, chalcones, lignans, flavonoids, and stilbenes. Main dietary sources of phyto-estrogens for humans are as follows: soy and other legumes for the isoflavonoids, alfalfa and clover sprouts for coumestrol (a coumestan), oilseeds and nuts for the lignans, grapes and some vegetables for a stilbene resveratrol; and hops for 8-prenyl narringenin.
Soy (*Glycine max*) is probably the most important dietary source of isoflavonoids, containing from 50 up to 300 mg of isoflavonoids per 100 g of beans. Main soy isoflavones are daidzein and genistein and their glycosides. The glycosides are cleaved during cooking and in the gastrointestinal tract and the aglycones are liberated. Moreover, daidzein is partly metabolised to a more potent estrogen, equol. Considerable amounts of isoflavonoids are also found in mung beans (*Vigna* sp.), chickpea (*Cicer* sp.), alfalfa (*Medicago sativa*) and clover (*Trifolium* sp.) Humans consume the last two items as sprouts almost exclusively, and there is an observation that the isoflavonoid content increases during sprouting.
The *in vitro* and *in vivo* laboratory experiments show a promising beneficial influence of isoflavonoids on bone metabolism, parameters of lipid and cholesterol metabolism, vaginal histology and other estrogen related parameters. At the same time, these compounds do not stimulate estrogen dependent cancer cells. Especially genistein was reported many times to inhibit the growth of both estrogen dependent and estrogen independent cancer derived cell lines, influencing also other signalling systems in the cell. Dietary intake of soybeans has been associated with lower incidence of several hormone dependent cancers, e.g. the breast and uterine cancer, prostate and colon cancer, and with lower risk of cardiovascular diseases. Seeds and nuts are rich in lignans, of which secoisolariciresinol and matairesinol are precursors of enterolactone, a weakly estrogenic lignan found in mammalian body fluids. The effect of enterolactone on estrogen dependent tissues is biphasic. While the concentrations 0.5–2μM slightly stimulate the growth of estrogen dependent cell lines in the absence of other estrogenic substances, the concentrations above 10μM are growth inhibiting. At concentrations about 1μM, enterolactone is able to decrease the effect of estradiol on MCF-7 breast cancer cells, supposedly by competition for estrogen receptors. Adlercreutz found that the risk of breast and uterine cancers was lower in Scandinavian women with high dietary intake of enterolactone than in those with low intake.
Resveratrol is a stilbene derivative found in some vegetables (e.g. cabbage, broccoli, onion) and in vine grapes. It became popular in connection with so-called "French paradox"– i.e. the statistically lower incidence of cardiovascular diseases in France when compared to other Western countries. An explanation of this phenomenon has been attributed to the French life style, namely to their regular consumption of wine and the vegetables rich cuisine. Despite the structural similarity to the strong synthetic estrogen stilbestrol, resveratrol is a very weak ligand of the estrogen receptor. Molecular modelling revealed that different amino acid residues are engaged in the interactions of the receptor with diethylstilbestrol and with resveratrol, respectively.
Beer has been mentioned as breast protecting and stimulating in the Central European folk tradition. A few glasses of beer per day used to be recommended to breast-feeding women in order to support lactation. Disputations have been held for a long time whether the frequent tendency to an apparent feminisation in strong beer drinkers (the "beer makes pretty bodies"

effect) may be attributed to some hormonally active compounds or is caused solely by the estrogenic potential of the adipose tissue.

Phytoestrogen isoflavonoids have been found in beer (i.e. daidzein, genistein, formononetin and biochanin A, but their concentrations did not reach 0.1µM, being by several orders of magnitude lower than in legumes. A flavonoid from hops, the 8-prenylnaringenin, was recognised as a potent phytestrogen (Milligan et al. 1999).



Structural type sof phytoestrogens:

Isoflavonoids: daidzein (1), formononetin (2), genistein (3), biochanin A (4) and coumestrol (5) are present in many legumes, *S*-equol (6) is synthesized from daidzein by the intestinal microflora; pyranoisoflavonoids glabridin (7) and glabren (8) are found in licorice, prenylated isoflavonoid isoerysenegalensein E (9) is an antiestrogen from seeds of *Milletia* sp. (Fabaceae)
Flavonoids: 8-prenylnaringenin (10), baicalein (11); **lignans:** enterolacton (12); **stilbenes**: resveratrol (13); **alkoxyphenols**: ethoxymethylfenol (14); **hydroxybenzoates:** ferutinin (15); **antraquinones**: 2,4 dihydroxyantraquinone (16); **arylbenzofuranes**: ebenfuran II (17); **phenolic alkaloids**: 10-hydroxycoronaridin (18)

Comprehensive reviews of epidemiological and clinical data regarding the phytoestrogen action indicate that the expectations originally connected with phytoestrogens could have been overestimated; however, the evidence of their expediency and safety has been repeatedly demonstrated. Dietary phytestrogens are neither panacea nor efficient enough to be a widely applicable alternative to HRT. Nevertheless, they may positively influence the quality of life by protective effects on the cardiovascular system and lipid metabolism, by reducing the frequency of menopausal symptoms and thus decreasing the need for medication.

6.4.

Terpenoids

1887 O. Wallach: Biochemical origin of terpenoids
- condensation of isoprene units followed by further reactions

1953 Leopold Ruzicka – The Isoprene Rule:

Each group of terpenes originates from condensation head to tail of different number of isoprene units. In each group of isoprenoids, different compounds may be derived from one precursor by an array of cyclizations, functionalizations and rearrangements

According to the degree of condensation terpenoids are categorized into:
Monoterpenoids (C10)
Sesquiterpenoids (C15)
Diterpenoids (C20)
Tritertpenoids (C30) and steroids (derived from a C30 precursor by truncation of the starting structure)
Carotenoids (C40)
Polyterpenes(large molecules of different size up to 10^6 Da)

Monoterpenoids

Monoterpenoids are formally deriveed from condensation of two isoprene units. They usually occur free, some are found also as glycosides. Depending on the functionalization (particularly on oxygen content) they are volatile liquids or easily sublimating solids. Pure hydrocarbons and less oxidized monoterpenes are soluble particularly in non-polar organic solvents. Their solubility in water is rather limited.
Monoterpenoids and sesquiterpenoids are often olfactoricaly active compouds, they belong to the most common constituents of essential oils, being the active principles of numerous spices, flowers scents and perfumes.

Esential oils
Definition:
Essential oils are products, generally of complex composition, comprising the volatile principles contained in the plants, and more or less modified during the preparation process. Only physical methods may be used for obtaining essential oils: distillation, steam distillation and expression.

Composition: Volatiles, namely monoterpenes, sesquiterpenes, low m.w. alcohols, aldehydes, volatile phenylpropanoids, coumarins, etc. Together with substances found in the intact plant, modified compounds may may be present as well, e.g. products of hydrolysis or partial decomposition. (For example, sulfur containing products of decomposition of glucosinolate aglycones in essential oils from Brassicaceae species).

Properties: Liquids, usually of lower density than water. Certain components may be crystalizable at lower temperatures.
Optically active. Soluble in organic solvents and in lipids (fat)

Virtually insoluble in water (but enough for flavouring)

Chemotypes : composition of essential oils from the same species may vary, sometimes despite their morphologic uniformity.

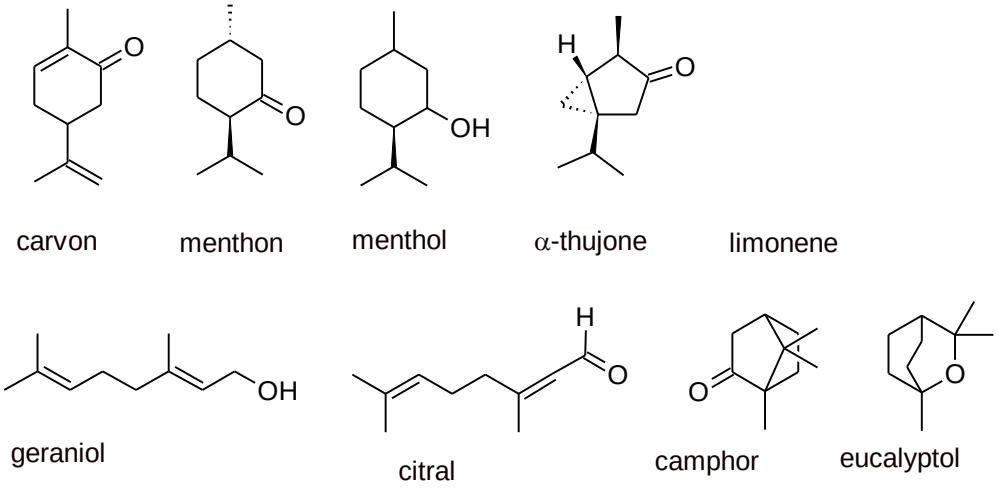


Fig. Examples of monoterpenoid structures

Taxa to be discussed:

Apiaceae: Caraway *(Carum carvi),* Anis (*Pimpinella anisum*), Fennel (*Foeniculum vulgare*),Parsley (*Petroselium hortense*), Celery (*Apium graveolens*), Dill (*Anethum graveolens*)
Compositae: Matricaria (*Chamomilla recutita*), Wormwood (A*rtemisia absinthum*)
Lamiaceae: Sweet basil (*Ocimum basilicum*), Oregano (*Origanum vulgare*), Lavender (*Lavandula spp*), Sage (*Salvia officinalis*), Peppermint (*Mentha x piperita*), Spearmint (*Metha spicata*)
Myrtaceae: Eucalyptus (*Eucalyptus globulus*), Clove (*Syzigium aromaticum*), Tea tree (*Melaleuca alternifolia*)

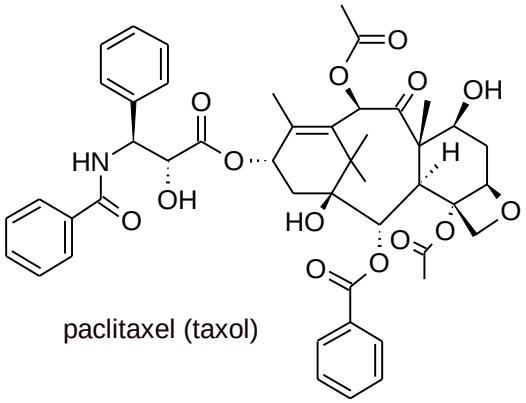
Diterpenoids

Taxa to be discussed**:**

***Taxus* spp., Taxaceae**

Toxicity of yew (Taxus sp.) has been known for centuries.
Traditional use – criminal and war poison, abortifaciens.
Anticancer activity in yew extracts was discovered in1967 and the active substance was isolated two years later. Structure of paclitaxel has been determined in 1971.
Molecular mode of action (i.e. the interaction with tubulin) has been elucidated in 1979.
Clinical Phase I studies initiated in1983. Proved effective cytostatic against ovarian and breast cancers, paclitaxel has been approved by US FDA for their treatment (1992 and 1994, respectively).

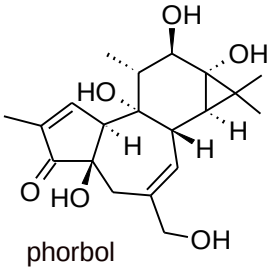
Total synthesis of paclitaxel was described in1994 independently by Nicolau and Holton, but none of them was economicaly feasible for an industrial scale production. Paclitaxel is obtained by isolation from leaves of *Taxus canadensis* or *Taxus baccata*, together with several related compounds which can be converted to paclitaxel by several simple synthetic steps.



paclitaxel (taxol)

***Croton* spp. (Euphorbiaceae)**

Toxic esters of diterpenoid phorbol are found in oils of *Croton* species. When applied to skin or mucosas, they are iritating, inflammatory and in some circumstances even carcinogenic. Some of them are used as drastic laxatives. Phorbol 12-myristate 13-acetate (PMA) known also as 12-O-Tetradecanoylphorbol 13-acetate (TPA) is used in biomedicinal research as a model iritant.



phorbol

Syllabus: Bioactive Natural Compounds

1. History of the subject, basic terms. Pharmacognosy and chemosystematics.
2. Cellular and molecular targets of biological activity.
3. Polysacharides: Fibers, gums, mucillages and pectins
   - plants: *Acacia sp., Astragalus gummifer, Ceratonia siliqua, Psyllium sp., Agar agar*
4. Specifically active proteins: Enzymes, lectins, protein sweeteners
   - plants: *Carica papaya, Canavalia ensiformis, Ricinus communis, Abrus precatorius, Thaumatococcus sp.*
5. Alkaloids I: Generalities, taxonomical occurance, protoalkaloids.
6. Alkaloids II. Selected types of alkaloids and their biological activities
   - plants: *Atropa belladona, Erythroxylon cocca, Nicotiana sp., Areca catechu, Conium maculatum, Papaver somniferum, Cataranthus roseus, Strychnos nux-vomica, Physostigma venenosum*
7. Alkaloids III. Ergot alkaloids, pseudoalkaloids, purine alkaloids.
   - plants: *Claviceps purpurea, Aconitum sp. Veratrum sp., Coffea arabica, Camelia sinensis, Theobroma cacao, Ilex paraguariensis*
8. Glycosylation, Specific examples of bioactive glycosides: Cardiac glycosides, saponins, glucosinolates, cyanogenic glycosides.
   - plants: *Digitalis sp., Drimia maritima, Glycyrrhiza glabra, Aesculus hippocastanum, Hedera helix, Brassica sp., Manihot esculenta.*
9. Phenolics I: Generalities. Phenolic acids and their esters, coumarines.
   - plants: *Myroxylon balsamum, Rosmarinus officinalis, Salix sp., Melilotus officinalis, Heracleum sp.*
10. Phenolics II: Flavonoids and lignans. Phytoestrogens.
    - plants: -*Silybum marianum, Fagopyrum esculentum, Glycine max., Trifolium sp., Linum usitatissimum*
11. Phenolics III. Tannins. Quinones and phloroglucinols.
    - plants: -*Quercus sp., Juglans regia, Lawsonia inermis, Cassia sp., Rhamnus frangula, Humulus lupulus, Cannabis sp.*
12. Terpenoids I: Monoterpenes and essential oils. Toxic diterpenes.
    - plants: -*Lavandula sp., Mentha sp., Thymus sp, Carum carvi, Citrus aurantium, Artemisia sp., Taxus sp., Croton sp., Daphne mezerum*
13. Terpenoids II: Triterpenoids, steroids and carotenoids. Vitamin A and retinoids.
    - plants: -*Betula sp., Cucurbita sp., Capsicum annuum, Crocus sp.,*
14. Excursion (Botany Garden).

Examination requirements: Written test followed by a short interview.

The test consists of two parts

a) multiple choice test

b) answer to a specific question

Course requirements: Students are expected to have basic knowledge of organic chemistry. Chemical structures will be explained on selected examples of individual types of bioactive natural compounds. All medical and botanical terms will be explained. Botanical names will be given in both Latin and English languages.

Additional literature:

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