ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
FAKULTA TROPICKÉHO ZEMĚDĚLSTVÍ

Bioactive Plant Products

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Bioactive Plant Products

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ISBN 978-80-213-2571-5

Bioactive Plant Products
Contents

Natural pigments

- structure diversity of pigments of plant or animal origin
- some have physiological effects (drugs)
- coloration of plant parts or body parts of some animals, signalling and communication especially in mating/fertilising period (attracting pollinators, significant sexual traits in animals, sexual dimorphism (birds) or warning/threat (**aposematic coloration**, easily remembered patterns)



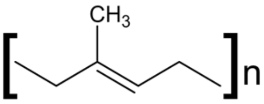

Aposematic coloration – threat

Some butterfly species are distasteful or toxic to birds. Such species invariably tend to "advertise" their toxic properties by the use of eye-catching and easily remembered patterns, usually comprising of bright orange markings on a contrasting black or white ground colour.

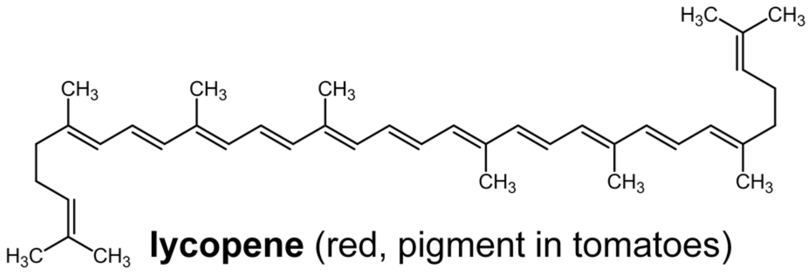
Any bird that attempts to eat one of these species will find the experience extremely unpleasant, and is likely to suffer an immediate attack of vomiting. Having tasted such a butterfly, the bird will quickly learn to associate the colour and pattern with the unpleasant experience, and will avoid eating similarly coloured butterflies in the future.

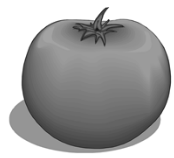
Physical properties of pigments

- absorption of light in visible range
- presence of chromophore in the molecule (conjugated double bonds, carbonylic groups, aromatics)



natural rubber (colourless)





lycopene (red, pigment in tomatoes)

Examples of traditional natural pigments

- **henna** – red/orange - *Lawsonia inermis* (tropical evergreen bush), young shoots with leaves; dyeing of skin, hair or beard, fabrics (rapping mummies in Egypt)
- **indigo** - blue – leguminous shrub - structure 1883
- **alizarin** - red - madder family Rubiaceae (Mediterranean, perennial plant), roots - structure 1868; ink
- **hematoxylin** - purple - logwood tree (tropics), heartwood; used in microscopic techniques
- **purple** - purple dye murex (sea snail)
- **carmine** - scale insect (females)

purple dye murex or the **spiny dye-murex**, is a species of medium-sized predatory sea snail, a marine gastropod mollusk in the family Muricidae,

Source of plant pigments – different plant parts (flowers, leaves, fruits, seeds, roots, rhizomes, heartwood).

Location in cells:
in **plastides** (pigments soluble in lipids)
in **vacuoles** (water-soluble pigments)

Nowadays mostly synthetic pigments (dyes) are used in industry.

A distinction is usually made between a pigment, which is insoluble in the vehicle (resulting in a suspension), and a dye, which either is itself a liquid or is soluble in its vehicle (resulting in a solution). The term biological pigment is used for all colored substances independent of their solubility. A colorant can be both a pigment and a dye depending on the vehicle it is used in. In some cases, a pigment can be manufactured from a dye by precipitating a soluble dye with a metallic salt. The resulting pigment is called a lake pigment.

Classification of natural pigments according to their structure

- **Polyenic pigments**
- in both plants and animals
- yellow, orange to red colour, lipophilic
- dominate in leaves in autumn (photosynthesis stops, chlorophyll is degraded

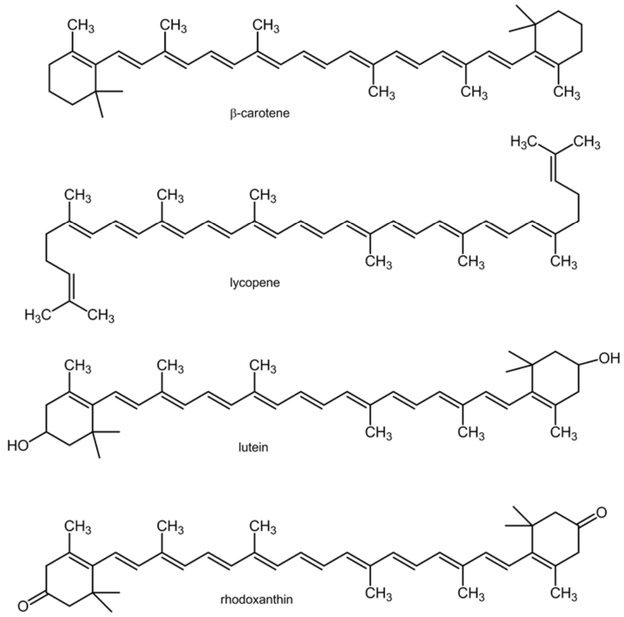
- system of conjugated double bonds, most often around 40 carbon atoms
- double bonds mostly in *trans*-configuration (*E*)
- carotenoids isolated first from carrots (1831), later separated to 3 isomers (a, b, g; liquid chromatography)

Carotenoids protect chlorophyll from oxidation.

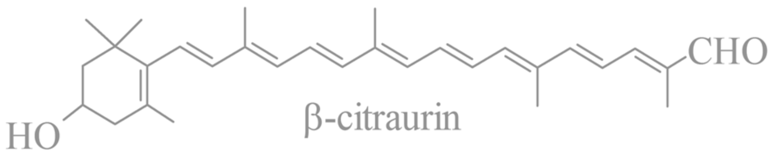
Carotenoids

carotenes (hydrocarbons)

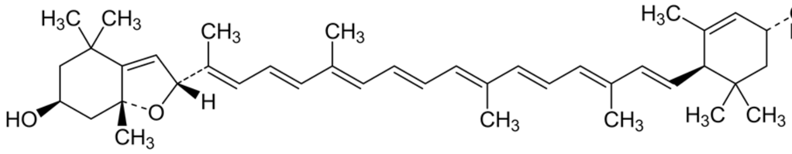
xanthophylls (oxy-derivatives)



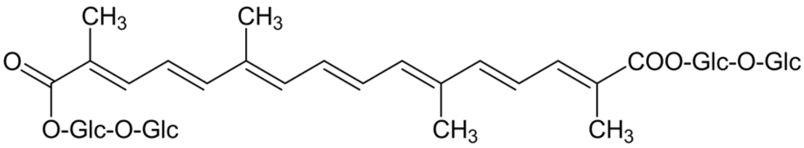
Xanthophylls – most common **lutein, zeaxanthin** (maize, flowers of tulips; both present in eye retina), **rubixanthin** (rose hip), **cryptoxanthin** (egg yolk, maize, butter), **rhodoxanthin** (autumn leaves), **astacin** (crayfish, lobster) or **capsanthin** (red pepper). **Bixin** from seeds *Bixa orellana* is used in butter and cheese (E160b). Saffron (spice) - **crocetin**. Structures with degraded cycle on one end: **β-citraurin** (orange peel).



β-citraurin



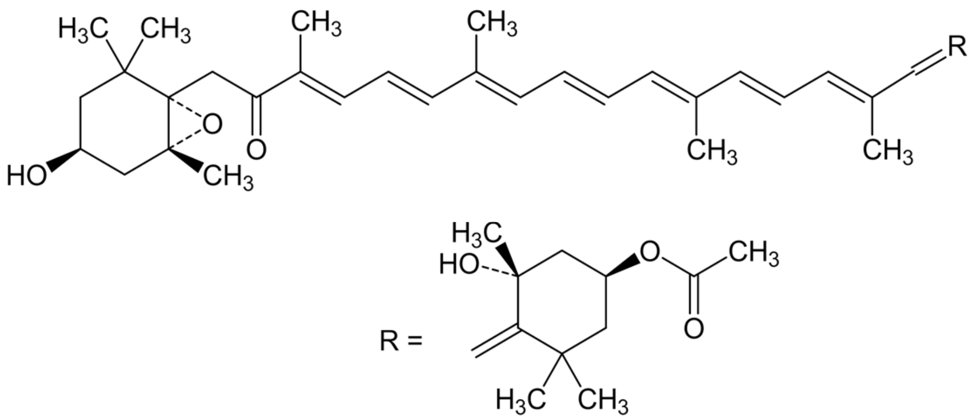
flavoxanthin (yellow chrysanthemum)



crocein (yellow saffron - *Crocus*; crocetin = free diacid)

Saffron – expensive spices (*Croccus* stigma), used maily for adding color to food

Other sources of polyenic pigments

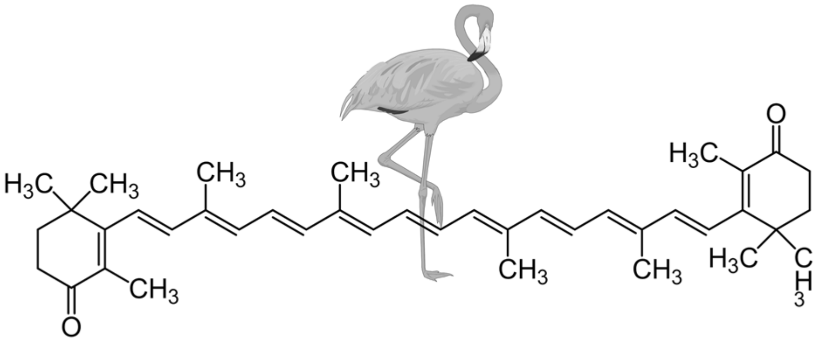


fucoxanthin – brown algae, *Phaeophyta* species
canthaxanthin - mushrooms, *Cantharellus*

(bacteria, birds, insects)

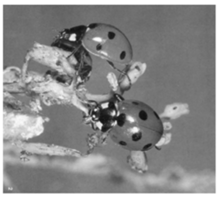
fucoxanthin – allenic moiety– interesting structure

flamingoes – **cantaxanthin** from their diet



insects:

Colorado potato beetle



ladybugs

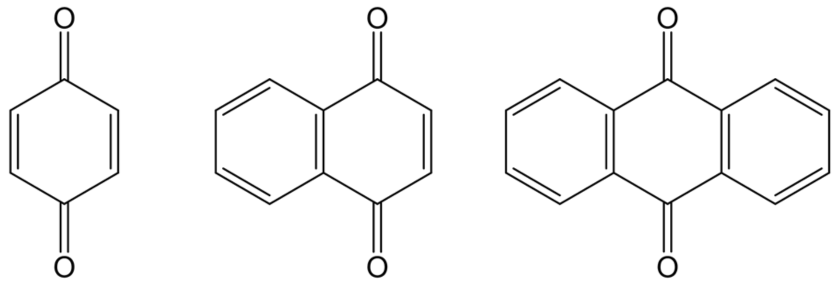
•in mammals: biological effects of carotenoids - provitamins A

Quinonic pigments

- most common natural pigments, often hidden (roots, bark)
- variety of colours, from light yellow, orange, red, brown to almost black
- in many species of mushrooms
- secretions of beetles when threatened

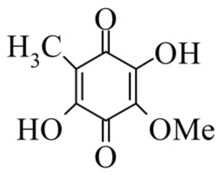
Quinonic pigments

- **structures** - derivatives of benzoquinone, naphtoquinone, anthraquinone...

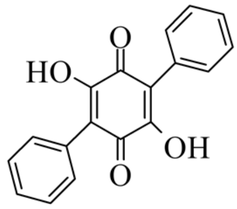


- often bound sugars (glycosides)

p-Benzoquinone derivatives

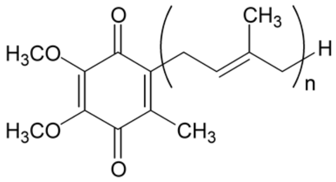


spinulosin



polyporic acid

- isolated from fungi
- purple **spinulosin** from *Penicillium spinulosum*
- dark purple **polyporic acid** from *Polyporus vidulans* (pathogenic fungi on oaks)
- **ubiquinones** – from many organisms (coenzymes Q)



n = 10
coenzyme Q_{10}
(ubiquinone 50)
antioxidants, co-substrates in respiration

combination of quinones and isoprenoids

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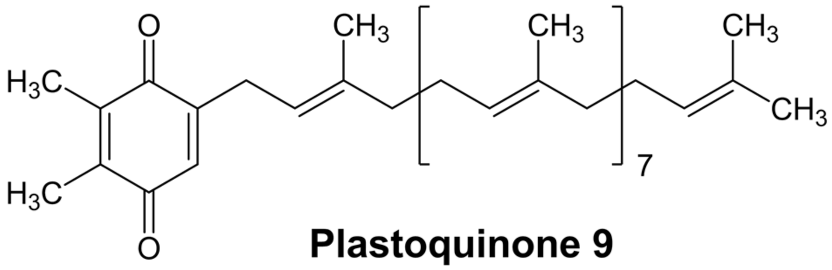
Polyporic acid makes up to 18 % dry weight of the fungus

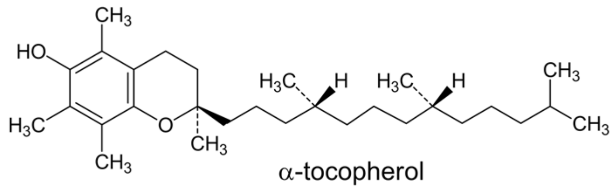
Other benzoquinones with biological activity



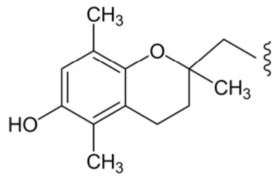
Plastoquinone 9

Plastoquinones - structurally related to ubiquinones, participate in reversible redox reactions (electron transfer) in plastides (photosynthesis)

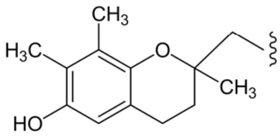
Tocoquinones, tocopherols - vitamins E, in wheat sprouts and plant oils, antioxidants



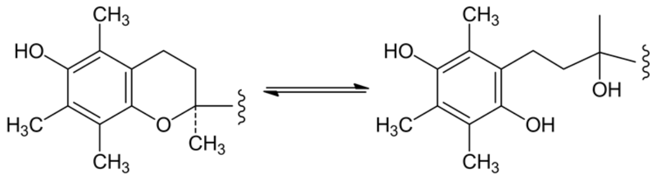
α-tocopherol



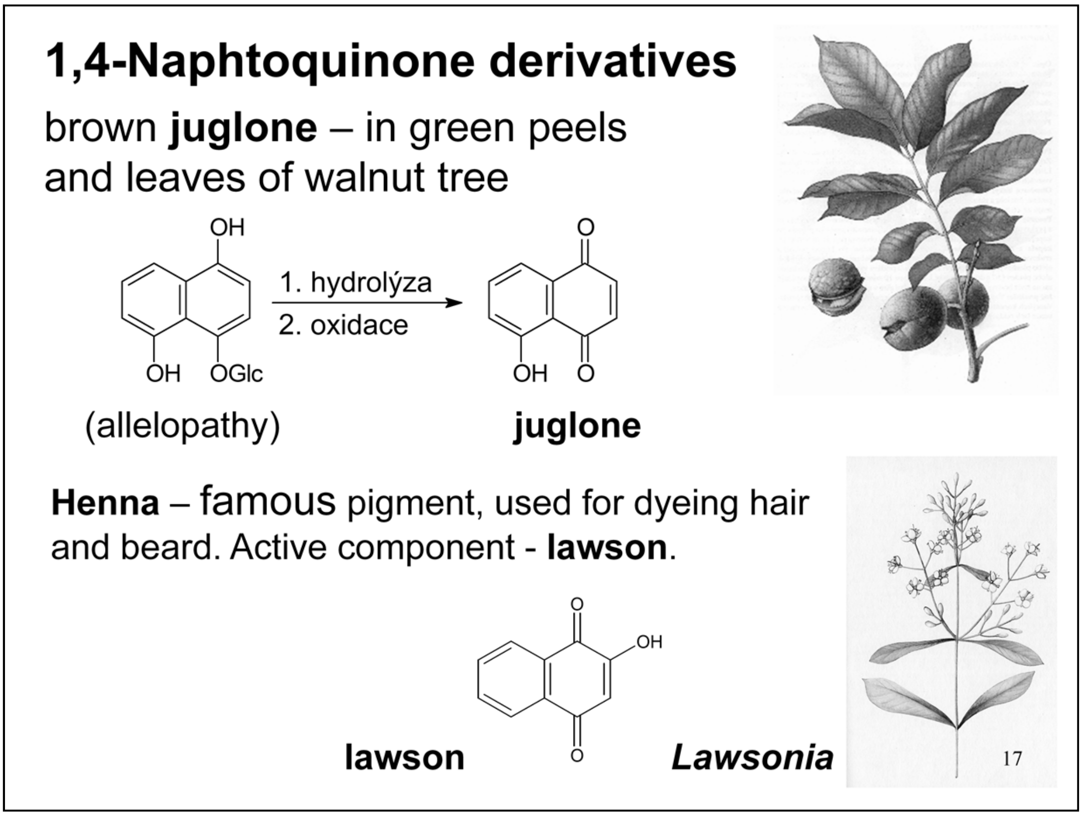
β-tocopherol



γ-tocopherol



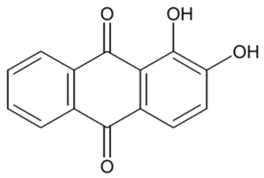
α-tocopherol tocopherol hydroquinone



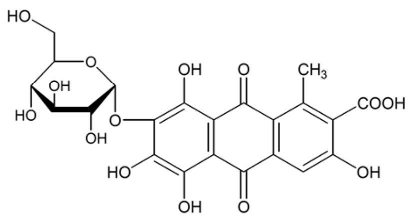
Lawsonia – evergreen bush, mostly used young sprouts with leaves

Other naphtoquinones – spinochromes, pigments from sea-urchins; different substituents on naphtalene rings

Anthraquinone derivatives



Alizarin – red pigment from roots of plants of the madder genus (common madder, *Rubia tinctorum*). Bound in glycoside **ruberythric acid.** Nowadays synthetic only.



carminic acid

- use as red pigment for alcoholic beverages (Campari), candy, indicator in cytology
- isolated from females of scale insects (*Coccus cacti*) occurring in Central and South America (content of pigment 10 % beside lipids and wax)

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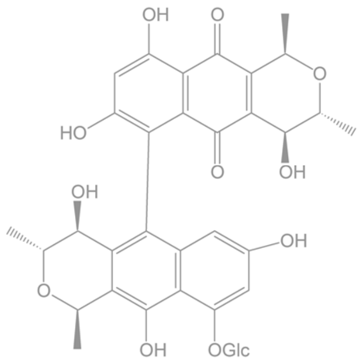
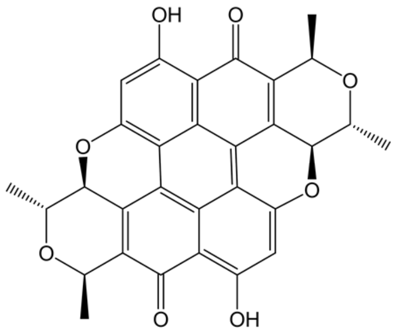
common madder, *Rubia tinctorum,* earlier used for making ink

Alizarin was used as a red dye for the English parliamentary "new model" army. The distinctive red color would continue to be worn for centuries, giving English and later British soldiers the nickname of "redcoat". In 1869, it became the first natural pigment to be duplicated synthetically.

Higher quinones (anthraquinone dimers)

aphins – from the haemolymph of different aphids





erythroaphin

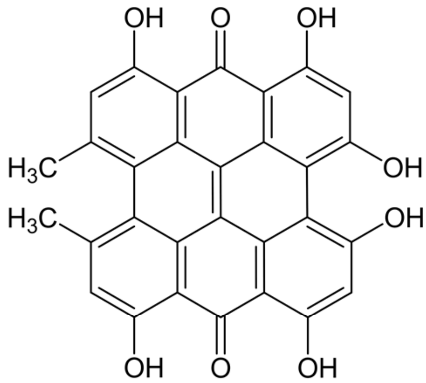
protoaphin

changes to more stable **erythroaphin** in dead and decomposed insect bodies (colour change)

Aphids – genus *Aphis*

Plants - St John's wort

hypericin



Hypericum perforatum

Properties of hypericin

- dark blue needle-like crystals
- red in basic solvents (pyridine)
- photosensitive – causes burns on skin exposed to light
- pharmacological effect - antidepressant

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St John's wort is the plant species **Hypericum perforatum**, and is also known as **Tipton's Weed**, **Chase-devil**, or **Klamath weed**.

Biosynthesis of quinones

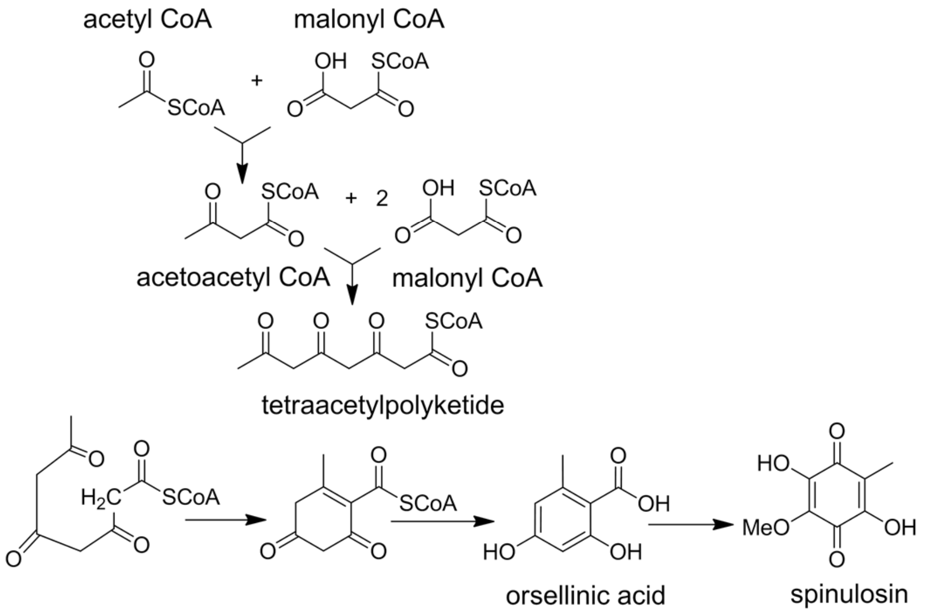
- 3 pathways, one compound can be synthesised by different pathways in different organisms

- **polyketide pathway**
- **shikimic pathway**
- **mevalonic pathway**

Polyketide pathway

- most common biosynthesis of quinone pigments
- close to biosynthesis of fatty acids
- starts from acetyl CoA and malonyl CoA, formation of acetoacetyl CoA, elongation by 2 carbon atoms in each step

Polyketide pathway:
formation of **naphtoquinones** in mushrooms, **anthraquinones** in insects, **higher quinones**.



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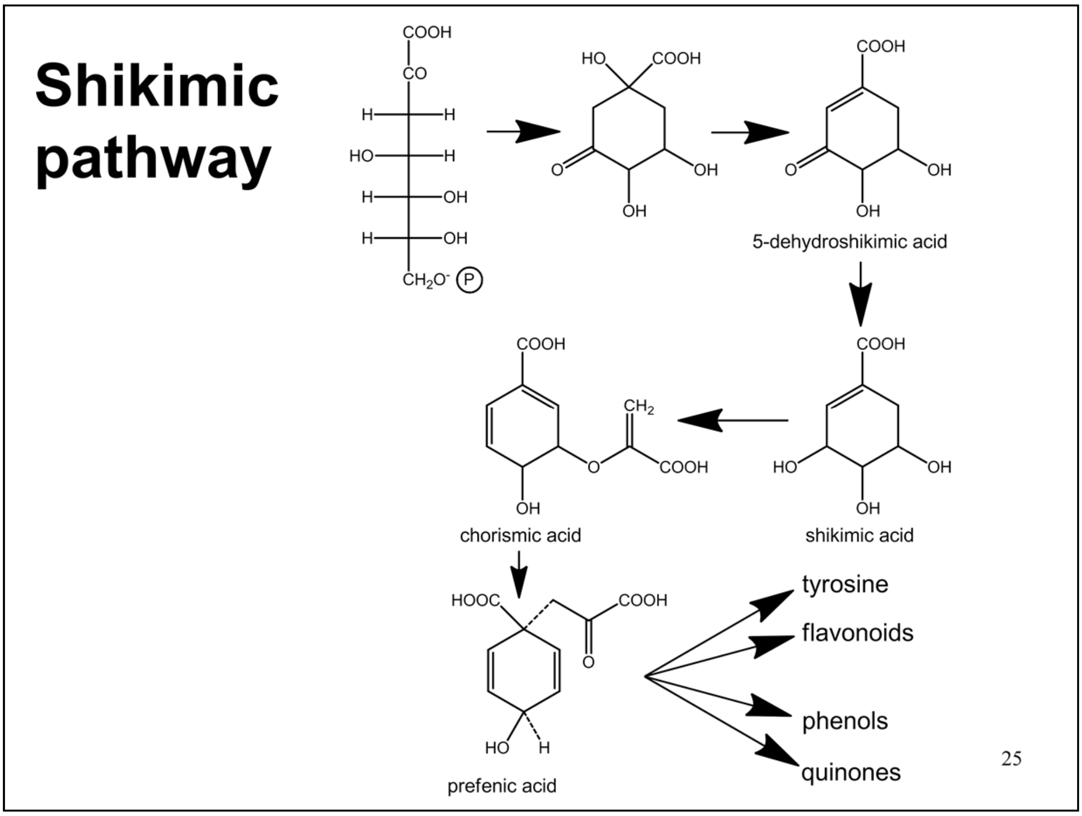
spinulosin isolated from fungus *Penicillinum spinulosum*

Shikimic pathway

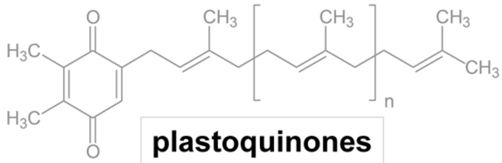
- second most common biosynthetic pathway in quinones
- close to biosynthesis of amino acids
- biosynthesis of important cell components e.g. ubiquinones, plastoquinones or naphtoquinone analogues (vitamins K - phylloquinone and menaquinone)



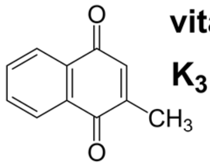
7-phospho-3-desoxy-D-arabinoheptulosic acid; Shikimic acid reacts further with a 3-carbon fragment (phosphoenolpyruvate) under formation of chorismic acid.

chorismic acid – prefenic acid (intramolecular rearrangement)

Shikimic pathway leads to:

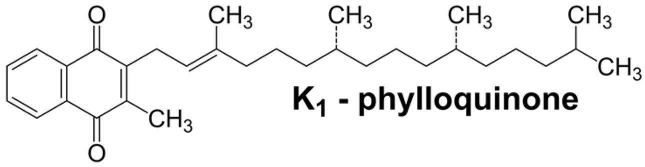


plastoquinones

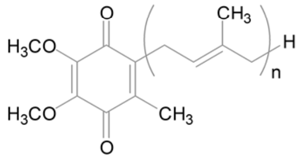


vitamins K

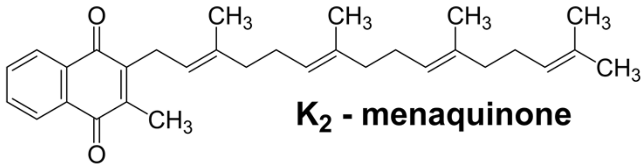
K_3 - menadione



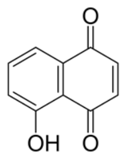
K_1 - phylloquinone



ubiquinones



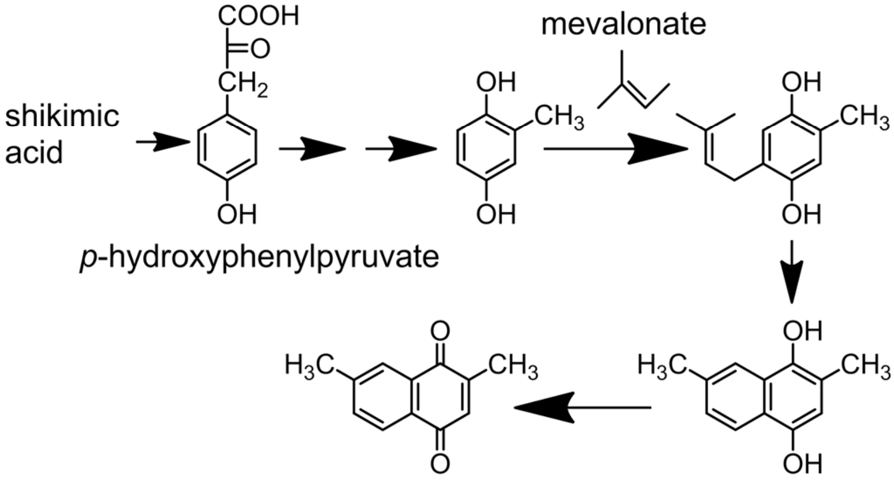
K_2 - menaquinone



juglone

Mevalonic pathway

- in combination with other pathways
- ubiquinones – side-chain arises from mevalonic pathway
- common combination of shikimic and mevalonic pathways – lead to naphtoquinones and anthraquinones



chimaphylline

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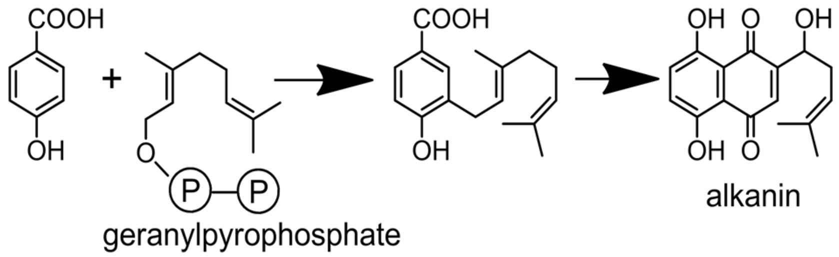
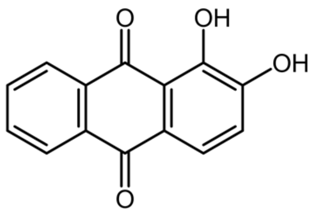
Step from p-hydroxyphenylpyruvate includes intramolecular rearrangement

chimaphylline – plant pigment (Pyrolaceae)



the same pathway leads to some anthraquinones (**alizarine**)



loss of one carbon atom from isoprene

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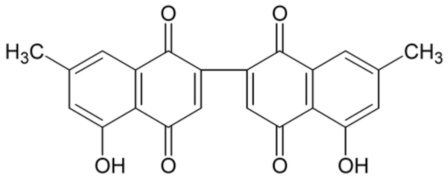
alkanin – plant pigment from roots of Boraginaceae

Phenolic condensation

radical reaction, dimerisation, formation of higher quinones (hypericine, aphins)



dimer of 7-methyljuglone - sundew (*Drosera*)

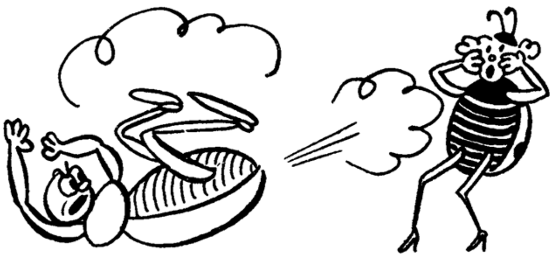
other dimers cause the black colour of ebony heartwood

Biological properties of quinones, not connected to absorption of light:

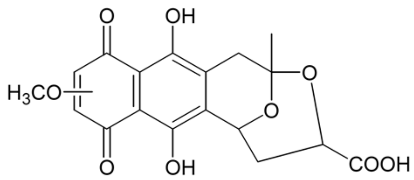
- electron transfer - redox reactions hydroquinone/quinone (ubiquinones – respiration co-enzymes; vitamins K; plastoquinones – photosynthesis in chloroplasts of green plants)

Quinones as semiochemicals:

- bombardier beetle - **benzoquinone** – defence compound
- **juglone** – walnut tree - toxic for neighbouring plants (allelopathy)

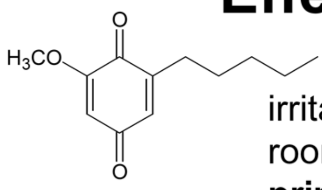


- some naphtroquinones and anthraquinones from mushrooms have antibacterial properties (protection from diseases)
- **spinochromes** (from sea urchins) inhibit growth of algae - algistatics, algicides
- pathogenic fungus *Fusarium martii* - **marticine** – causes wilt of host plant



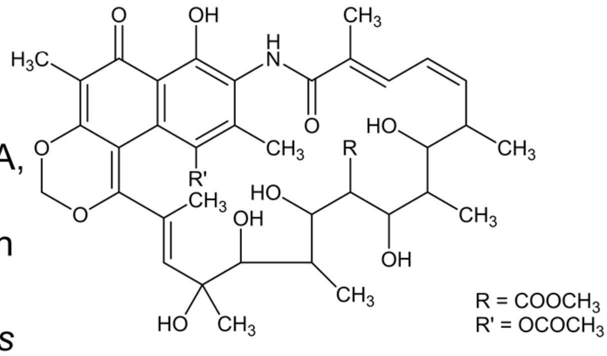
spinochrome D, a brownish red pigment that occurs naturally in the shells and spines of sea urchins

Effects on humans



irritant, alergene (e.g. common room plant *Primula obconica* - **primine**)

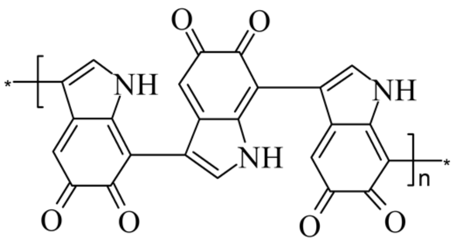
- **hypericine** - photosensible, antidepressant



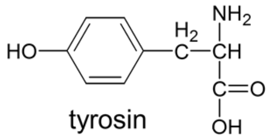
Streptovaricin C

- **Streptovaricin**. Antibiotic comples of streptovaricins A, B, C, D, E, F, G, J and K (streptovaricin C is the main component).
- Isolation from *Streptomyces spectabilis*, effective against *Mycobakterium tuberculosis*

Melanines



- structurally related to quinones
- macromolecules formed by enzymatic oxidation of **tyrosine** (irregular structure)

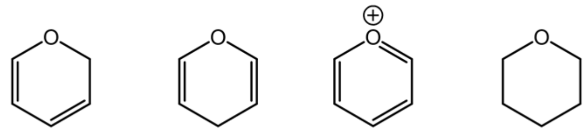


tyrosin

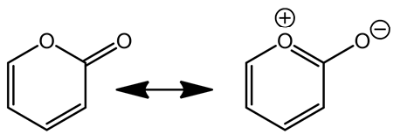
- yellow to red/brown
- present in skin and hairs of mammals (absent in albinos)
- bird feathers, insect cuticle
- fungi and bacteria
- pathological presence in human urine (melanoma)
- in plants - allomelanines
  (biosynthesis from precursors not containing nitrogen)

Don’t mix up melanines with melamine (plastics) or melatonine (regulator of circadian rhythm in vertebrates)

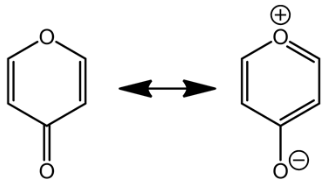
Pyran-based pigments, flavonoids



α-pyran 2*H*-pyran | γ-pyran 4*H*-pyran | pyrilium cation | tetrahydropyran (pyranose)



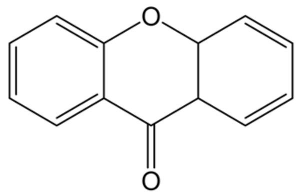
α-pyrone
2*H*-pyrone



γ-pyrone
4*H*-pyrone

Pyran-based pigments

- almost exclusively in higher plants
- especially in flower and fruit tissues
- classification according to basic skeleton:
- xanthons, flavanoids (flavons, isoflavons, flavanols, anthocyanins)
  and complex pyran-based pigments



xanthon

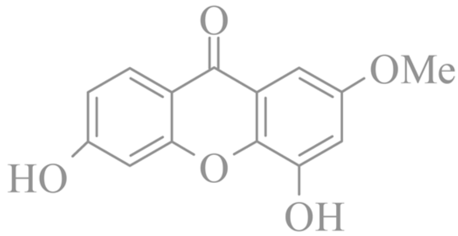
Classification of living organisms

Linnaeus 1735 2 kingdoms	**Haeckel** 1866 3 kingdoms	**Chatton** 1925 2 empires	**Copeland** 1938 4 kingdoms	**Whittaker** 1969 5 kingdoms	**Woese** 1977 6 kingdoms	**Woese** 1990 3 domains	**Cavalier-Smith** 2004 6 kingdoms
					Eubacteria	**Bacteria**	
		Prokaryota	**Mychota**	**Monera**			**Bacteria**
					Archaebacteria	**Archaea**	
(not treated)	**Protista**						
							Protozoa
			Protoctista	**Protista**	**Protista**		
							Chromista
		Protoctista	**Plantae**	**Plantae**	**Plantae**	**Eukarya**	**Plantae**
Vegetabilia	**Plantae**						
			Fungi	**Fungi**	**Fungi**		**Fungi**
Animalia	**Animalia**		**Animalia**	**Animalia**	**Animalia**		**Animalia**

Pyran-based pigments

- in plants most often in form of glycosides
- colours from yellow to red to blue
- most of them have also a biological activity

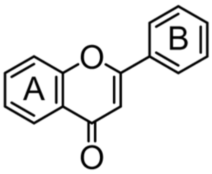
gentisin – xanthone derivative,
yellow colour of *Gentiana* roots



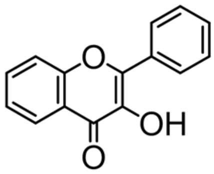
gentisin

gentisin – color of flowers of yellow Gentiana

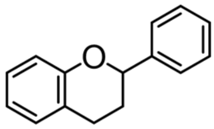
Typical structures and their nomenclature



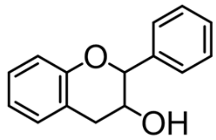
flavone



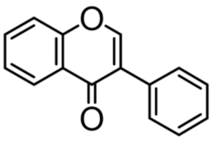
flavonol



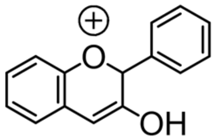
flavane



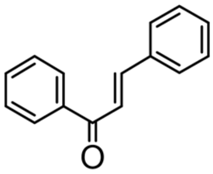
flavanol



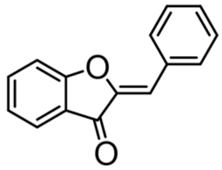
isoflavone



anthocyanidin



chalcone



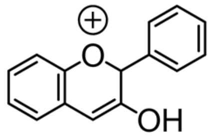
aurone

Anthocyanins

- base of red, blue, and purple colours of flowers and fruits
- colour depends on pH and presence of ions of some metals (iron, aluminium)
- glycosides, aglycone is called anthocyanidin

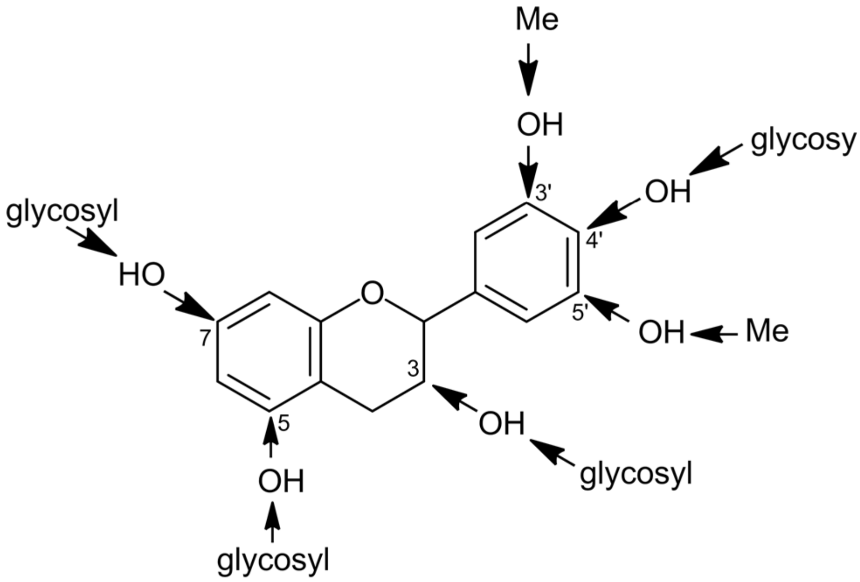






anthocyanidin

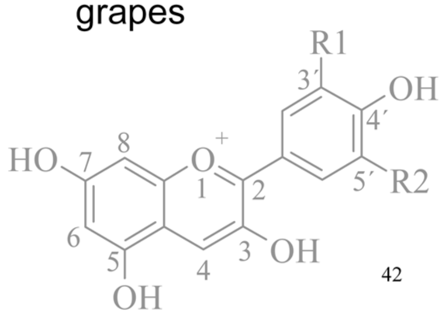
Substitution on flavane skeleton



Substitution on flavane skeleton

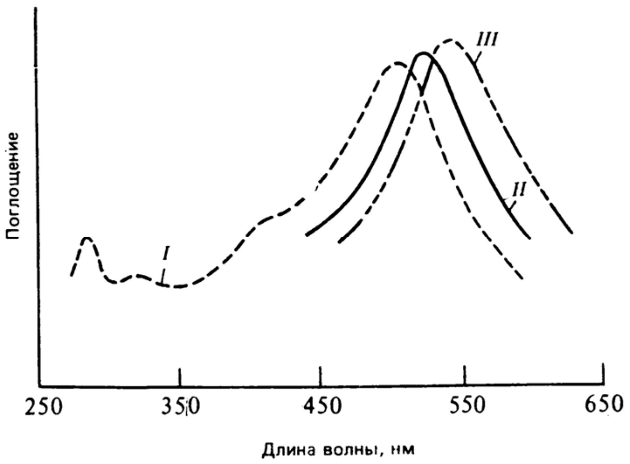
- aglycone contains at least 4 hydroxy groups available for glycosylation, thus chemodiversity of anthocyanins
- sugar unit bound always in position 3, often also 5, less frequently positions 7, 3´and 4´
- beside glucose, often presence of rhamnose, arabinose and disacharides rutinose (α-L-rhamnosyl-(1$\rightarrow$6)-D-glucose) or sophorose (β-D-glucosyl-(1$\rightarrow$2)-D-glucose)

R1	R2	Name	Colour	Occurrence
H	H	pelargonidin	red	*Pelargonium*
OH	H	cyanidin	red (H^+)	rose, cherries, cranberries, *Hortensia*
			blue (OH^-)	cornflower, *Hortensia*
OMe	H	peonidin	pink	peony
OH	OH	delphinidin	purple	pansy, red grapes
OMe	OH	petunidin	red	grapes
OMe	OMe	malvidin		



only 6 aglycones

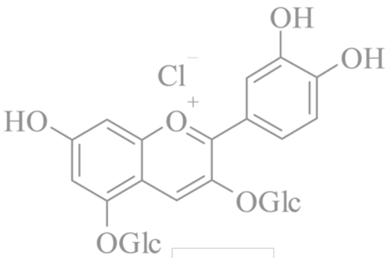
The more hydroxy groups in B cycle, the longer waves (higher wavelength λ) are absorbed.



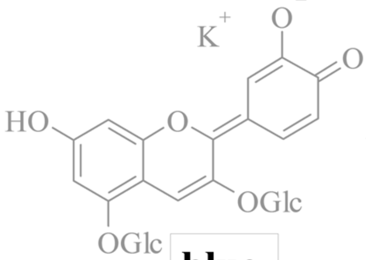
I, pelargonidin, R1 = R2 = H
II, cyanidin, R1 = OH, R2 = H
III, delphinidin, R1 = R2 = OH

Influence of pH on colour

- **cyanin** (cyanidin-3,5-di-β-D-glucopyranoside)
- **red** in rose flowers (acidic)
- potassium salt is **blue** colour of cornflower (alkalic)



red



blue

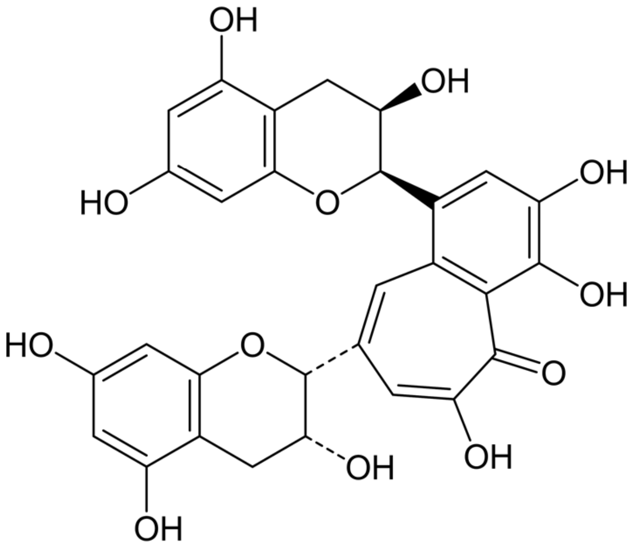
Occurrence of flavonoids

- almost exclusively in higher plants
- reports in animals – sequestration/accumulation from food (butterflies)
- microorganisms (*Aspergillus candidus*)
- in plants – all tissues, in vacuoles, soluble in water; their composition is species-specific (**chemotaxonomy**)

Occurrence of flavonoids

- Both carotenoids and anthocyanins cause autumn leave colouration of trees (chlorophyll degradation).
- **Flavons** and **flavonols** don't absorb in visible light range (white flowers), they absorb in near UV range (bees). **Co-pigments -** stabilise anthocyanins (complexes with metals).

Flavonoids form dimers and oligomers – brown colour (black tea)



teaflavin

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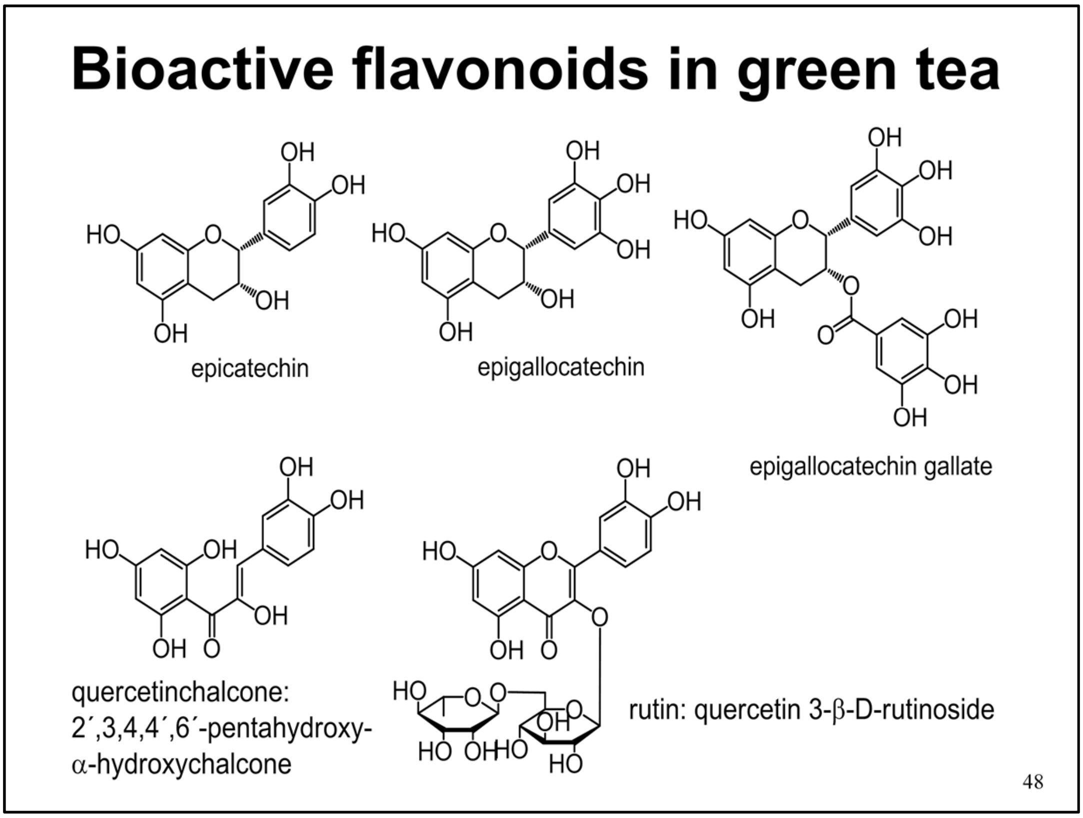
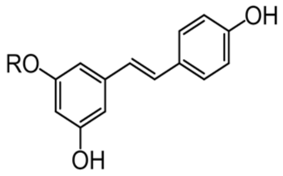
dimer is formed by fermentation from monomer, present in green tea

Green tea is a rich source of polyphenols known as flavonoids. The predominant flavonoids in green tea are catechins. Fermented green tea, also known as black tea, offers a powerful polymerized catechin known as teaflavins.

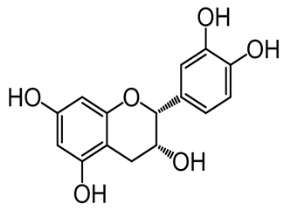


Bioactive flavonoids are present in the green tea. They act as antioxidants, stabilize vitamin C (avoid dehydrogenation). Prevention of cancer.

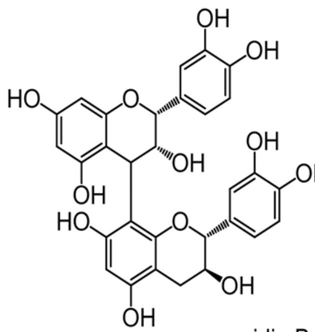
Bioactive stilbenes and flavonoids in wine



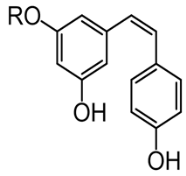
trans-resveratrol



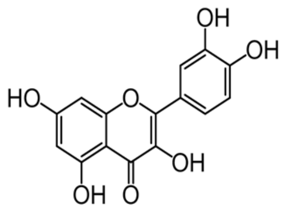
epicatechin



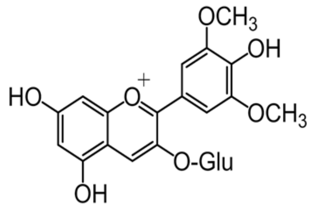
procyanidin B_1



cis-resveratrol



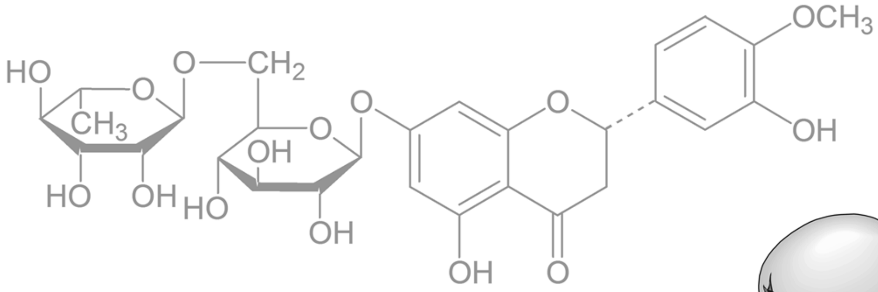
quercetin

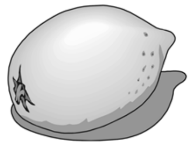


malvin-3-glucoside (enoside)

Other examples

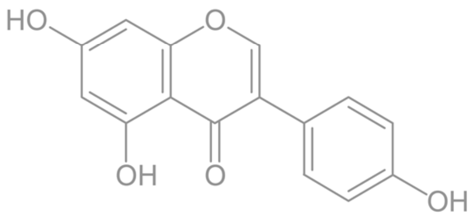
glycoside **hesperidin** – yellow colour of citrus fruits (lemons, oranges)





Other examples

- glycoside **genistin** (yellow aglycone **genistein**) – flowers of Dyer's Broom (*Genista tinctoria)*

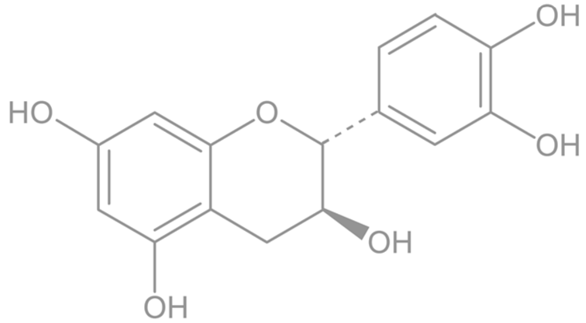


isoflavonoid; occurring also in soy - phytoestrogenic activity, specific inhibitor of protein kinase

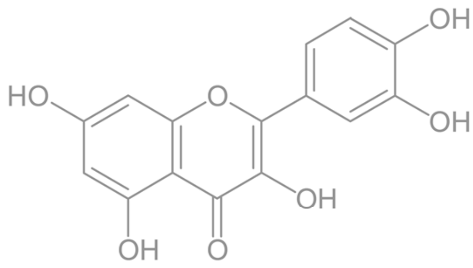
Genista tinctoria, with common names: Dyer's Broom, Dyer's Greenweed, Dyer's Whin, Furze, Greenbroom, Greenweed, Waxen Woad, Woad Waxen and Waxen Wood

Catechin - flavonoid from wood

- (+)-catechin occurs with (-)-epicatechin together, *cis*-form; mahogany wood; forms oligomers (procyanidins)



Orange-brown **quercetin** – very common (hops, tea, maize, garlic, onion, chestnut fruits; peels and barks)

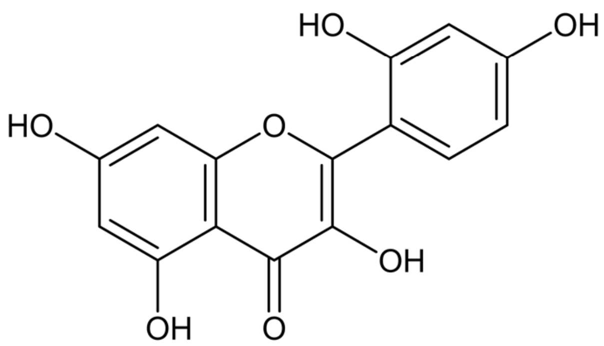


- aglycone of **quercitrin**, **rutin**, and other glycosides (glycosylation in different positions); also in flowers of clover; **quercetin** protects capillaries (vitamins P)

Bioflavonoids

- complex of vitamins P (**P**ermeability); citruses
- contribute to healthy state of capillaries
- reduce permeability of capillaries, contribute to elasticity of capillary walls
- present in higher plants
- extracted commercially in large scale from citrus peel, rosehips, black current
- synergic effect with ascorbic acid
- inhibit autooxidation of adrenalin

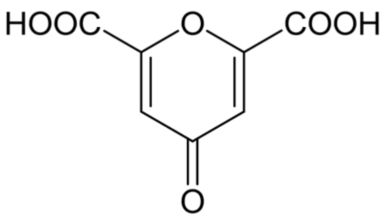
Morin – from wood of mulberry tree; used for dyeing of fabrics, in laboratories as luminiscence indicator (preparative TLC)



morin

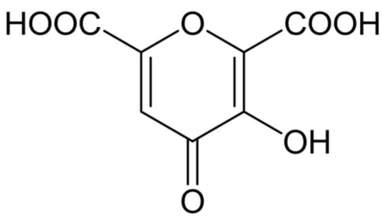
Other pyrane derivatives

co-occurrence with alkaloids



chelidonic acid

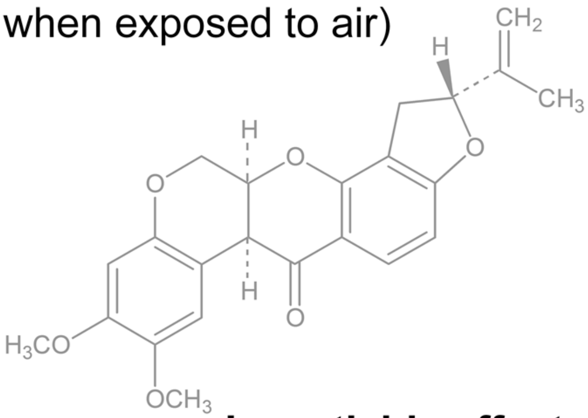
celandine (*Chelidonium*)



meconic acid

poppy - opium (4-6 %)

Other pyrane pigments - papilionaceous plants (Africa) - **rotenon** (colourless, but quickly oxidises when exposed to air)



Insecticide effect; model for synthetic insecticides; paralytic effect on fish; African tribes use it for fishing. Higher doses are toxic for humans when inhaled (little perorally).

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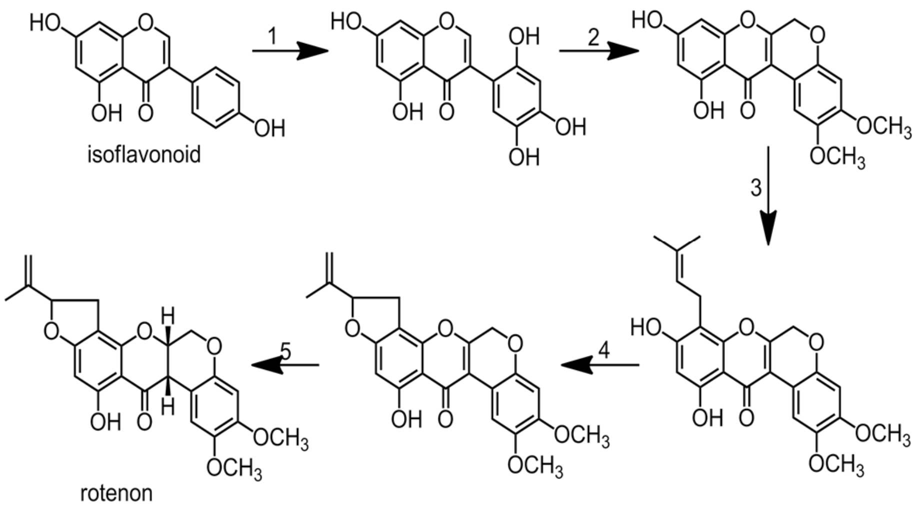
The structure is basically an isoflavonoid

Rotenone biosynthesis

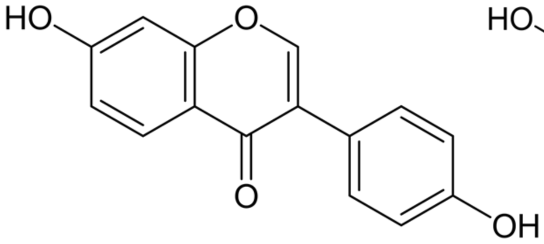


1. hydroxylation, 2. methylation, 3. dimethylallyl pyrophosphate addition, 4. cyclization,

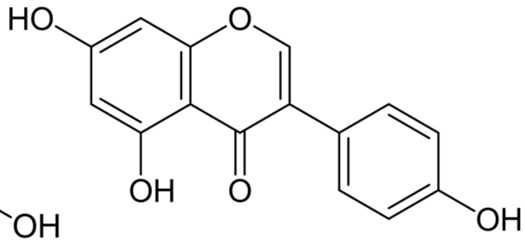
5. hydrogenation

Phytoestrogens

- ◆ compounds of plant origin, binding to estrogenic receptor,
- ◆ isoflavone derivatives (**genistein, daidzein**)



daidzein



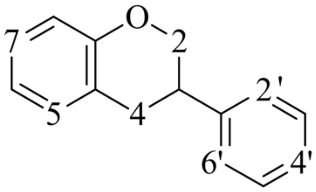
genistein

Phytoestrogens

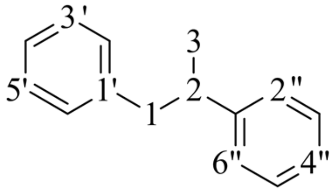
- present in soy and other legumes, full corn breads, seeds, nuts, also in beer
- in human guts metabolised to compounds similar to precursors of estrogens, metabolites have a weak estrogenic and antioxidative activity
- effects of phytoestrogens on humans:
- metabolism of sexual hormones
- influence on proteosynthesis in cells
- influence on proliferation of cancer cells
- statistically lower number of cases of breast cancer in Asia, less problems in menopause women

Metabolism of phytoestrogens

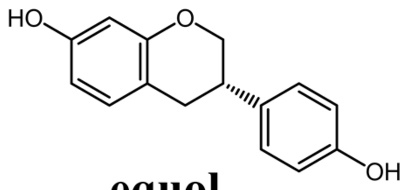
Intermediates: angolensin derivatives (detected in urine)



ISOFLAVANE



ANGOLENSIN



equol

Equol (4',7-isoflavandiol) is an isoflavandiol[1] metabolized from daidzein, a type of isoflavone, by bacterial flora in the intestines.[2] While endogenous estrogenic hormones such as estradiol are steroids, equol is a nonsteroidal estrogen. However, only about 30-50% of people have intestinal bacteria that make equol.[3] Equol may have beneficial effects on the incidence of prostate cancer[4] and physiological changes after menopause.[5] Other benefits may be realized in treating male pattern baldness, acne, and other problems because it functions as a DHT blocker.[6] S-Equol preferentially activates estrogen receptor type β.

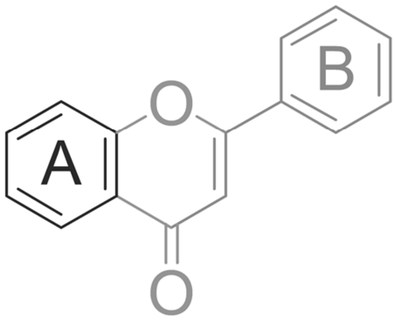
Potential use of phytoestrogens

- prevention of cancer
- physical, mental, or emotional issues of menopause in women
- prevention of osteoporosis (?)

!!! influence of genetic *vs* nutrition factors is not known!!!

Biosynthesis of flavonoids

combination of polyketide and shikimic pathways



ring A - polyketide pathway (from acetate blocks)

ring B - shikimic pathway (from phenylalanine or tyrosine, deamination, formation of cinnamic or *p*-coumaric acid)

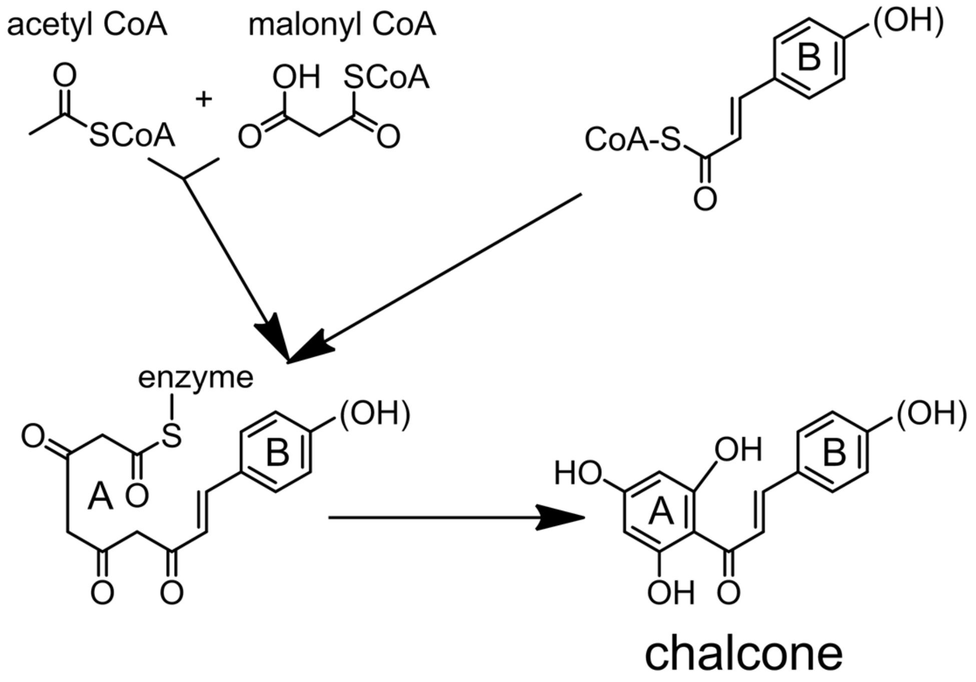
Influence of the flavonoid biosynthesis:

Light - speeds up formation of the ring B (cinnamic acid), no influence on formation of the ring A

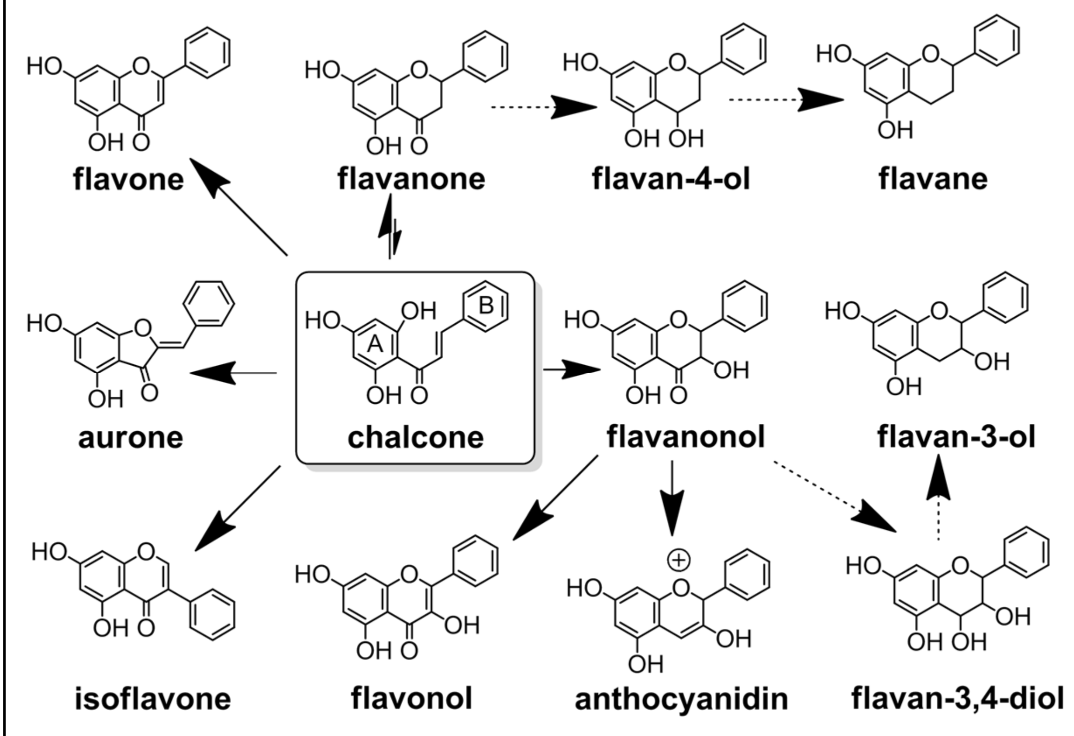
Mechanical wounding or virus infection cause increased production of flavonoids

Some flavonoids are phytoalexins

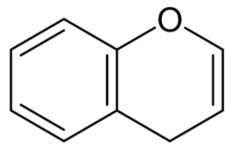
Apples infested by insects get red color earlier (biosynthesis of flavonoids) – can be distinguished from healthy ones



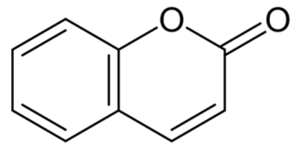
Chalcone plays a central role in biosynthesis.


flavone
flavanone
flavan-4-ol
flavane
aurone
chalcone
flavanonol
flavan-3-ol
isoflavone
flavonol
anthocyanidin
flavan-3,4-diol

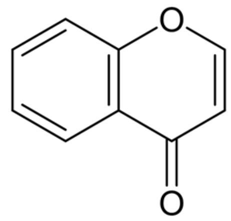
Other oxygen-containing heterocycles



4H-chromene
1,4-benzopyrane

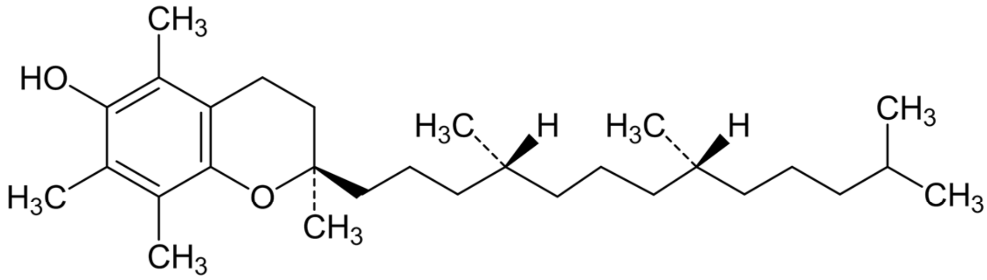


coumarin



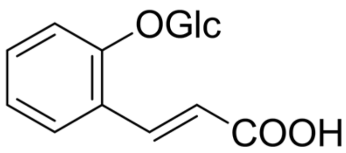
chromone
4-benzopyrone

Other oxygen-containing heterocycles

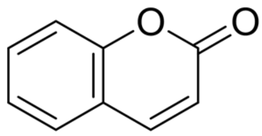


α-tocopherol, vitamin E
in cereal sprouts, lettuce, oily seeds

Coumarin and its derivatives



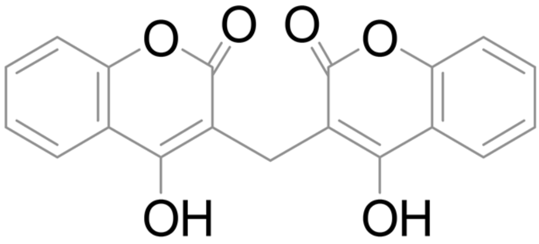
glykoside of
***o*-coumaric acid**



coumarin

- present in lavender oil, clover
- pleasant fragrance similar to vanilla

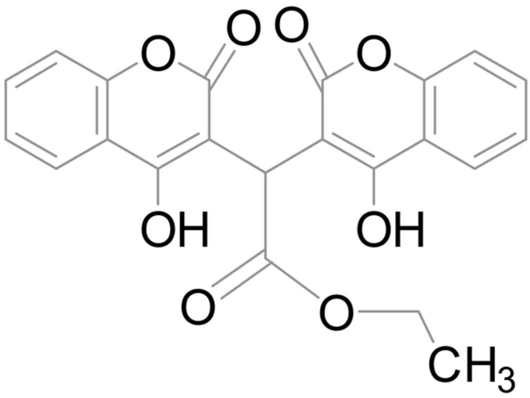
Coumarin and its derivatives



melitoxin
(dicoumarol,
bishydroxycoumarin)

- in carious hay containing clover
- inhibits coagulation of blood (anticoagulant, „antivitamin K“)

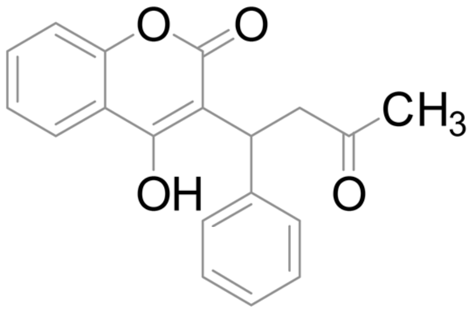
Coumarin and its derivatives



Pelentan
(ethylbiscoumaracetate)

synthetic analogue, used for treatment of thrombosis or heart attack as anticoagulant

Coumarin and its derivatives



Warfarin
anticoagulant

- rat poison (rodenticide) – internal bleeding (inhibits formation of prothrombin, increases fragility of capillary walls)
- used for human patients with thrombosis
- tested in human medicine - antimetastatic effect in lung cancer

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Antagonistic effect to vitamins K and P (the same skeleton slightly modified had an adverse effect!)

Main functions of flavonoids in plants

- pigmentation of tissues
- protection from UV light
- defence from insects (repellents)
- defence from fungi (phytoalexins)
- stored in vacuoles
- detoxication - binding ions (sulphates, positions 3 or 3’) – plants growing on seashore

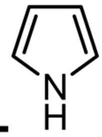
Ecological function of colours

- attracting pollinators
- attracting seed spreaders
- defence from predators (warning)

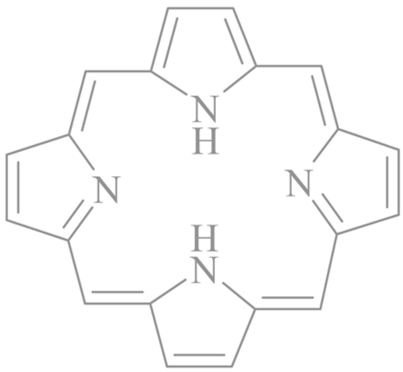
Use of flavonoids

- food additives (from strongly pigmented fruits); extraction with water or alcohol
- at pH>4 colour not stable (stabilisation)
- vitamins
- food supplements

Pyrrole-based pigments



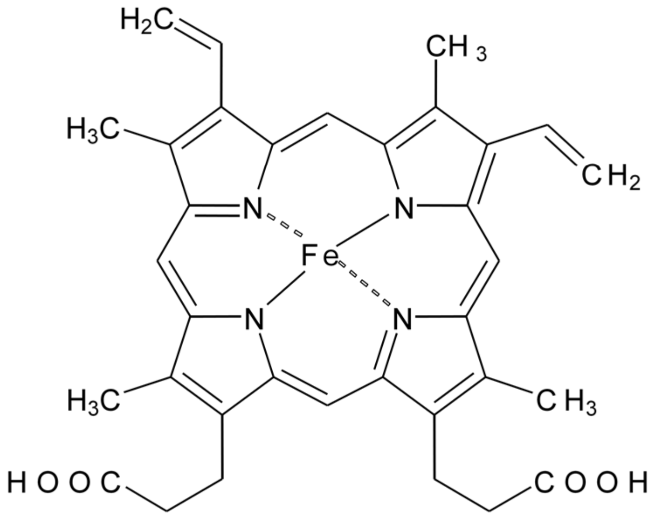
- **pyrrole ring** – base of 2 pigments necessary for plants and animals - blood pigment **haemoglobin** and leaf green **chlorophyll**



porphyrin skeleton – 4 pyrrole rings connected by methine bridges

looses easily acidic hydrogens from nitrogen atoms and forms anion (binds metal cations)

Heme (porphyrin) - **haemoglobin** (heme + protein) - **erythrocytes** (red blood cells containing haemoglobin)



heme (haem)

HEME

heme – coordination of oxygen to iron atom (NOT oxidation of iron atom)

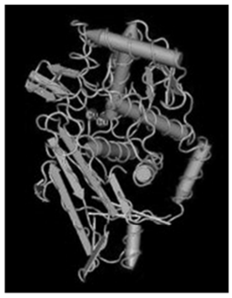
Heme is present in all vertebrates and some invertebrates

Heme-like compounds (different color) are present in some animals (e.g. chlorocauroheme) – green blood

Other blood pigments – haemerythrins and haemocyanins

- **Haemerythrins**
- metalloproteins of molluscs
- red blood
- Fe(II) → Fe(III)
- colour transition: light yellow → red
- coordination of oxygen
- lower importance than haemocyanins

Haemocyanins

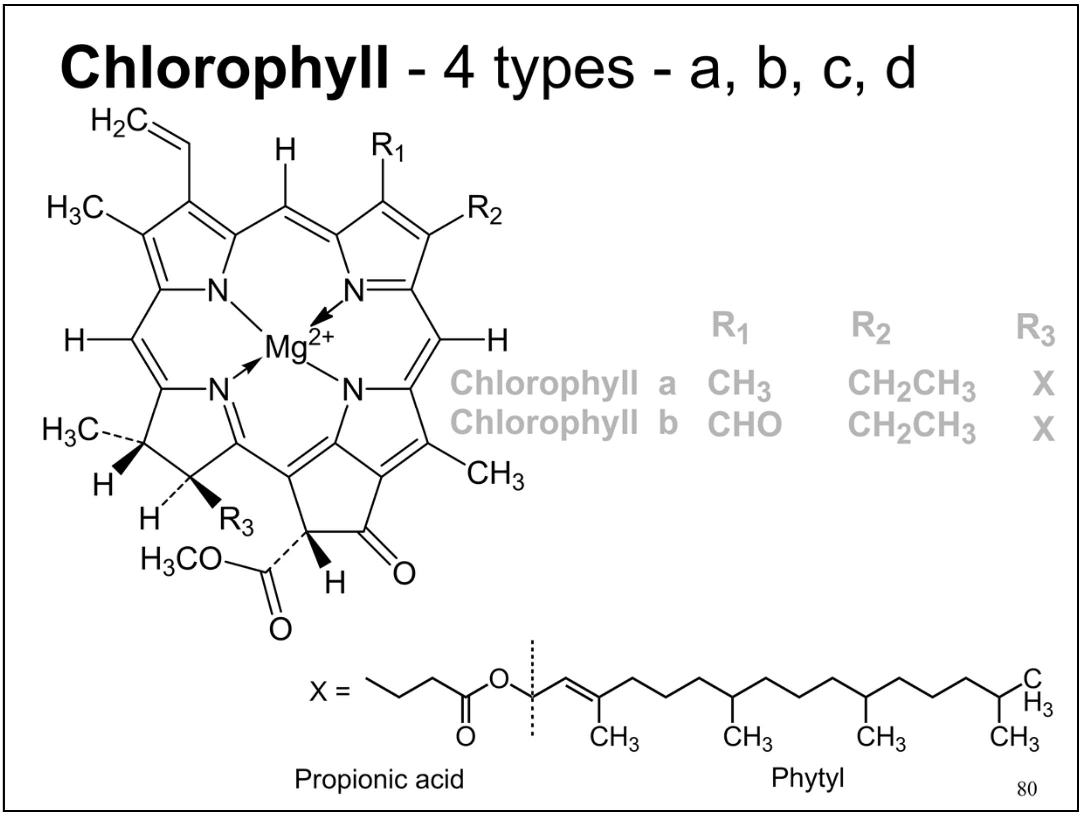


- in molluscs and some arthropods
- blue blood
- Cu(I) → Cu(II)
- colour transition: colourless → blue
- free in haemolymph (not bound to blood cells)
- coordination of oxygen

Species using hemocyanin for oxygen transportation are commonly crustaceans living in cold environments with low oxygen pressure. Under these circumstances hemoglobin oxygen transportation is less efficient than hemocyanin oxygen transportation.

Cytochromes – structurally related to haemoglobin

- similar to heme
- electron transfer in organisms
- in all animals, plants,
  and aerobic microorganisms
- main cytochrome groups - a, b, c, d
- cytochrome P450 - absorbs at 450 nm
- coordinated metal is iron
- oxidases - cofactor is oxygen, reaction
  $RH \rightarrow ROH$



CHLOROPHYLL

phytol – diterpenic alcohol

Total synthesis by Woodward (1944 quinine, 1951 steroid skeleton, 1954 strychnine, 1960 chlorophyll, 1965 Nobel prize)

Chlorophyll

- higher plants and green algae - chlorophyll a:b 3:1
- red algae - chlorophyll a, chlorophyll d
- total synthesis of chlorophyll a - Woodward 1960
- industrial isolation and use for dyeing of cosmetic products, food, leather
- in colour photography
- source of phytol (production of vitamins E and K)
- anti-knock additive for fuel
- accelerator in vulcanisation of rubber
- odour adsorber
- healing of skin injuries (lesions)

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CHLOROPHYLL

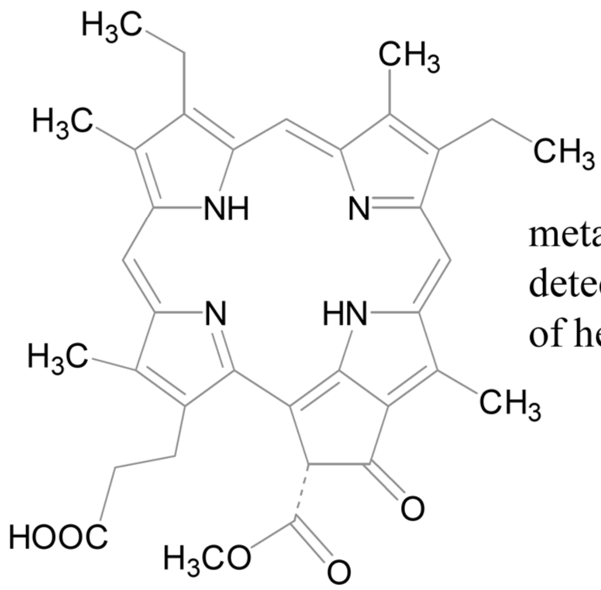
In some less frequent types of chlorophyll (e.g. in green algae) is bound geranylgeraniol or farnesol instead of phytol (bakteriochlorofyl)

Isolated chlorophylls are instable, easily undergo isomerization and change color

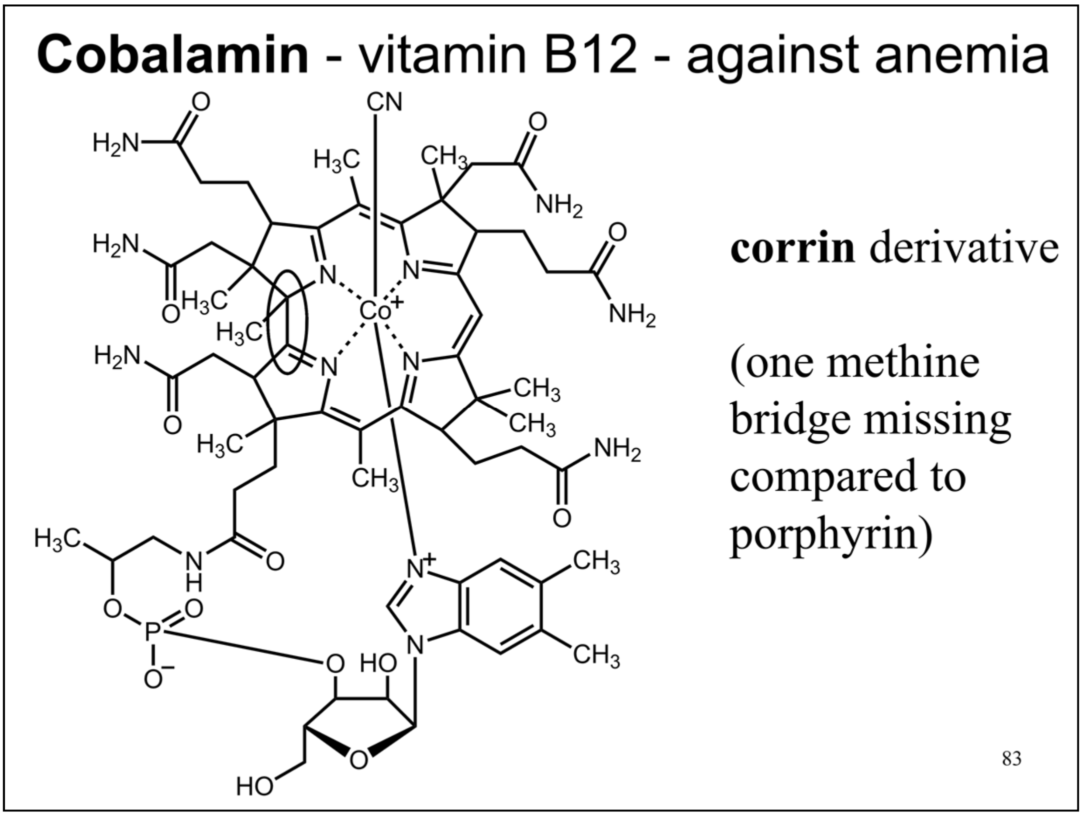
Chlorophyll can be extracted by polar solvents.

Chlorophyll in animals – reported a couple of case, but it is produced by symbionts (algae). Some animals are able to transfer functional chloroplasts from algae, accumulate them in its body (e.g. mollusks). Chloroplasts then function in the new organism exactly as in the original algae. Such animals are of green color.

Phylloerythrin



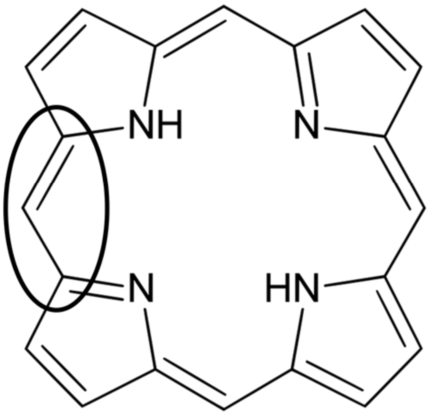
metabolite of chlorophyll detected in digestive tract of herbivorous animals



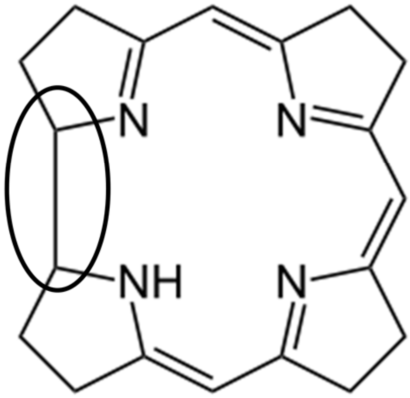
PORFYRINS AND THEIR PRACTICAL USE

Synthetic derivatives with exchanged pyrrol rings are used as photosensitisers (treatment of cancer, targeted transport into the tumor followed by laser irradiation, release of singlet oxygen that damage cancer cells).

Porphyrin and corrin



porphyrin



corrin

Cobalamin - vitamin B12

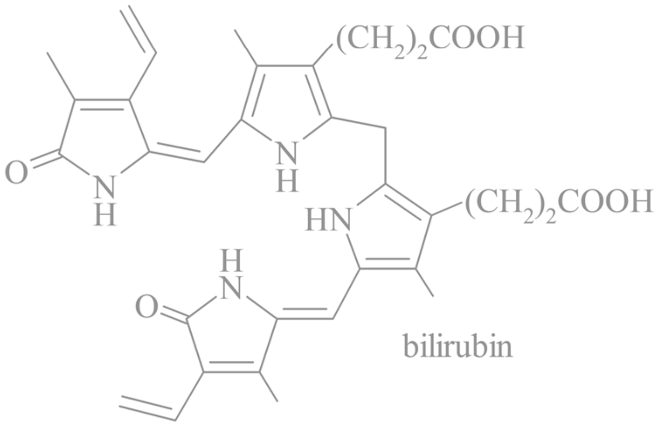
- cobalamins are natural complexes of cobalt
- present in fish, meat, liver, diary products
- plants don’t produce cobalamins
- first isolation from liver in 1948, structure 1955, total synthesis 1973 (Woodward)
- biotechnological industrial production (bacteria)

Bile pigments

- linearly bound pyrrole rings by methine or methylene bridges - basic structure of bile pigments
- present in both animals and plants
- in animals – products of heme catabolism
- role in photosynthesis in some algae

Bilirubin

- orange-red, present in bile as glucuronide (glykoside of D-gluconic acid)

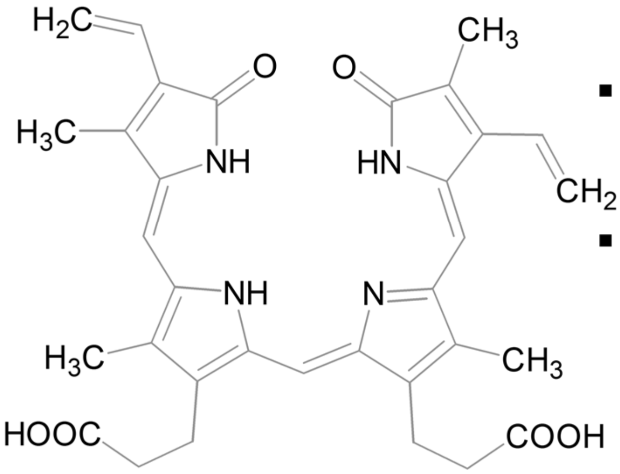


bilirubin

Other bile pigments

- **biliverdin** (green) and **urobilin**
- Similar structure present in the bird egg shells (green, blue), in insect wings (green, earlier incorrectly thought to be chlorophyll) and in sea algae.
- In vertebrates not visible on body surface, bile acids present in bile and excrements. Visible in illness - hepatitis (increased bilirubin level in blood).

Biliverdin



- body surface of caterpillars (large white, *Pieris brassicae*)
- in bile of amphibians and birds, in humans precursor of bilirubin, not detected in healthy people (high levels in liver carcinoma or cirrhosis)

BILIVERDIN

Bile pigments differ in positions of substituents at terminal pyrrol rings and by number of double bonds (therefore shifts of colors from red to green – absorption at higher wavelength)

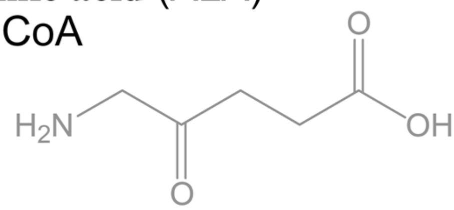
Other bile pigments

- similar compounds in algae - **phycobilins** (phycobiloproteins)
- phycobilins are classified depending on absorption maximum: **phycocyanins** (blue pigment) **phycoerythrins** (red pigment) **allophycocyanins** (light blue pigment)

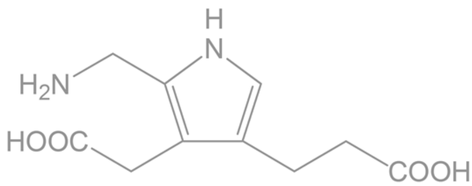
Biosynthesis of tetrapyrroles

5 basic steps, in common for formation of heme and chlorophyll

1. formation of **δ-aminolevulinic acid** (ALA) from glycine and succinyl CoA



2. formation of pyrrole ring from two molecules ALA, formation of **porphobilinogen**

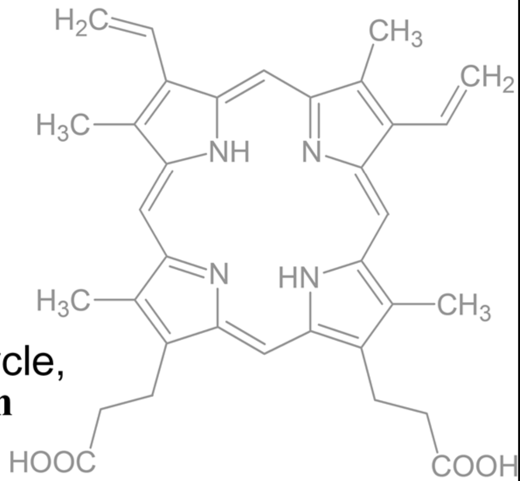


Biosynthesis of tetrapyrroles

3. closing of tetrapyrrole cycle – formation of **uroporphyrinogen**

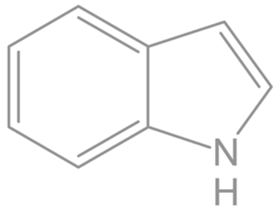
4. modification of tetrapyrrole cycle by introducing side chains

5. dehydrogenation of tetracycle, formation of **protoporphyrin**



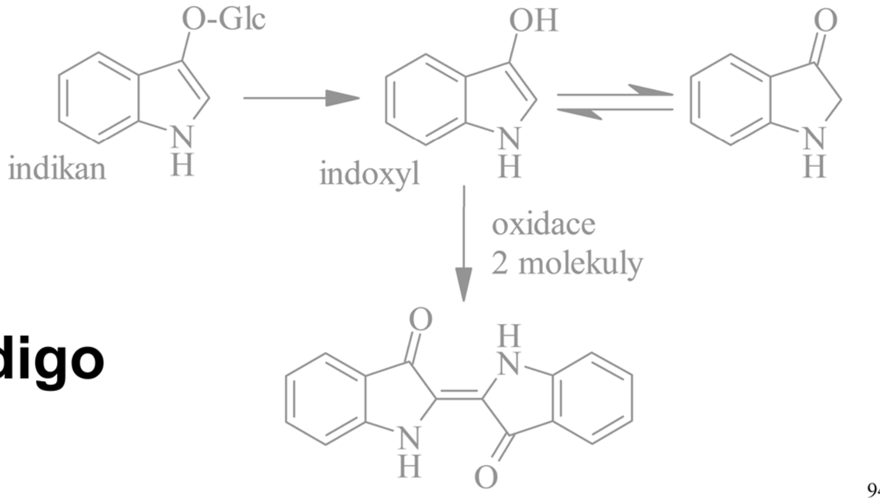
Indole-based pigments

- **indole** derivatives – flower odour of jasmine and citruses
- crude indole smells like faeces, pure indole has a pleasant odour
- **indigo** (indigotin) – most famous indole-based pigment, probably the oldest pigment known



- in leaves of *Isatis tinctoria* and *Indigofera tinctoria* (Fabaceae) as glucoside **indican** (colourless)
- enzyme emulsin breaks glycosidic bond, yellow aglycone **indoxyl**
- oxidation by air to blue **indigo**

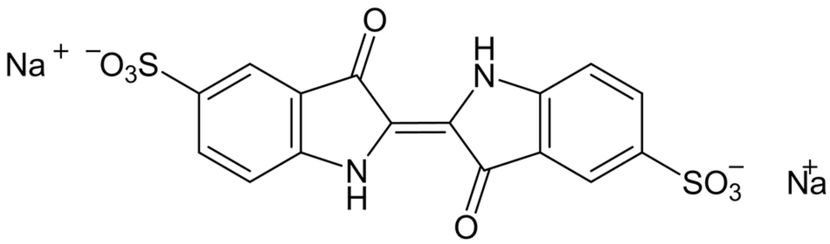
Indigo



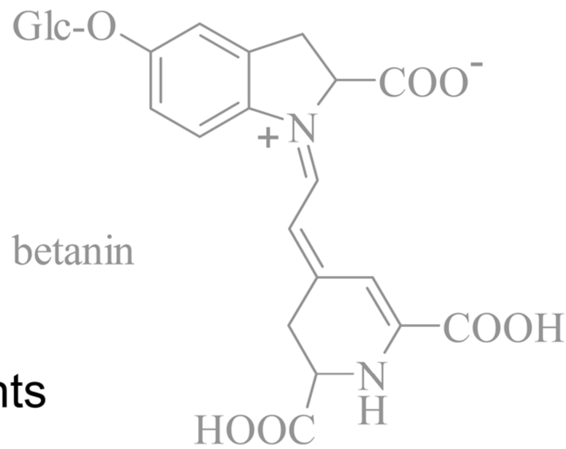
indigo (*trans* isomer)

Indigo

- insoluble in water and polar solvents
- used for dyeing fabrics and in medicine
- nowadays, phenanthrene dyes used instead
- **indigo carmine** – sodium salt of disulphate, soluble in water, approved in food industry and in medicine

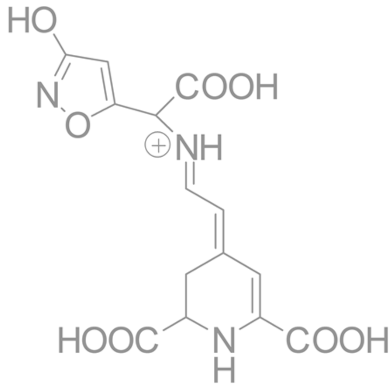


Betalains



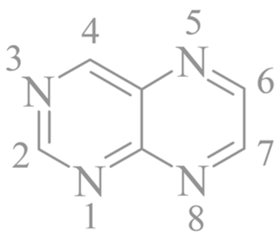
- ◆ indole-based pigments
- ◆ salts - **betanin**
- ◆ water-soluble pigment of red beets (*Beta)*; glucoside with aglycone **betanidin**
- ◆ produced exclusively by plants

- the same colours as anthocyanins, never occur together
- 2 main groups - **betacyanins** (**red-purple**) and **betaxanthins** (**yellow**)
- betaxanthins contain proline or another amino acid instead of indole (**muscaaurine**, *Amanita muscaria*)

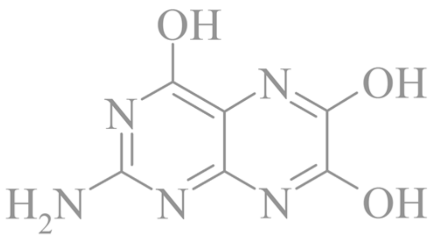


Pterines

- hydroxy and aminoderivatives of **pteridine** (pyrimidopyrazine)



pteridin

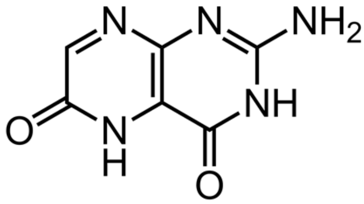


leukopterin

Pterines

- pigments of wings of butterflies and other insects (**pteros** = **wing** in Latin), bodies of wasps and bees, skin and eyes of fish, liver, yeast, also spinach
- colourless or yellow, fluorescent solutions
- **leucopterin** – wings of large white

Xanthopterin – yellow colour of wasps

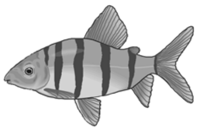


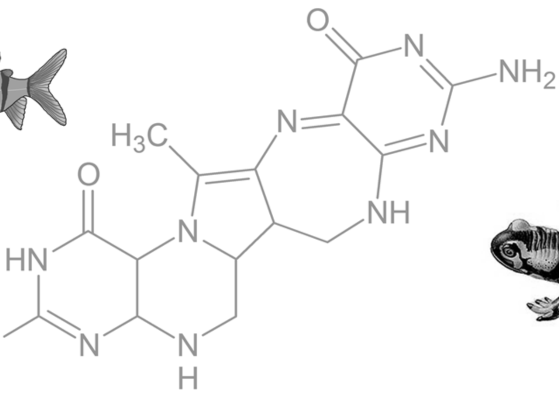
warning (threatening) coloured signal
yellow/black

(signalisation undertaken by humans)

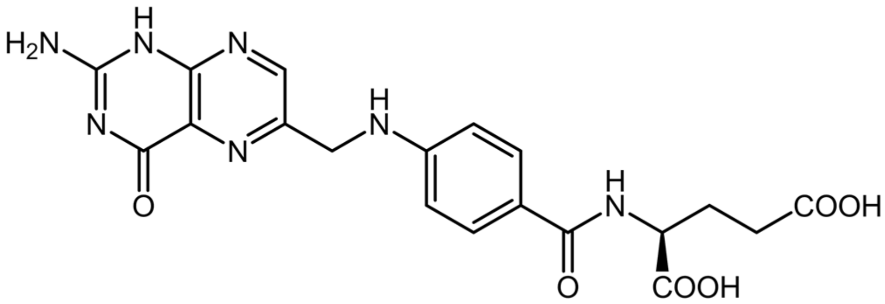
Dimeric pterines

- **drosopterin** (eyes of *Drosophila melanogaster*), similar compounds in fish, salamander, frogs (yellow-orange-red)





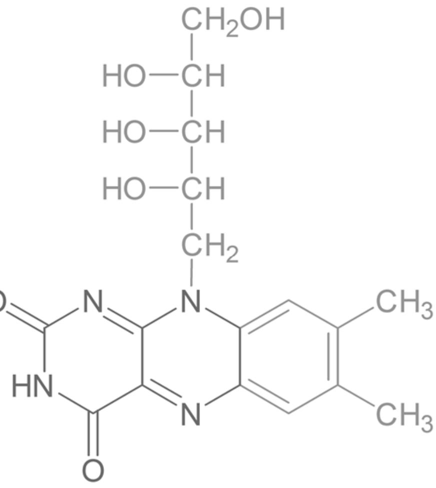
Folic acid is also a pteridine derivative (pteroylglutamic acid, PGA)



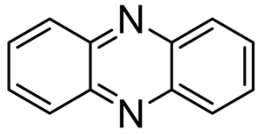
- yellow-orange crystals
- vitamin Bc, vitamin M
- necessary for growth of some microorganisms
- free or in combination of more molecules of (*L*)-(+)-glutamic acid present in liver, kidneys, mushrooms, spinach, and leaf vegetables

Yellow-orange colour of **riboflavin** (vitamin B2)

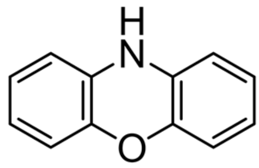
- coenzyme in redox reactions
- basic skeleton **isoalloxanthine**
- in milk, eggs, malt, liver, kidneys, leaf vegetables, yeast
- lack causes mucous inflammations



Phenazines and phenoxazines

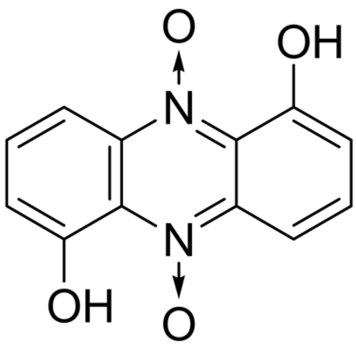


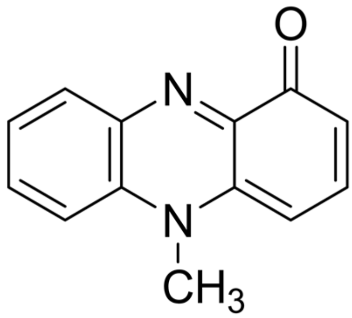
phenazine



phenoxazine

- phenazines are produced by microorganisms (*Pseudomonas*, *Streptomyces*); sometimes in ill animals (sheeps' hair coat)
- phenazines – first prove of antibiotic effect of one microorganism against another

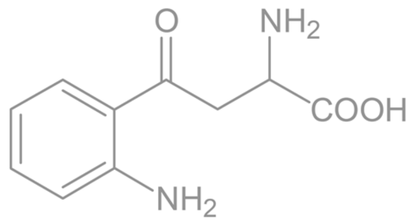




- **iodinin**
- purple pigment from *Chromobacterium* with antibiotic properties

- **pyocyanin**
- dark blue pigment from *Pseudomonas* with antibiotic properties

Phenoxazines form 2 groups –
ommatins and **ommins**
dimers and oligomers of **kynurenin** derivatives

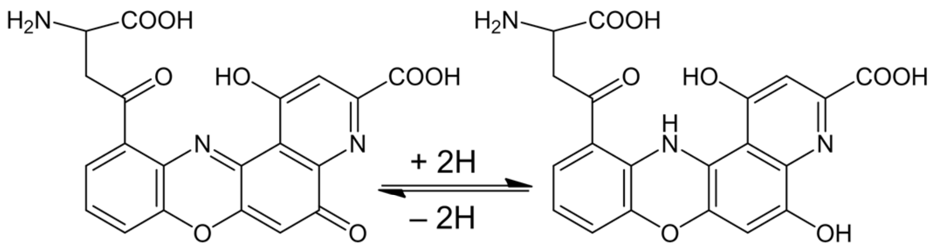


kynurenin

amino acid, formed in animal bodies
from tryptophan

Phenoxazine pigments – general term ommochromes

Ommatins

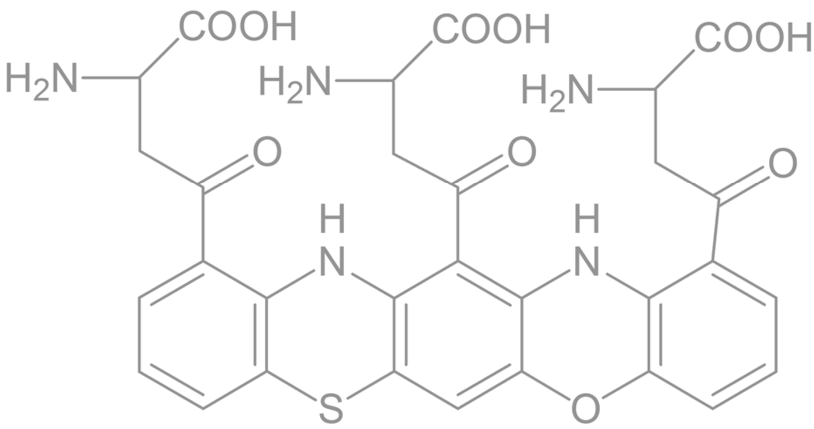


xanthommatin **dihydroxanthommatin**

yellow red

basic ommatin structure
(phenoxazine skeleton + 2 molecules of asparagic acid, one is cyclised, forms forth ring)
reversible dehydrogenation

Ommins are complex oligomers containing sulphur



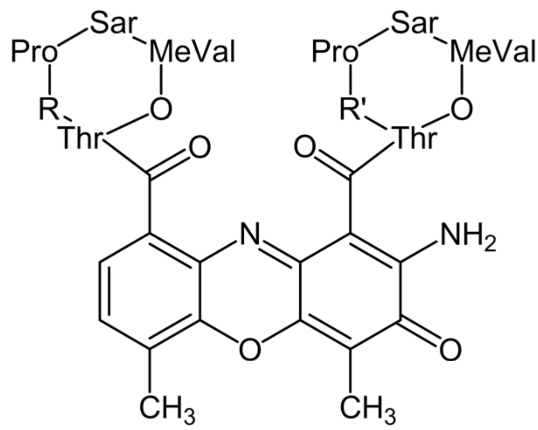
ommin A

Occurrence of ommochroms

- eyes of insect ant other invertebrates, complexes with proteins
- function as filters (protection of photoreceptors from intense light)
- not present in vertebrates
- biosynthesis *via* tryptophan
- structurally related papiliochroms are white and yellow pigments of butterflies' wings of a family Papillionidae (swallowtail)

Actinomycins

- ◆ antibiotic complex produced by *Streptomyces* fungi
- ◆ red microbial pigment
- ◆ phenoxazine skeleton bearing cyclic pentapeptide
- ◆ biosynthesis similar to xanthommatin



Actinomycin C_2 **R = D-Valin**
R' = D-Alloisoleucin

Actinomycin C_3 **R = D-Alloisoleucin**
R' = D-Alloisoleucin

Chemistry of pollination

- **pollinators:**
- insects
- bats
- birds (humming birds)
- small rodents
- wind in small number of Magnoliophyta (grasses)

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Plants (ferns, gymnosperms – conifers) approx. 230 mil. years

Angiosperms (flowering plants) approx. 60 mil. years

Insect pollinators approx. 60 mil. years

Examples

bat (cacti)

humming bird (*Fuchsia*)

honey bee (*Digitalis*)

Chemistry of pollination

- both partners (plant and pollinator) profit (**synomone**)

- biochemical factors playing role in pollination:
- odour, fragrance
- colour
- nutrition value of nectar and pollen

Chemistry of pollination

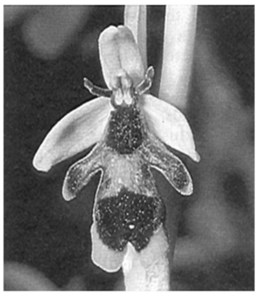
- **temperate zone** – main pollinators – Hymenopteran insects (bees, bumblebees), maximum activity during day
- **tropics** - broad spectrum of pollinators (humming birds, tropical butterflies, wasps, beetles, bats, moths, mice), activity both days and nights

- activity on flower – visitor
  pollinator
  nectar robbers

Nectar robbers – most often ants, but sometimes also bees (feed on nectar without touching stamen and transfer of pollen)

Co-evolution and its consequences

- majority of plants needs pollinators
- male and female flowers often on different individuals
- immunological barriers of self-pollination (self-incompatibility)
- co-evolution – adaptation of shape, colour, and fragrance of a flower and body shape of a pollinator to reach highest efficiency
- extreme cases – flower is pollinated by one insect species only (pollination syndrome) - orchids and solitary bees



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pollination syndrome – mimesis of flower fragrance and shape

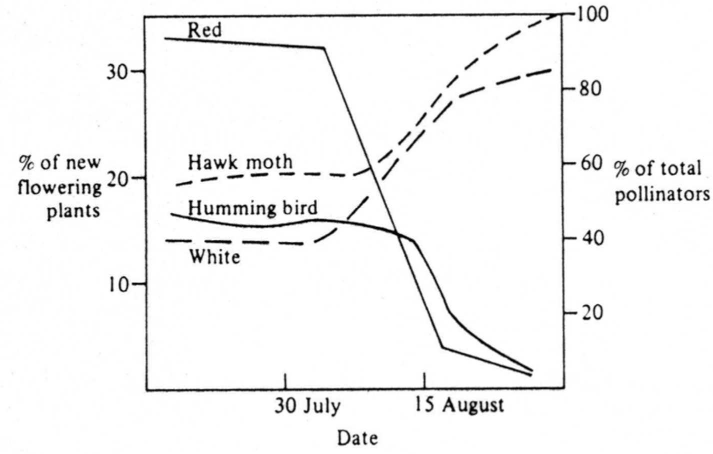
self-incompatibility – genetic variability in a population (plants need cross-pollination, transfer of pollen from another plant of the same species)

Fidelity of pollinators is due to their ability of learning and due to the flower color, shape, and fragrance (evolutional adaptation)

Pollinator	Colour preference		Note	Pigment
bees	yellow and intense white	blue	insensitive to red, vision in UV	carotenoids, flavonol, delphinidin
butterflies	bright colours, red and purple			pelargonidin
moths	red and pink		night activity	pelargonidin, peonidin
flies	pale, brown, purple, green		„chequered“ patterns	cyanidin, carotenoids, chlorophyll
beetles	pale, cream-coloured, green		bad colour vision	flavones, chlorophyll
wasps	brown			cyanidin, carotenoids
bats	white, green, pale		no colour vision	flavones, chlorophyll
mice	pale colours		night activity	
birds	bright colours, bicolour red/	yellow	sensitive to red	pelargonidin

Colour preferences of pollinators

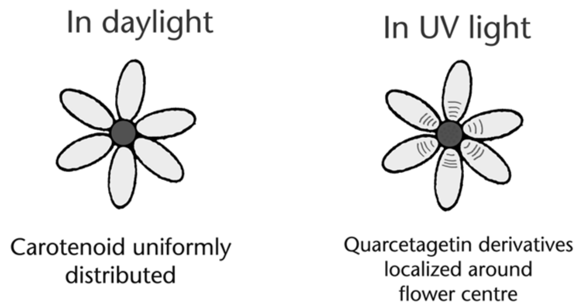
- each group of pollinators is sensitive to „their“ colours according construction of their vision



- seasonal change of flower colour (from red to pink to white) – adaptation to activity of pollinators during season (*Ipomopsis*, Arizona)

Different function of colours

- *Rudbeckia* – pollinated by bees (yellow flowers)
- 2 types of pigments:
- **carotenoids** – yellow colour (day light), large flower visible from long distance
- **flavonoids** – guide the bee in UV light directly to nectar sources (honey guide)



Combination of pigments is a common strategy of flowers.

Flower fragrance

- smell is adapted to type of pollinators
- maximum production (release) is timed to ripe pollen and maximum activity of pollinators
- „diurnal cycle“

Spathiphyllum

Flower fragrance

- industrial use in perfumes (esencial oils – rose oil, lavender oil)
- first perfume manufacture founded by Dominican monks in Florence (1608)
- nowadays, perfume industry uses more synthetic compounds
- perfume – usually a mixture of around 100 compounds

Animal perfumes

- **musk** – gland secretion of muskrat or Siberian musk deer
- **ambergris** – intestines of the sperm whale
- **civet** – perineal glands of African civette, similar odour also in beaver

- gas chromatograph with olfactodetector

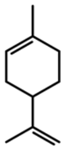
Patrik Süskind: Parfume; interesting book and movie on influence of human odors to other people

Flower scent (bad smell)

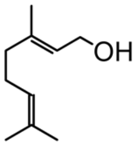
- bad smelling flowers are less investigated
- typical chemical mimics: flower smells faeces or rotten meat to attract carrions and some fly species

Scent of flowers and seeds

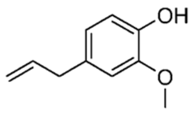
scent - monoterpenes, sesquiiterpenes, aromatics, aliphatic alcohols and aldehydes



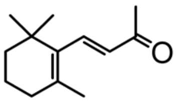
limonene
(+) citruses
(–) pine



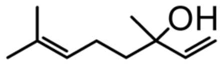
geraniol
Geranium



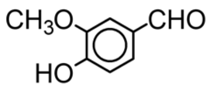
eugenol
clove



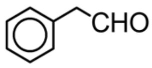
β-ionone
Viola



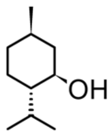
linalool
Daphne, coriander



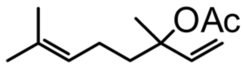
vaniline
vanilla beans



phenylacetaldehyde
hyacinth, lilac



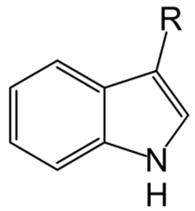
menthol
peppermint



linalool acetate
lavender

Bad smelling flowers

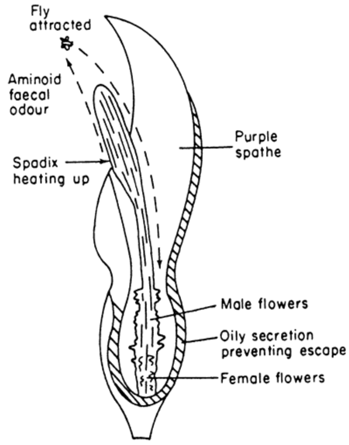
- **smell** - amines (methyl- to hexylamine, fish smell), sometimes NH_3, α,ω-diamines (smell of degraded proteins), nitrogen-containing heterocycles - indol, scatol (faeces), sulphides and polysulphides (dimethyl disulphide, dimethyl trisulphide)



R = H, indol
R = CH_3, scatol

Amorphophallus (Araceae)

- temperature on spadix surface increases to 30 °C $\Rightarrow$ increased evaporation of volatiles attracting pollinators (flies)



- temperature increase is due to fast respiration (burning of large amounts of pool starch)
- signal = salicylic acid

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Salicylic acid – the same (pyretic) effect in snowdrop, antipyretic effect of aspirin, SOS signal (parsimony)

Bad smelling flowers

- carnivorous plants sometimes produce bad smell to attract insects, not for pollination
- **pitcher plant** (*Sarracenia*) - produces alkaloid coniine (mousy smell)
- **coniine -** 2 roles: attraction of flies and paralysis to keep them from escape

Pitcher plant (*Sarracenia leucophylla*) – 8 carnivorous *Sarracenia* species (Northern America), flower shape is a trap for insects

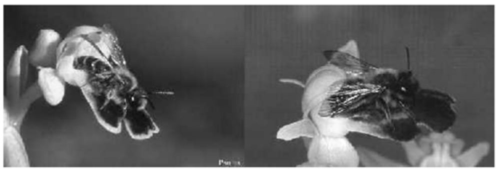
Bad smelling flowers

- relation between pollinator and flower is partially based on learning (habit)
- **datura** (*Datura*) produces hallucinogens or drugs; insect feeds on nectar containing drug and becomes addicted to tropane alkaloids

Flower scent and insect pheromones

- may contain identical compounds (co-evolution); insect is attracted by flower that smells like a mate (pseudocopulation – mating behaviour released by sex pheromone)
- typical example – solitary bees and orchids (*Ophrys*)

Flower shape and colour, flower scent



- *Psithyrus vestalis* a *Ophrys chestermanii* (Sardinia)
- flower scent contains components of female sex pheromone
- males is attracted and pollinates (pseudocopulation), but he is not rewarded with nectar

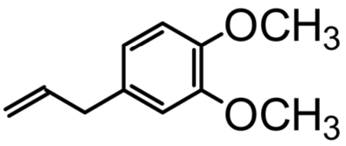
Deceit pollination in orchids


Bombus terrestris queen
on a flower of *Orchis pauciflora*

- flower scent contains 2,3-dihydrofarnesol
- (*S*)-enantiomer present in both, male pheromone and flower scent
- bumblebee queens are main pollinators (*B. terrestris*)
- are queens attracted by pheromone? (in *Ophrys*, males are attracted)

Flower scent and insect pheromones

- fruit fly *Dacus dorsalis* - sex pheromone is **eugenol methylether**



Eugenol methylether

- produces by inflorescences of golden shower tree (*Cassia*, Fabaceae, Egypt)
- flower scent attracts different moths
- flowers used as attractants in traps
- 0,1 mg elicits a strong response in insects
- compound stimulates males to feed (**fagostimulant**) – continuous exposure leads to males' death from overfeeding

Nectar and pollen

- function of **nectar** is attraction of pollinator, award for flower visit (experience)
- nectar components:
- **sugars**
- **amino acids**
- **lipids**
- **toxins**
- **nectar** is mainly water solution of **sugar** (15–75 weight %) - glucose, fructose, and sucrose

Nectar

- **amino acids -** minor component (1–9 %); all essential amino acids; sufficient source of nitrogen for most insects; nectar with high content is sought out by carrion insects
- **lipids –** present in some families only (bee-pollinated); triglycerides and free fatty acids;
  rich energy source
- **toxins –** rarely in nectar, reported intoxications by honey collected on *Rhododendron* (acetylandromedol) or ragwort (*Senecio,* pyrrolizidine alkaloids)

Rhododendron

Senecio

Nectar and pollen

- function of **pollen** is a transfer of male genetic material for reproduction of plants
- main food source for Hymenopteran insects and beetles

Pollen

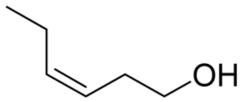
- ***Components:***
- **proteins** (16–30 %)
- **polysaccharides** (1–7 % škrobu)
- **monosaccharides** (0–15 %)
- **lipids** (3–10 %)
- **trace components** (vitamins, salts)
- sometimes **odorants**, different from flower scent
- overproduction of pollen has ecological significance for pollination, but also for reward and nutrition for pollinators

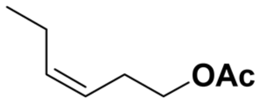
Host plant signals

- control important developmental phases of insect life – host finding (**primer attractants**), feeding (**fagostimulants**), and egglaying (**oviposition stimulants**)
- primer attractants lead “specialised” insects to their host plants (**kairomone**)
- herbivorous insects are usually specialised to one or a few related plant species

Host plant signals

- volatile attractants (perceived by smell) – two types:
- **non-specific** - aliphatic alcohols (**3-hexenol**) - „green leave volatiles“





- **specific** - often monoterpenes in typical composition in each species
- for host finding, the complex mixture (pattern) of volatiles is important (qualitative and quantitative composition)

electroantennography – sensitivity of antennae to host plant volatiles is 1-2 orders of magnitude lower than to pheromones

hexenol or hexanol (green leave volatiles) is used in electrophysiology as standards – confirmation that the antenna is in a good physiological state able to respond to tested compounds

Host plant volatiles

carrot - *Daucus carota* – pest carrot psyllid (*Trioza apicalis*), lays eggs and feeds on Apiaceae

carrot
(*Daucus carota*)

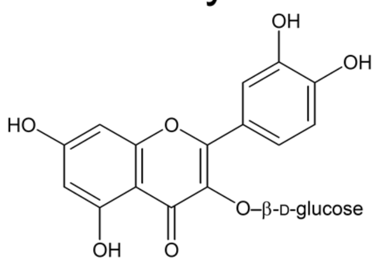
parsley
(*Petrosenimu crispum*)

fennel
(*Foeniculum vulgare*)

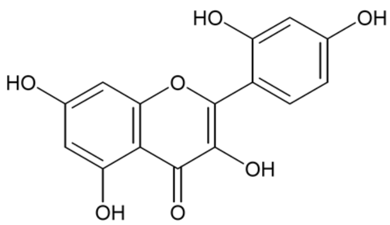
host plant is found even surrounded by weeds
volatiles composition is different in different species

Fagostimulants

- stimulate insects to feeding; some are necessary for growth and development (sugars, sitosterol), others are recognition signal (taste stimuli)
- silk moth (*Bombyx mori*) – feeds exclusively on mulberry



isoquercitrin



morin

Silk moth

- **3 types of signals from host plant:**
- **smell attractants** – essential oils (citral, linalool, 3-hexenol)
- **taste attractants** – flavonoids; morin is present almost exclusively in mulberries (host recognition)

- **thickening factors** – nutrition (cellulose), minerals

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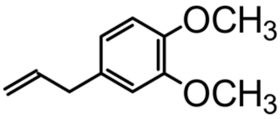
Mombyx mori – in tests when receptors were removed (antennae, palpae), the insect refuses to feed (missing stimuli).

If the monosacharide moiety in the flavonoid is changed (e.g rhamnose instead of glucose), the insect stops feeding (confirmed on artificial diet).

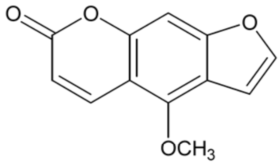
Oviposition stimulants

- stimulate phytophagous insects to egglaying on the „right“ plant
- signal – both volatile and non-volatile compounds from the plant surface
- combination of olfactory and taste cues, taste receptors often on tarsae (feet)

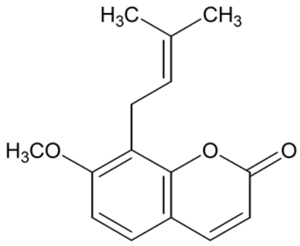
Carrot fly, *Psila rosae*



methyleugenol



bergapten



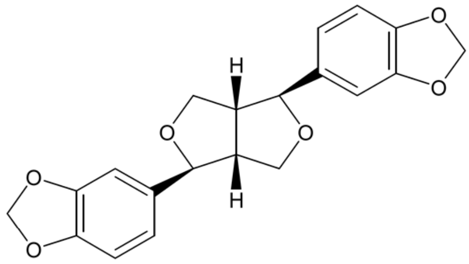
osthol

Food deterrents (antifeedants)

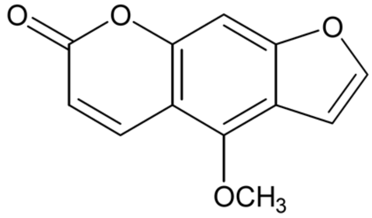
- not toxic, but cause avoiding contaminated food (prevents from feeding)

- ***types of compounds:***
- lignans
- terpenoids
- steroids
- heterocycles (alkaloids)
- chromenes
- quinones
- flavonoids
- tanins

Food deterrents

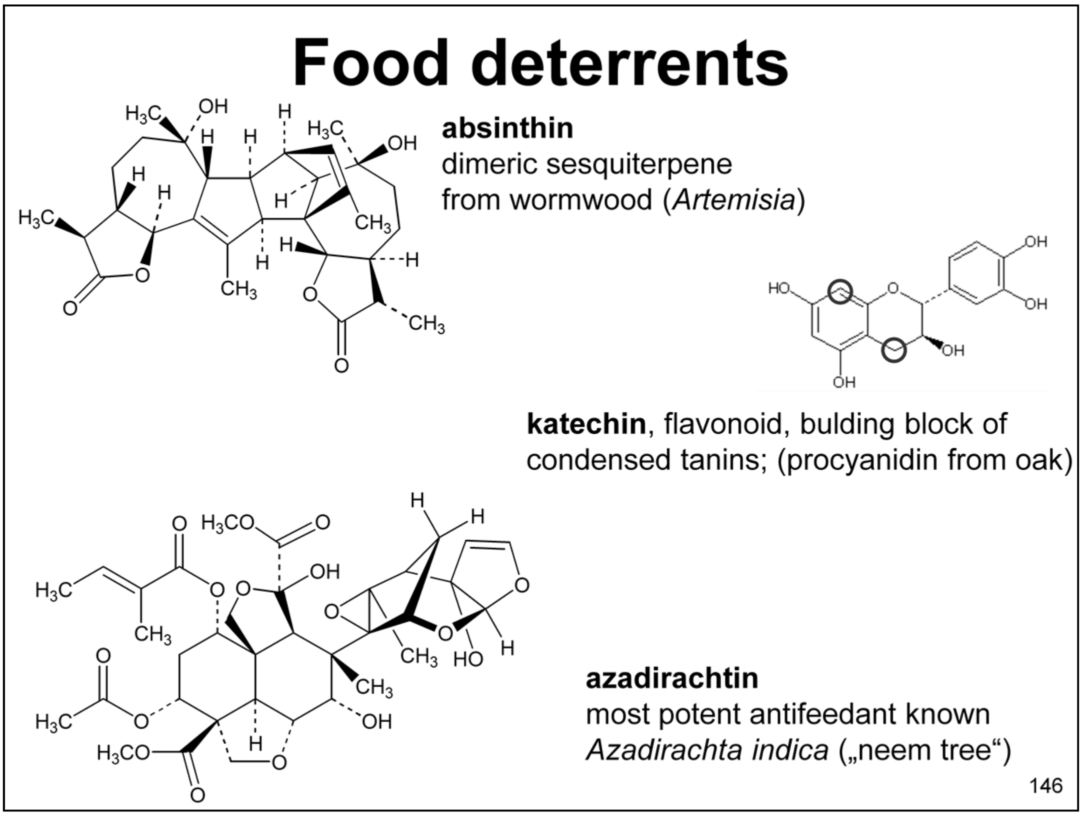


sesamin,
lignan from magnolia seeds



bergaptene,
from bergamot oil
(*Citrus bergamia*)

dichotomy of effects!



azadirachtin – triterpenoid, registred for use in organic growing, success in orchards (apples) or vegetable growing

application – 1% solution in organic plant oil, further diluted to 0.3% water emulsion, spraying 1000 l/hectar

Other deterrents – solanin, alkaloid from potatoes *Solanum tuberosum*, Colorado potato beetle (CPB) is however adapted to solanin

but:

demisin (a small change in the structure) from *Solanum demisum* is repellent for CPB (this potato species is resistent to CPB)

Food preferences of vertebrates and humans

- food choice depends on **taste** and **smell** regardless its nutritive value
- lack of reliable experiments
- expensive research in domestic animals
- unreliable data in wild animals
- all vertebrates significantly prefer sweet taste

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Difficulties of such research:

Research of effect of some plant products on herds of cattle would be too expensive (e.g. toxic effect of alkaloids)

Refusing of some plants as food may have two causes – absence of feeding stimuli or presence of a feeding deterrent

Wild animals feed on large areas, there is no control of plants they feed on

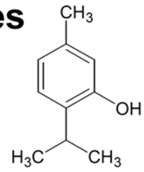
Humans - 5 basic types of taste

- **sweet** (tip of tongue)
- **bitter** (root of tongue)
- **sour** (sides of tongue)
- **salty** (tip and edge of tongue)
- **umami** (meet taste)

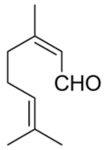
- threshold for perception of salty taste - 0,05 %
- bitter taste - 0,0001 %

Chemical basis of some aroma

Terpenes

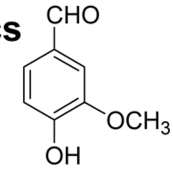


thymol
(mandarines)

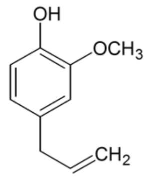


citral
(lemon)

Aromatics

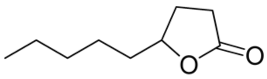


vanillin
(vanilla)

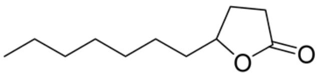


eugenol
(banana)

Lactones

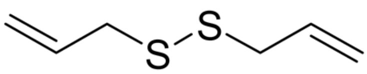


α-nonalactone
(coconut)

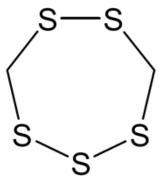


undecalactone
(peach)

Sulfur-containing compounds

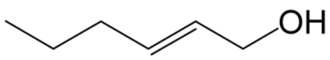


di-2-propenyldisulfide
(garlic)

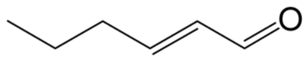


lenthionine
(mushrooms)

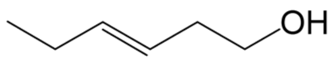
Green leaf odour



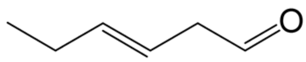
2- a 3-hexen-1-ol



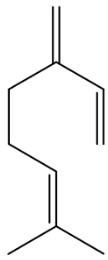
2- a 3-hexenal



fresh leaf odour

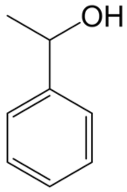


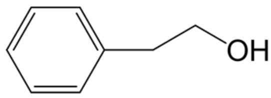
intense grass odour

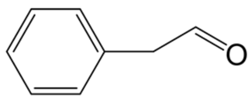


myrcene
hops odour

Flower odour



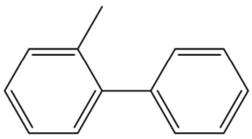


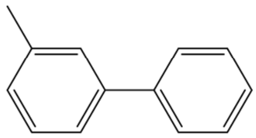


1- and 2-fenylethanol, fenylacetaldehyde

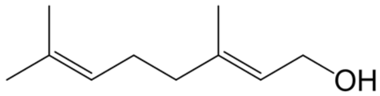
rose

hyacinth

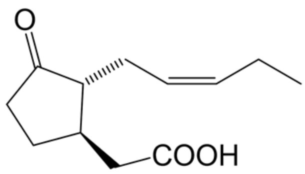




2- and 3-methylbifenyl
rose

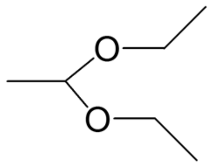


geraniol

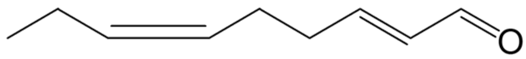


methyl jasmonate
jasmin odour

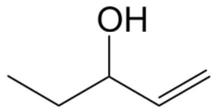
Fruit odour



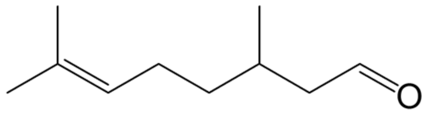
1,1-diethoxyethane



(2*E*,6*Z*)-2,6-nonadienal
melon odour



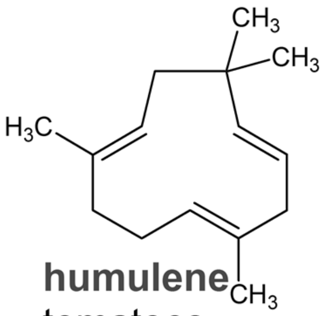
pent-1-en-3-ol
fresh fruits
(strawberries, blackberries)



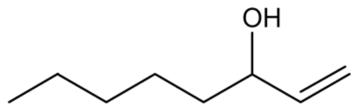
citronellal
lemon odour

aliphatic ketones - peach, banana
alkanes C_{11}-C_{15} - light fatty fruit odour

Vegetable odour



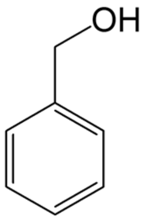
humulene
tomatoes



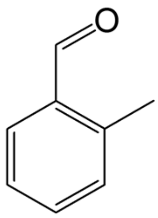
oct-1-en-3-ol
mushrooms

(*E*)-2-hexenal ..... raw potatoes
(*E*)-2-nonenal ..... fresh cucumber odour
dimethylsulfide ... fresh cabbage

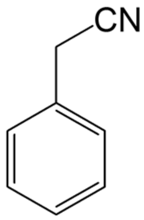
Nut odour



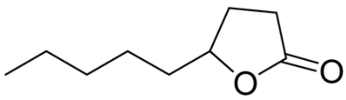
benzylalcohol
light almond odour



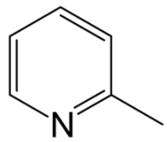
2-methylbenzaldehyde
bitter almond odour



fenylacetonitril
honey almond odour

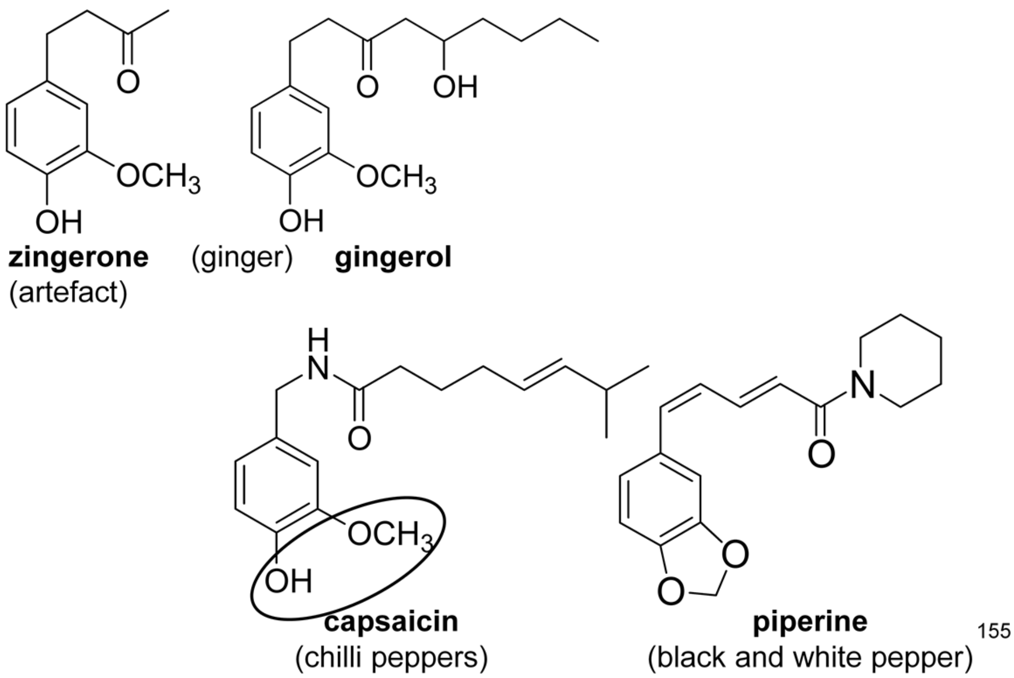


α-nonalactone
coconut



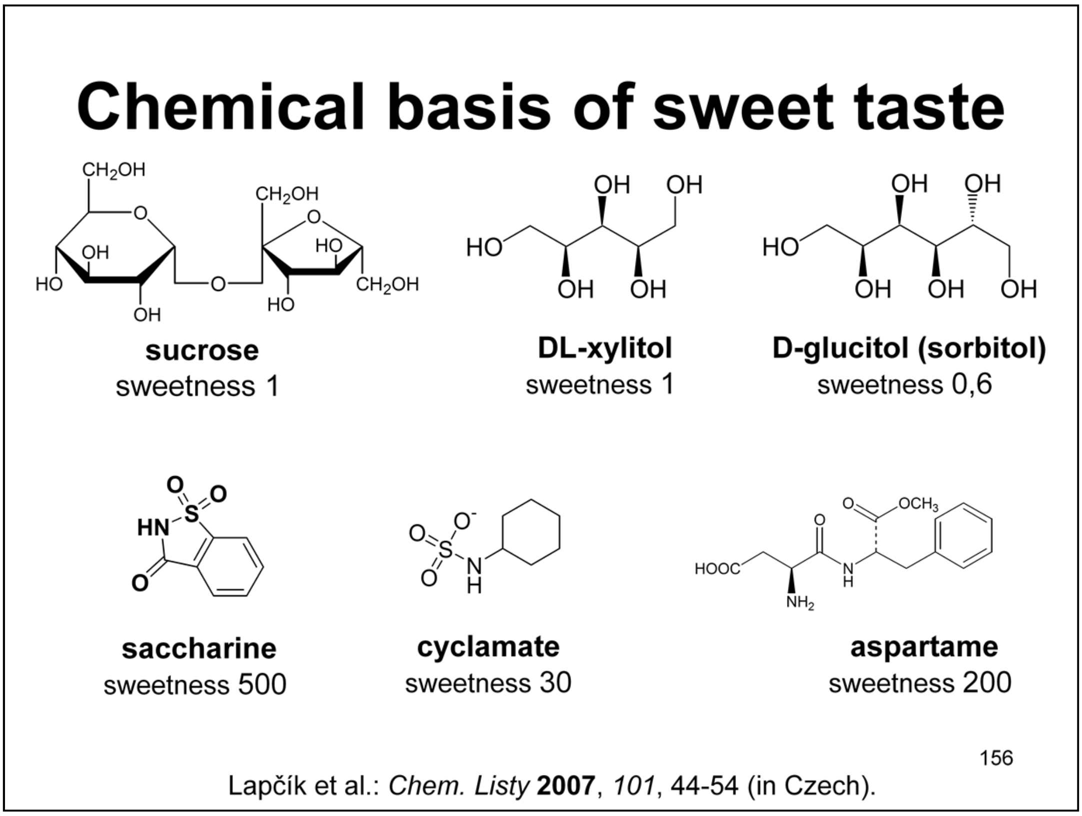
α-picoline
nut and rhum odour

Chemical basis of hot (spicy) taste



Zingerone is artefact

Free hydroxy- and methoxygroup are probably a cause of the spicy taste (pepper is less hot)



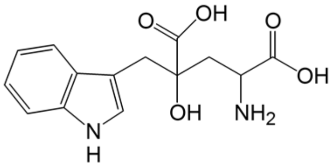
Cyclamate and saccharine are tentative carcinogens when used for a long time

xylitol – used in chewing gum, it has a cooling taste (high endothermic dissolving enthalpy)

Natural sweeteners

proteins – from seeds of different plants; sweetness 10^3

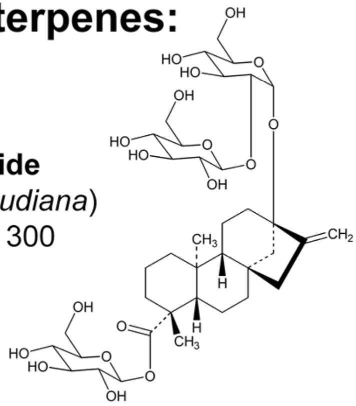
amino acids derivatives:



monatin
roots of South African bush
sweetness 10^3

terpenes:

stevioside
(*Stevia rebaudiana*)
sweetness 300

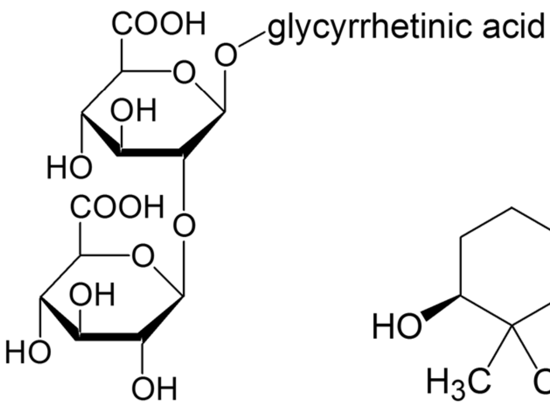


stevioside - diterpene

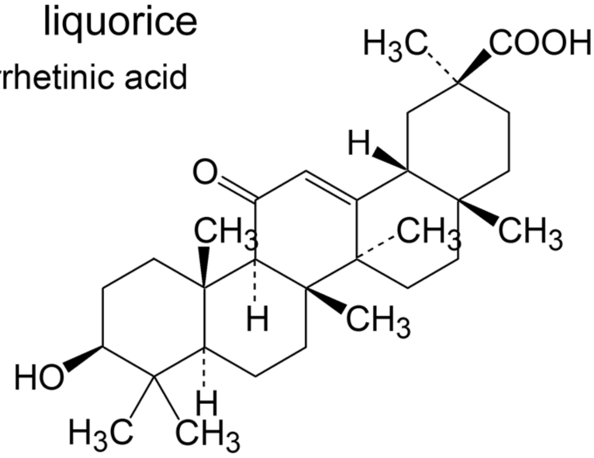
Natural sweeteners

triterpenes:

liquorice



glycyrrhizic acid



glycyrrhetinnic acid

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source: *Glycyrrhiza glabra*

Other non-sacharide sweeteners – steroids, some flavonoids, chalcones, and coumarine derivatives

Bitter agents

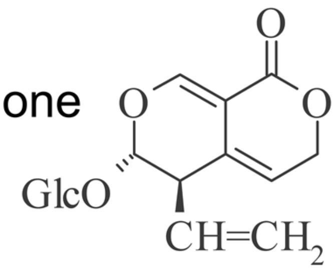
- bitter plant products without further pharmacological activity
- common occurrence, limited use
- different structural types (often glycosides)
- in some of them, chemical structure is not known
- **bitterness number** – the lowest concentration of a compound or extract that has a bitter taste

Bitter agents

- used in the form of extracts, tincture, or wine
- bitter agents - **amarae** – in small doses increase the appetite, secretion of gastric juice and influence its acidity
- **cholagogues** – stimulate secretion of bile or the gall bladder contraction to promote the bile flow
- used in food industry for production of liquors, aperitifs, and other bitter drinks

- ***Gentiana lutea*** (hořec žlutý) – perennial plants (up to 60 years old)
- roots harvested from 4th year
- protected, grown for medicinal effects
- dried roots
- content - glykosidic bitter agents – pyrane derivatives - **gentiopicrin** (bitterness number 12 000), **amarogentin** (bitterness number 58 millions), and **gentiamarin**, essential oil, tannins, and sugars (gentiobiose and sucrose)

- yellow pigment **gentisin** (xanthone derivative)
- the drug stimulates secretion of gastric juice and digestion of nutrients, division of blood cells
- **gentiopicrin, erythaurin** and its aglycone **erythrocentaurin** are present in ***Centaurium erythraea*** (zeměžluč menší), annual or biannual herb (harvest of flowering aerial parts)



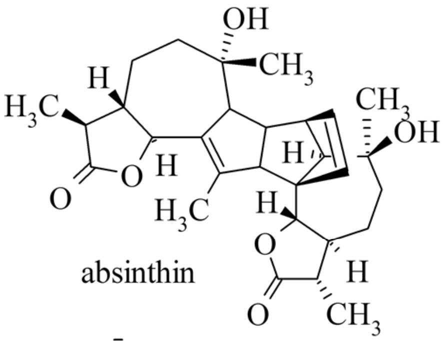
gentiopikrin

- **Absinthe wormwood** (*Artemisia absinthium,* pelyněk pravý) - perennial (South and Middle Europe)
- harvest of flowering aerial parts in full sun

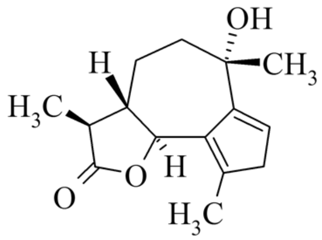
Absinthe wormwood

- content - 2 % essential oil (more than 20 components)
- main component - **β-thujone** (60 %)
- toxic, causes irreversible neurodegenerative damage
- other components – bitter agents **absinthin** and **artabsin**, bitterness number 10 mil.

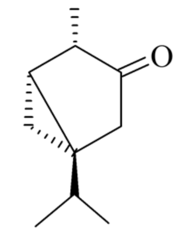
Components of absinthe wormwood



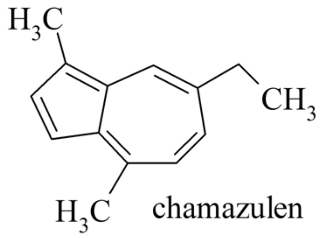
absinthin



artabsin



β-thujon



chamazulen

Essential oil of absinthe wormwood

- hydrodistillation – decomposition ⇒ **chamazulencarboxylic acid** ⇒ decarboxylation, the final product is **chamazulene** (blue colour)
- drug stimulates secretion of gastric juice (digestive)
- essential oil is toxic, causes nausea, dizziness, spasm, heady state, congestion of organs in pelvis (misused for illegal abortions)
- in low concentration additive in absinth or vermouth (nowadays prohibited in almost all countries)

- ***Achillea millefolium*** (řebříček obecný) – flowering aerial part contains 0,3 % essential oil (40 % chamazulene)

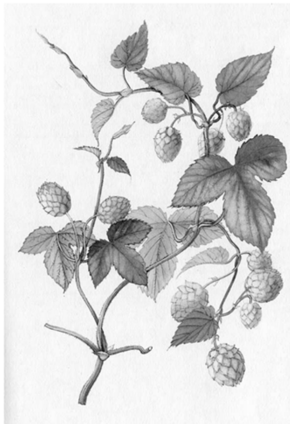
Bitter agent **achillin** oxidizes on air to chamazulencarboxylic acid that decarboxylizes to blue **chamazulene**

Achillea millefolium

- use - amarum, stomachikum
  and cholagogue
- in traditional medicine as anti-inflammatory agent

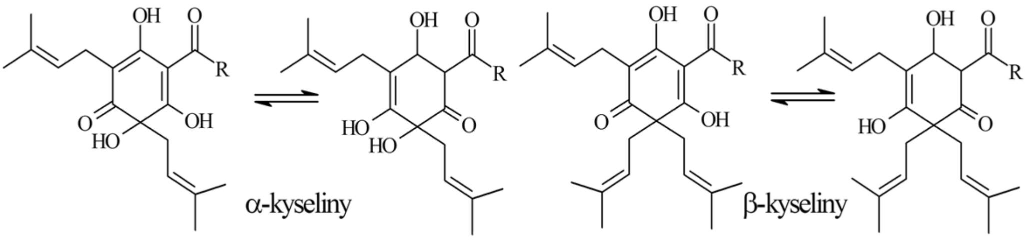
Acidic bitter agents

- **Hops** (*Humulus lupulus,* chmel otáčivý)
- drug - female burs, source of **lupulin**
- essential oil (1-3 %) consists of myrcene, caryophyllene, farnesene, humulene, and other components



Hops

- resin forms up to 80 % mass, contains mainly bitter agents (50 %), α- and β-bitter acids
- **humulone** (2–6 %) and **lupulone** (8–12 %)



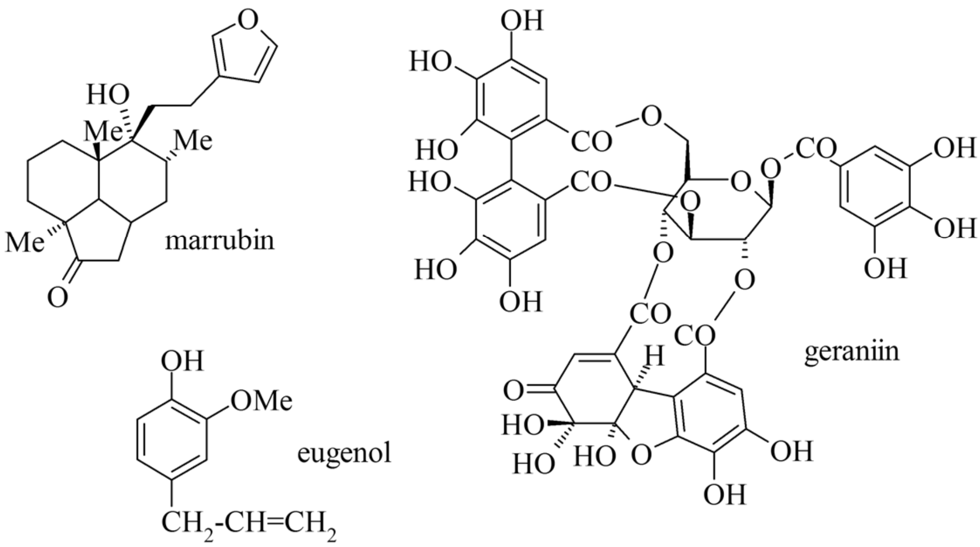
	R		R
humulon	CH_2-$CH(CH_3)_2$	lupulon	CH_2-$CH(CH_3)_2$
kohumulon	$CH(CH_3)_2$	kolupulon	$CH(CH_3)_2$
adhumulon	$CH(CH_3)CH_2CH_3$		

Bitter acids

- chemically instable, degradation in light and air (storage max. 1 year)
- taste and bacteriostatic effect - **isocompounds** arise from bitter acids in brewing process
- sedative effect, sleepiness, aphrodisiacs, amarum and stomachics
- hops extract has antibiotic and estrogenic effect

- ***Marrubium vulgare*** (jablečník) – perennial plant, origin in South Europe and Asia, occurring in Moravia as weeds, grown as medicinal plant
- flowering aerial part contains 6 % of diterpenic bitter agent **marrubiin**, further contains tannins, saponins, and ursulic acid
- effect - choleretics, expectorans, earlier used as substitution for quinine in treatment of malaria

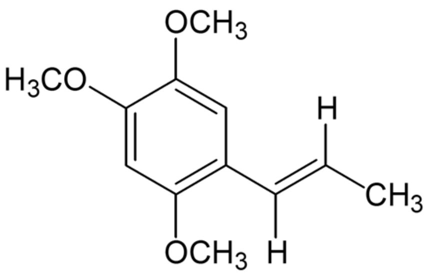
Bitter agents of *Marrubium* and *Geranium*



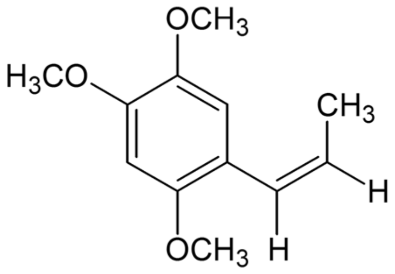
- ***Geranium robertianum*** (kakost smrdutý) – flowering aerial part contains bitter agent **geraniin**, 5–10 % tannins and essential oil
- drug stop diarrhea, used as diuretics in kidney stones

- ***Geum urbanum*** (kuklík městský) – harvest of rhizome or flowering aerial parts. Contains essential oil, tannins, yellow resinous pigment, and glycosidic bitter agent **gein**, degraded by fermentation to **eugenol**. Eugenol has antiseptic and light anaesthetic effect.

- ***Acorus calamus*** (puškvorec obecný) spread from Asia to Europe; grows in wetlands, harvest of rhizome
- content – essential oil (2–4 %)
- **β-asarone** and minor **α-asarone**

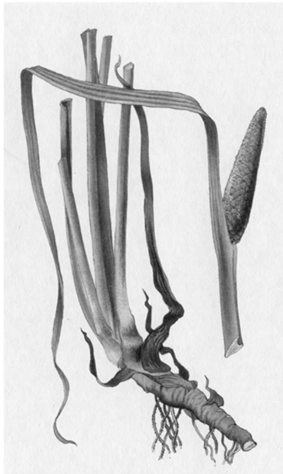


α-Asarone

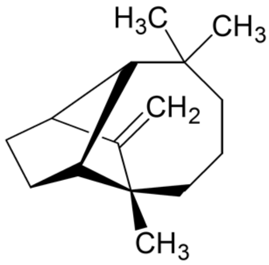


β-Asarone

- ***Acorus calamus***
- bitter agents **acorine** and **acoretine**; **choline**
- stimulates metabolism
- used for production of stomachic liquor (*Acorus* +*Gentiana*+*Angelica*+ *Centaurium*+fennel+caraway)
- asarone is suspected cancerogene, prohibited use in USA



- ***Juniper*** (*Juniperus communis,* jalovec obecný) – evergreen bush occurring in all Northen temperate zone, protected in CR
- drug – dried fruits, contains essential oil (2 %, terpenes - pinenes, cadinene, terpineol), up to 30 % invert sugar, inositol, 9 % resin, phytoncides, and bitter agent glycoside **juniperin**



longifolene (juniperene)

Juniper

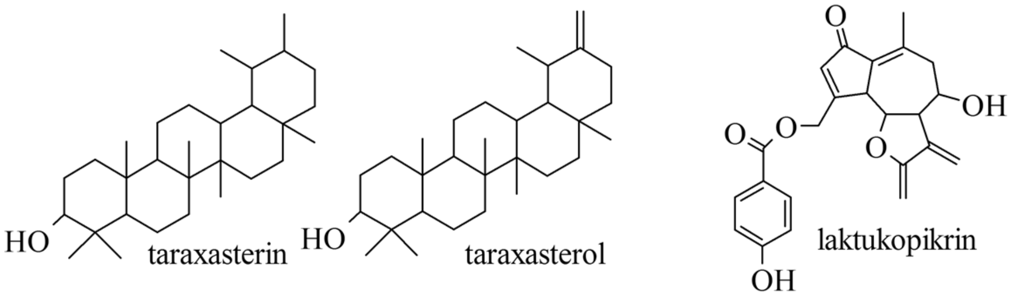
- effect - diuretics, stimulates metabolism, external application – skin congestion
- use in food industry (liquors, spice)

- **Dandelion,** *Taraxacum officinale* (smetanka lékařská – pampeliška)
- flowers - honey
- leaves – spring salad

- drug – root, leaves and unopened flowers; strong bitter taste – bitter agent **taraxin** (**taraxacin**), complex of water-soluble compounds

Dandelion

- other compounds - **lactucopicrin** (sequiterpenic lactone, guajanolide), bitter, sedative effect



Dandelion

- other compounds - phytosterols, terpenic alcohols (**taraxasterin**, **taraxasterol**), organic acids and fatty acids
- leave – high content of vitamin C
- flowers - vitamin B2
- cations - sodium, potassium, manganese
- drug stimulates digestion, heals urinary tract inflammations, kidney stones, liver and metabolic issues
- supporting drug in diabetes

Plant toxins

- **plant defence** from herbivores (insects, birds, mammals)
- **mechanical** (physical) - thorns
- **chemical** - toxins, repellents
- **toxins** in a broader sense are present in most plants

- **secondary metabolites**, secondary plant compounds, bioactive natural products – earlier considered waste products of primer metabolism without any function (**primer metabolites** = sugars, lipids, amino acids, and proteins, organic acids)
- some are stored in a form of **precursors** – in separate organelles there are enzymes that release a toxin in case of herbivore feeding
- **ecological function** of plant compounds – theory of **plant-animals-coevolution** was formulated in 60th; first studies of possible ecological significance of secondary metabolites

Plant defence and reactions of herbivores (Theory of co-evolution)

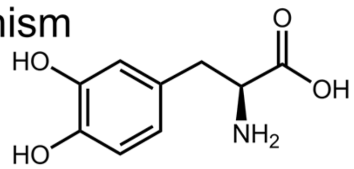
A plant produces **toxins** to defend itself from **herbivore** feeding (most often **insects**). Insects get adapted and develop a **detoxification mechanism**. Only limited number of species succeed, thus, a limited number of herbivorous **specialists** (plant species survives). Toxin becomes a **fagostimulant** for the adapted species. Sometimes it is sequestered to a **communication signal** (pheromone). If too many species feed on one plant species, the plant responds by production of new toxins (different from original ones). Evolutionary young plant species produce toxins that are more difficult to detoxify.

In case that too many herbivorous species get adapted to a toxin, the plant responds by biosynthesis of other toxins, different from the original one. Evolutionarily younger plants produce toxins that are difficult to metabolize.

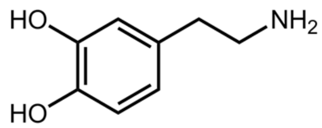
Classification of plant toxins

Nitrogen-containing toxins

- **anomalous** (non-proteinaceous) **amino acids**
- (dihydroxyphenylalanine - L-DOPA, β-cyanoalanine)
- in Fabaceae
- incorporated into proteins of the intoxicated organism



L-DOPA



dopamine

Parkinson's disease – lack of dopamine in brain;
L-DOPA used for inhibition of progress of the disease

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About 300 structures of plant amino acids are known. They are typical for Fabaceae (seeds). When an anomalous amino acid is incorporated into proteins, these proteins/enzymes are non-natural and thus inactive.

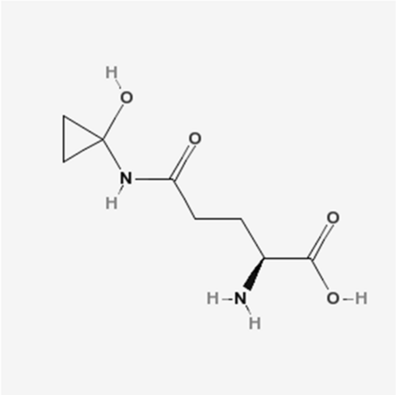
L-DOPA is not toxic for mammals, but it is toxic to insects (plant constitutional defense)

L-DOPA – structure similar to tyrosinu and dopamine

Schizophrenia patients suffer from higher levels of dopamine in the brain; schizophrenia treatment may lower levels of dopamine too much and thus cause symptoms of Parkinsonism.

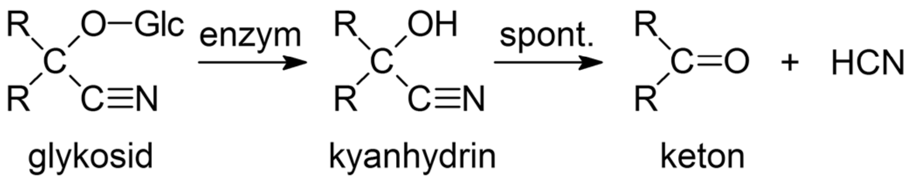
Anomalous (non-proteinaceous) **amino acids**

- **coprine** (common ink cap, *Coprinopsis atramentaria*)
- unusual amino acid inhibiting enzyme alcohol-dehydrogenase
- „antabuse syndrome“ when mushroom eating combined with alcohol



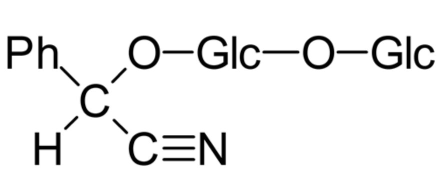
Cyanogenic glycosides

- defence from mollusc feeding (clover)



toxic principle of cyanogenic glycosides

Cyanogenic glycosides



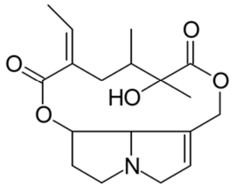
- **amygdalin**
- (bitter almonds)

Cyanogenic glycosides

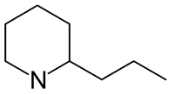
- Some animals can detoxify cyanogenic glycosides (molluscs, sheep, cattle).
- *detoxification principle:*
- $CN^- + S \longrightarrow CNS^-$
- sulfur comes from mercaptopyruvate ($HSCH_2COCOOH$) that is transformed to pyruvate by means of enzyme
- the same mechanism used for clinical treatment of cyanide intoxication (sodium thiosulfate)
- $CN^- + Na_2S_2O_3 \longrightarrow CNS^- + Na_2SO_3$

Other nitrogen-containing toxins

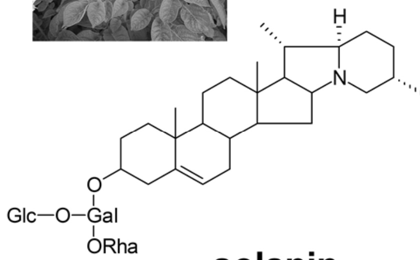
- **alkaloids** - 6500 known structures



senecionin
pyrrolizidine alkaloids, *Senecio* leaves



coniin
hemlock, *Conium* (Socrates)
isolation 1827,
synthesis 1886



solanin
Solanum
green tomato, potatoes

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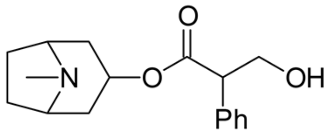
Accumulation of alkaloids in some insect species (e.g. Lepidoptera)

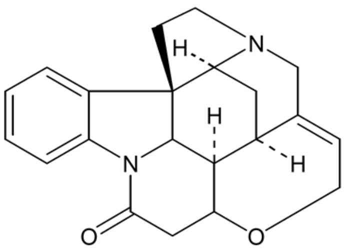
Alkaloid is transformed to pheromone, at the same time it protects butterflies from birds or spiders

During mating, the accumulated alkaloid is transferred from male to the female for protection of eggs

Alkaloids



atropine
Deadly Nightshade (*Atropa,* rulík), berries



strychnine
South Indian tree (*Strychnos,* kulčiba dávivá), fruits
one of first alkaloids isolated in pure state

isolation	1818
structure determined	1946
total synthesis	1954 (Woodward)
absolute configuration	1956
stereoselective synthesis	1993

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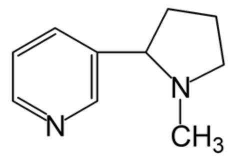
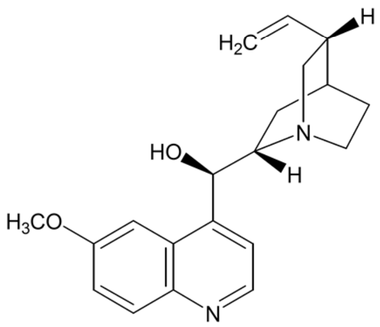
Alkaloids



nicotine
isolation 1828
synthesis 1904
Nicotiana tabacum



quinine
isolation 1820
synthesis 1944
tree bark of genus *Cinchona*

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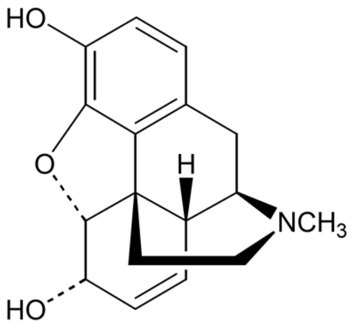
Cvět published principles of chromatography in 1903



Alkaloids

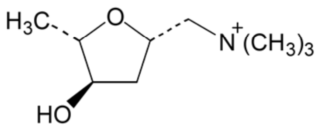
morphine
main component of opium (9-14 %)
dried sap (latex) from unripe seedpods of poppy (*Papaver*)
mixture of opium alkaloids

isolation	1820
synthesis	1944

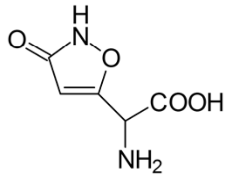
dried sap (latex) derived from shallowly slicing the unripe seedpods of the opium, or common or edible, poppy, *Papaver somniferum*

Quarternary ammonium salts

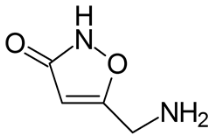
- fly agaric or fly Amanita (*Amanita muscaria*)
- Asian drug „soma“
- (Afghanistan and Siberia)
- hallucinogenic effect



muscarine (minor)



ibotenic acid



muscimol (main)

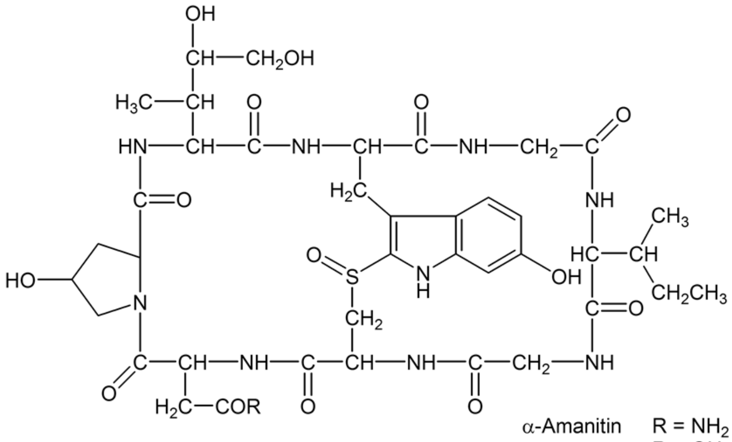
muscarine binds with muscarinic acetylcholine receptors, 2 other components bind to GABA (γ-aminobutyric acid) receptors

binds with <u>muscarinic acetylcholine receptors</u> leading to the excitation of neurons bearing these receptors

Other nitrogen-containing toxins: Peptides

- Death Cap, *Amanita phalloides* (muchomůrka zelená)
- 2 types of toxins - α-amanitin and phalloidin
- both are cyclic oligopeptides
- phalloidin - low toxicity for mammals
- **amanitin** – lethal dose 0,1 mg/kg body weight (cyclic heptapeptide)

Peptides



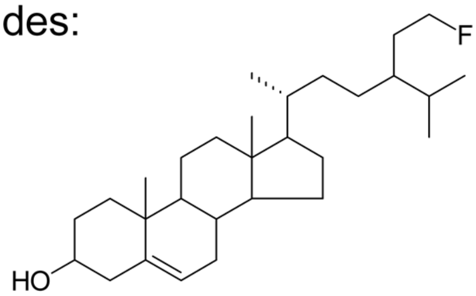
α-Amanitin R = NH_2
β-Amanitin R = OH

Other nitrogen-containing toxins: Proteins

- common bean, *Phaseolus vulgaris* (toxic for insects) - glycoproteins
- **abrin** – lucky bean, tropical legumes, lethal dose 0,5 mg/kg
- denaturises at 65 °C (detoxication by boiling)

Simple organic acids

- **fluoroacetic acid** - FCH_2COOH – in South African plant (shrubs or small trees) *Dichapetalum cymosum*; it enters Krebs' cycle (citric acid cycle) as substrate, but the cycle is inhibited by fluorocitric acid formed, inhibition of respiration
- fluoroacetic acid is a model for one type of insecticides:



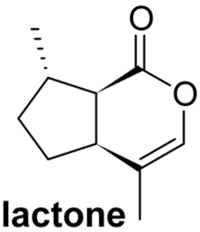
fluorinated sitosterol $\xrightarrow{\text{enzyme}}$ cholesterol + **FCH_2COOH**

Simple organic acids

- **oxalic acid** - HOOC-COOH – in many sour-tasting plants (sorrel, rhubarb, oxalis); only plants containing more than 10 % oxalic acid are dangerous; toxic principle is not fully clear

Other toxins

- **iridoids** - monoterpenic lactones
- **nepetalactone** – catnip or catmint (*Nepeta*) – repellent or toxic for some insects and birds
- attractant for other insects, even host plant signal for some insects

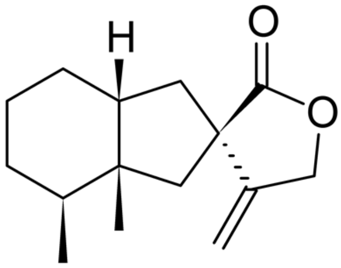


nepetalactone

Nepetalactone is a female sex pheromone of aphids

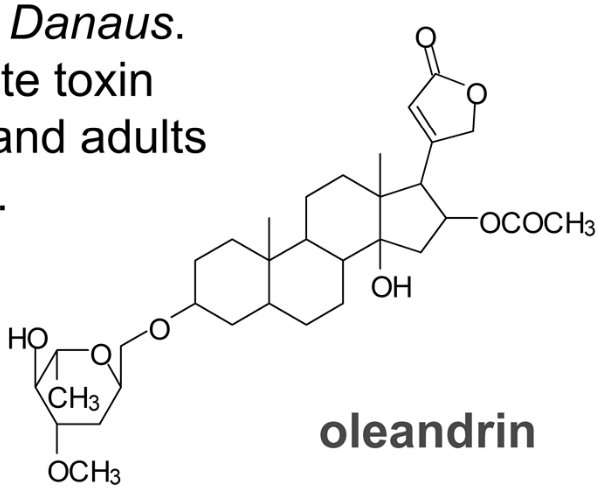
Other toxins

- **sesquiterpenic lactones** – composite plants (*Eupatorium*), Butterbur (*Petasites,* devětsil); some lactones have antitumor activity
- **bakkenolide A** from *Petasites*, deterrent of storage pest insects



Other toxins

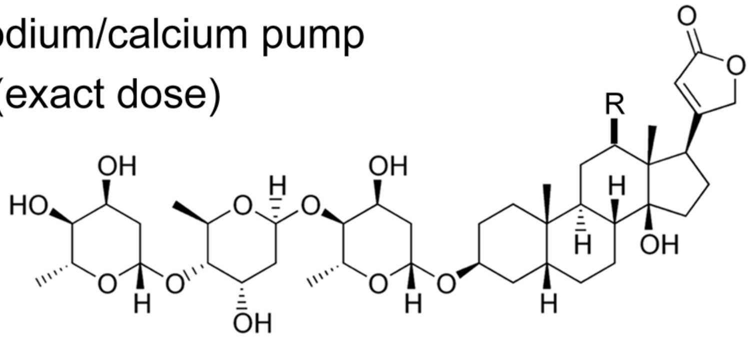
- **cardiac glycosides** (cardiotonics) - milkweeds (*Asclepias*), bitter taste to higher animals and humans. Feeding of butterflies genus *Danaus*. Caterpillars cumulate toxin that protects them and adults from bird predators.



oleandrin

Other toxins

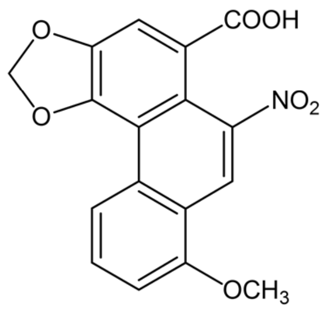
- **cardiac glycosides** – foxglove (*Digitalis purpurea, lanata,* náprstník), digoxin (aglycone digoxigenin), digitoxin (digitoxigenin)
- an antiarrhythmic agent to control the heart beat
- influences sodium/calcium pump
- high toxicity (exact dose)



R=OH, digoxin
R=H, digitoxin

Other toxins

- **aristolochic acid** – in plants family Aristolochiaceae
- 1-fenantrencarboxylic acids, 14 structures known
- sequestered by butterflies feeding on Aristolochiaceae



Aristolochia.elegans

acute nefrotoxicity, causes cancer of kidneys and urinary tract, forms covalent adducts with DNA

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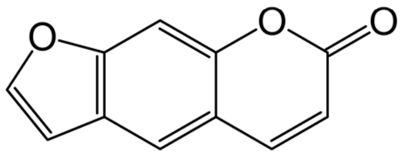
Used in traditional medicine in Balkan countries

In Netherlands, affair with a reduction program based on Asian plants, by mistake, the plant mixture contained species producing aristolochic acid. Women in this program got kidney cancer in high percentage.

Other toxins

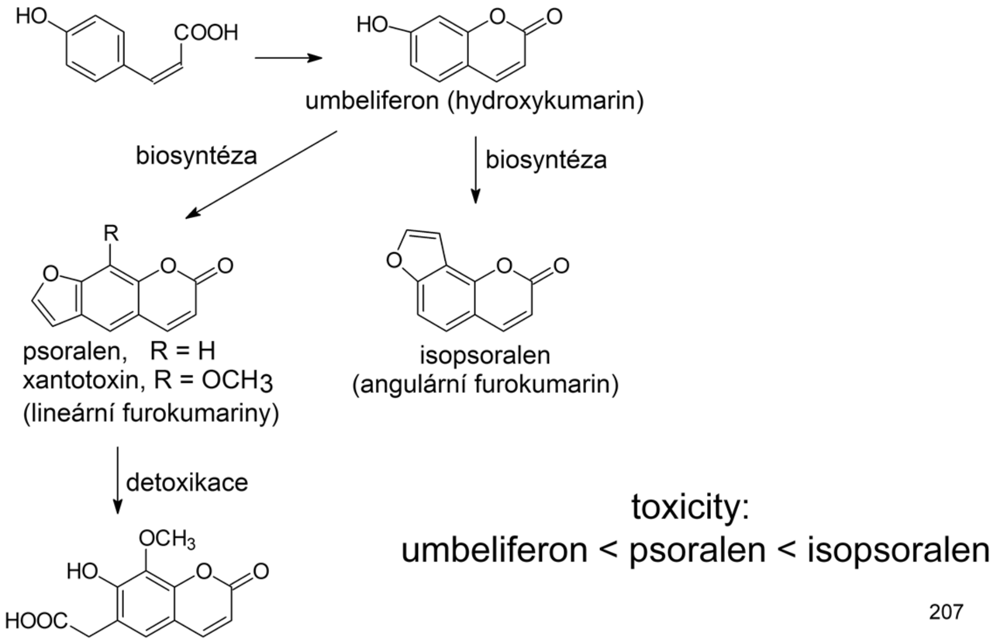
- **furocoumarins** - bergamot essential oil, hogweed, parsley; toxin degrades in UV (sunlight) to more toxic products, irritates skin, toxic to insects



psoralen

Even the content bellow 1% causes in daylight 100 % mortality in *Spodoptera uridania*.

Biosynthesis and metabolism of substituted coumarins in Apiaceae



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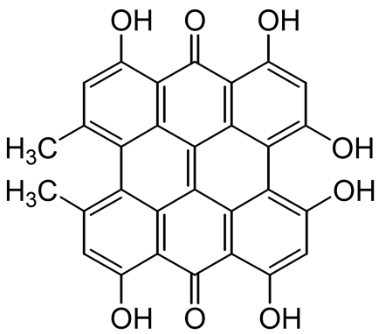
UV-induced phototoxicity

Papilio butterflies are very resistant, the caterpillars are able to detoxify a high dose in 2 hours

Evolutionarily higher plants in Apiaceae produce angular toxins, more difficult to degrade

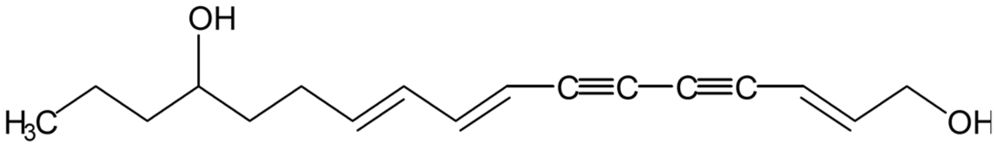
Other toxins: Quinones

- **hypericin** from *Hypericum*
- after feeding followed by exposition to sunlight, animals develop sunburns and skin damage



Other toxins: Polyacetylenes

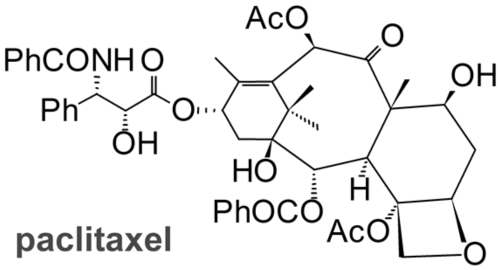
- **Oenanthotoxin**
- central nervous system poison
- also photolabil
- from hemlock water dropwort (*Oenanthe,* halucha*)*



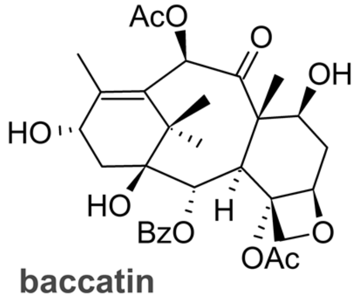
Other toxins: Diterpenes

- **acetylandromedol** - diterpene from *Rhododendron* (leaves and flowers – intoxications by honey!)

- **paclitaxel** (Taxol®)
- yew (American *Taxus brevifolia*, European *T. baccata*), efficient cytostatic agent, treatments of breast and ovarian cancer



paclitaxel

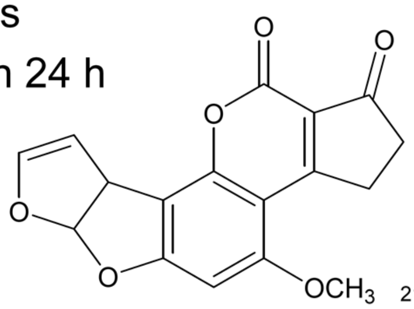


baccatin

- sufficient supply of **paclitaxel** from yew bark is problematic – slow growth, low content
- semisynthesis from **baccatin**, higher content in plant, especially in needles (grows up more quickly)

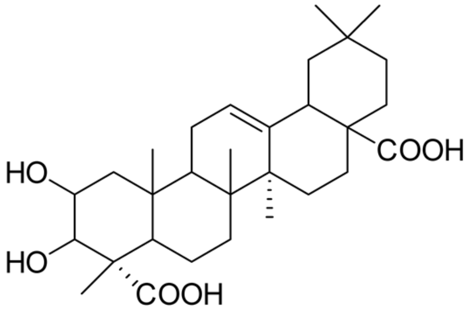
Other toxins

- **aflatoxins -** group of toxins of related structures, produces by microorganisms (mycotoxins)
- **aflatoxin B1 –** produced by fungus *Aspergillus flavus*
- grows on peanuts
- discovered in USA (ducks fed by mouldy peanuts)
- lethal dose 24 mg in ducks
- liver insufficiency, death in 24 h



Other toxins

- **saponins** - alfalfa (*Medicago sativa,* vojtěška)
- **medicagenic acid**
- toxic to fish and some insects



Derivative of β-amyrine

Saponins

- water solutions strongly foam when shaken (surfactants)
- use - in pharmaceutical industry, food industry, cosmetics, earlier for washing laundry
- **haemolytic activity** (toxicity, blood poisons)
- water animals – high toxicity – increase permeability of skin and gills, organism looses life-important electrolytes

Saponins

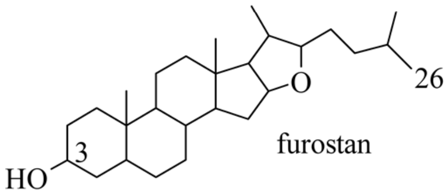
- saponin drugs - expectorants (surface activity)
- influence resorption of other compounds (emulsions), enables absorption of active components (medicaments)
- irritate mucous tissues
- chemical structures - **glycosides**, lipophilic aglycone (**sapogenin**) and hydrophilic sugar moiety
- classification - **steroid** and **triterpenic** saponins

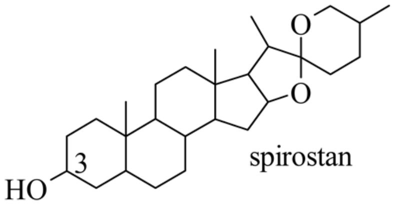
Steroid saponins

- monocotyledonous plants (monocots), seldom in dicotyledonous (*Digitalis*)
- in Liliaceae, e.g. yucca, aloe) or Amaryllidaceae (e.g. agave) are very common
- suitable source of steroids for production of steroid hormones
- small amounts of steroid saponins – in lilly-of-the-valley (*Convallaria,* konvalinka, convallamarin) or *Hellebore*



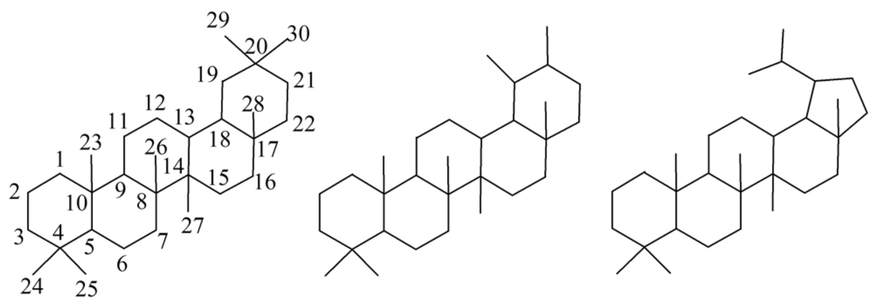

- basic skeleton of steroid saponins - **cyclopentanoperhydrofenanthrene** – 2 types: **furostane** and **spirostane**
- sugar always in position 3, furostane also in position 26 (**bidesmosids**)





Triterpenic saponins

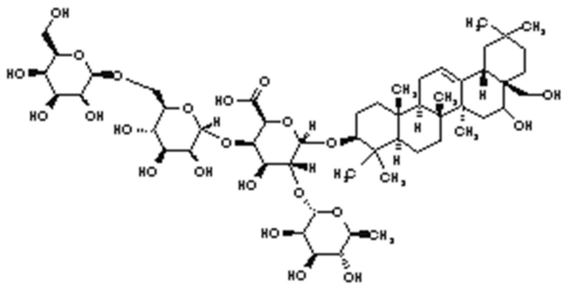
- common in dicotyledonous plants
- basic types (skeletons): β-amyrin, α-amyrin, and lupeol
- sometimes carboxylic group (COOH) in position 17



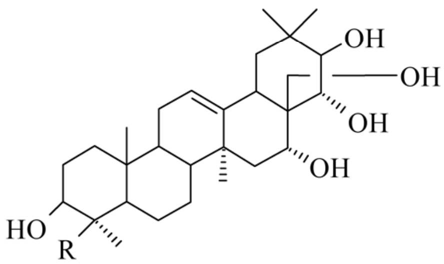
β-amyrin **α-amyrin** **lupeol**

- **liquorice** (*Glycyrrhiza glabra,* Fabaceae) – perennial plant
- harvest of dried roots
- main component (5-15%) - sweet saponin **glycyrrhizin** (glycoside), 50 times sweeter than sucrose
- breaking of glycosidic bond leads to loss of sweet taste

- **primrose** (*Primula veris*) - saponins in rhizome and roots (5–10 %)
- main component **primulic acid**



- horse chestnut tree (*Aesculus hippocastanum*) contains saponins (15 % in fruits)
- **aescine**, mixture of saponins with aglycones
- in some cosmetic products



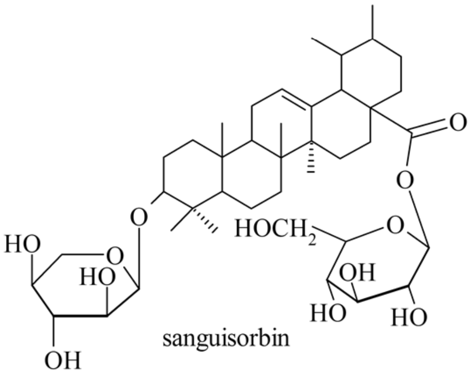
protoaescigenin   R = CH_2OH
barringtogenol   R = CH_3
cukry: glukosa, xylosa, kys. glukuronová

- **soap-wort** (*Saponaria officinalis,* mydlice) gave name to a group of glycosides
- perennial, harvest of roots
- 5 % of saponin mixture

- **common horsetail** (*Equisetum arvense,* přeslička rolní) – medicinal plant
- harvest of aerial parts in summer
- 5 % of saponin **equisetonin** (haemolytic effect) and many more compounds of different chemical types
- mostly used as diuretics
- in traditional medicine used for healing tbc, but this effect was not proved

Fungicidal effect

- **Great Burnet** (*Sanguisorba officinalis,* krvavec toten) – rhizome contains tannins (17–20 %) and a saponin **sanguisorbin** (4 %)
- against diarrhoea (tannins), healing wounds, bleeding of teeth gum or nose



sanguisorbin

Plant induced defence

Outlines

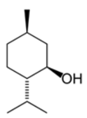
1) Chemical diversity of plant products
2) Plant defence and multitrophic interactions
3) Herbivore induced plant volatiles (HIPVs) and their detection by parasitoids and predators of herbivores
4) Importance of HIPV – 3rd trophic level of interactions

Plant defence and reactions of animals

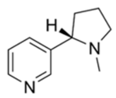
Plant defence: **static** (constitutive)
dynamic (induced)

Localisation of toxins in plants – often in trichomes, bearing exocrine glands. The most vulnerable plant parts (young leaves, shoots) are usually protected by highest toxin levels. Presence of toxins and **timing** of their production correlates with usual occurrence of herbivores.

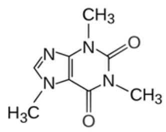
>30 000 secondary metabolites known



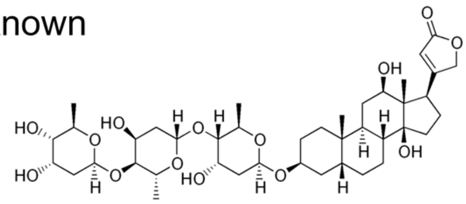
Menthol



Nicotine

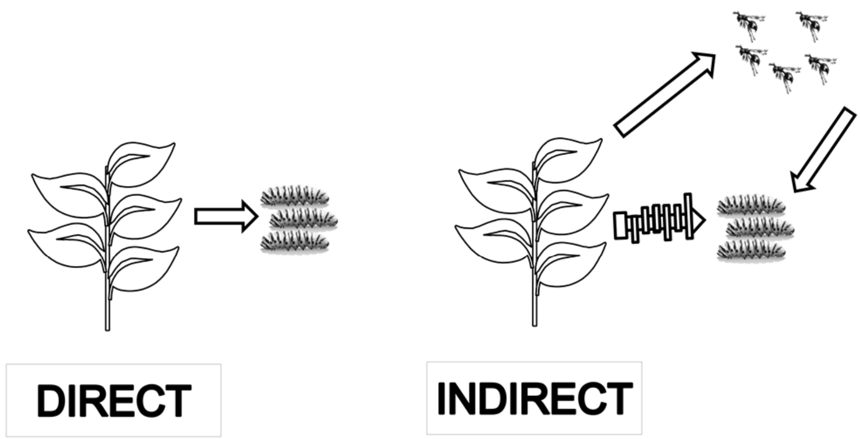


Caffeine



Digoxin

Plant defence



Interaction with predators and parasitoids

Plant stress, elicitors of plant defence

- **abiotic**
- light (UV)
- temperature
- salty soil
- draught
- heavy metals

- **biotic**
- pathogens
- herbivores
- neighbouring plants
- parasites
- symbionts

Induced defence from herbivores

- **Consequence of herbivore feeding:**
- ***a)*** higher production of constitutive toxins,
- ***b)*** some toxins are stored in a form of **precursors** – in separate organelles there are enzymes that release a toxin in case of herbivore feeding
- ***c)*** induction of proteins that inhibit insect proteases and block insect's digestion
- ***d)*** gene expression and *de novo* synthesis of new compounds/toxins

Induced defence from pathogens and herbivores

- history – defence from pathogens has been studied since beginning of 20th century, defence from herbivores only since seventies
- the same mechanism or 2 different plant responses?
- common principles, some transduction signals are identical
- more mechanisms, influence one another

Specific or non-specific response?

- abiotic and biotic stress factors studied
- examples exist of both, specific *vs* general response
- plant starts to produce compounds both within the plant (non-volatile) and those released to environment (volatile)

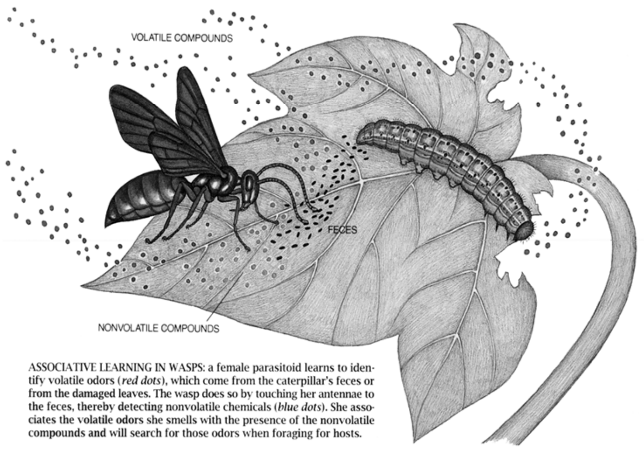
Plant’s talk - function

- fungicidal (fungi - phytoalexins)
- antibiotic (bacteria)
- induction of defence compounds in neighbouring plants
- influence on growth of neighbours and germination capacity of seed around (allelopathy)
- calling for help of parasitoids (tritrophic system, „SOS“ signals)

Plant enduced defence

tritrophic system

Parasitic wasp *C. marginiventris* × Caterpillar *S. exigua* × Maize *Zea mays*



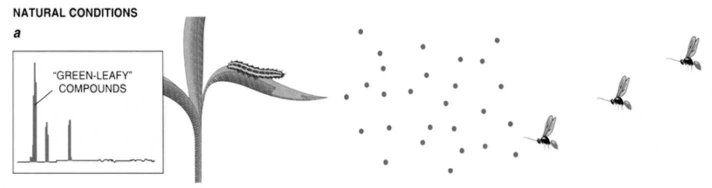
ASSOCIATIVE LEARNING IN WASPS: a female parasitoid learns to identify volatile odors (*red dots*), which come from the caterpillar's feces or from the damaged leaves. The wasp does so by touching her antennae to the feces, thereby detecting nonvolatile chemicals (*blue dots*). She associates the volatile odors she smells with the presence of the nonvolatile compounds and will search for those odors when foraging for hosts.

Turlings T. C. J., Tumlinson, J. H., and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251-1253.

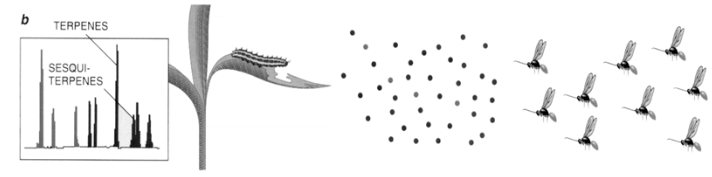
http://www.ars.usda.gov/Research/docs.htm?docid=7501

Parasitic wasp × Caterpillar × Maize

start of feeding



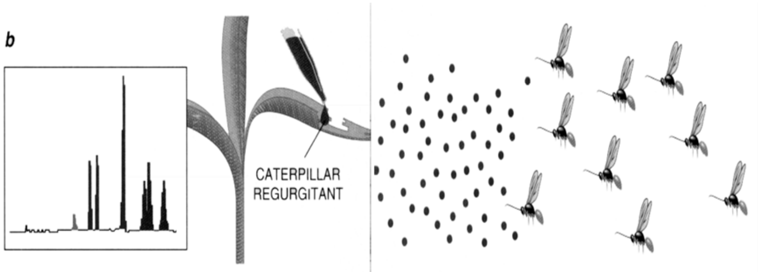
after 12 hours

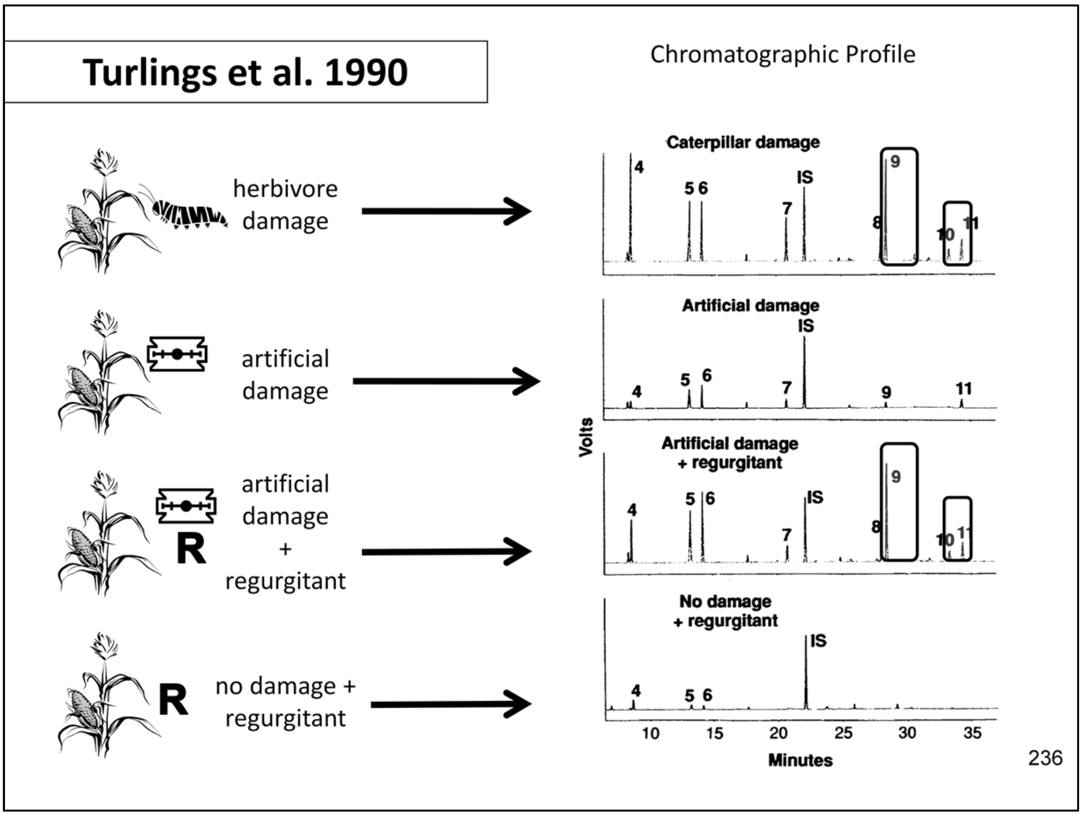


Plant produces chemical signals even after feeding ended, induced volatiles attract more predators.

Parasitic wasp × Caterpillar × Maize

Caterpillar's saliva induces similar compounds as released while caterpillar feeding.





Turlings T. C. J., Tumlinson, J. H., and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251-1253.

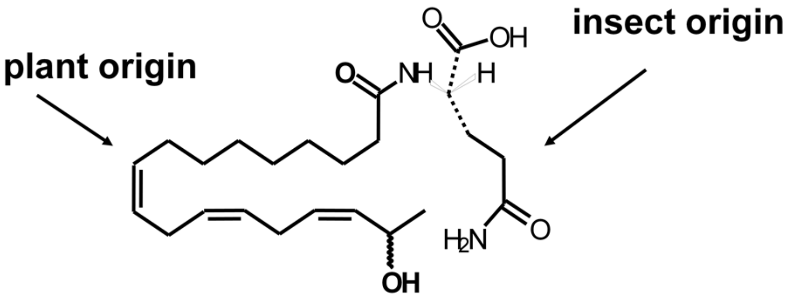
Elicitors of defence reaction

- Compounds present in saliva of herbivores start/stimulate:
- a) production of volatiles (SOS)
- b) synthesis of toxins

Structure of stimulus?

Volicitin

active compound from saliva of caterpillars
(Alborn *et al.*, *Science* **1997**)



isolated and synthesized compound:
***N*-(17-hydroxylinolenoyl)-L-glutamine**

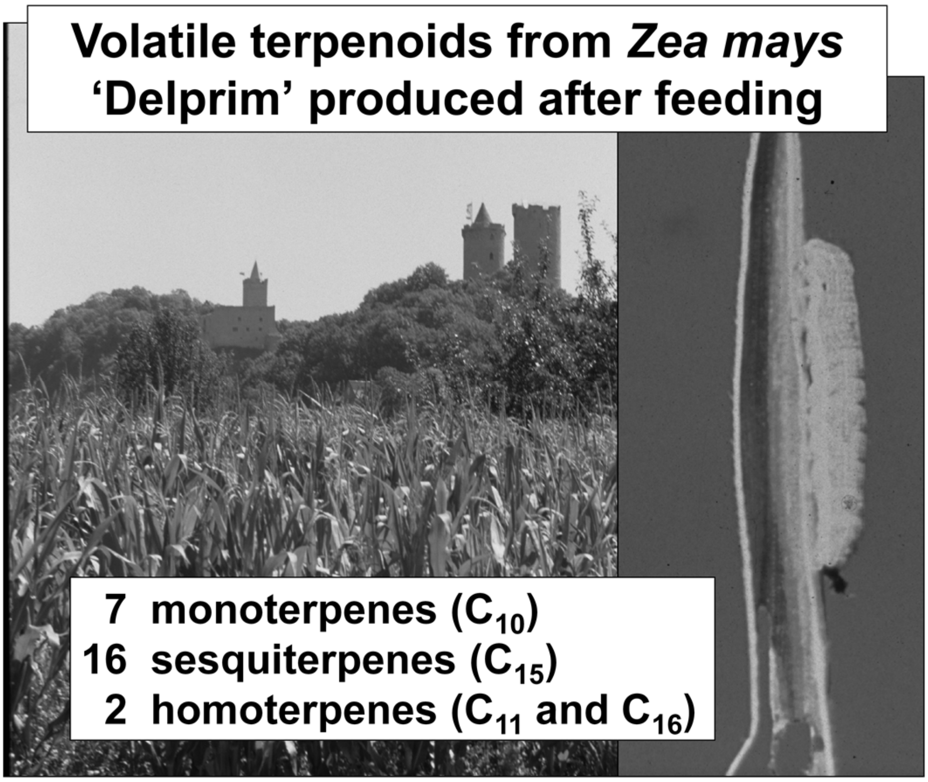
Paré *et al., PNAS* **1998**

Other elicitors of *de novo* synthesis of volatiles

- **β-glukosidase**, applied to mechanical wound, elicits synthesis of homoterpenes

Boland *et al.*, *Naturwissenschaften* **1992**

Volatile terpenoids from *Zea mays* 'Delprim' produced after feeding

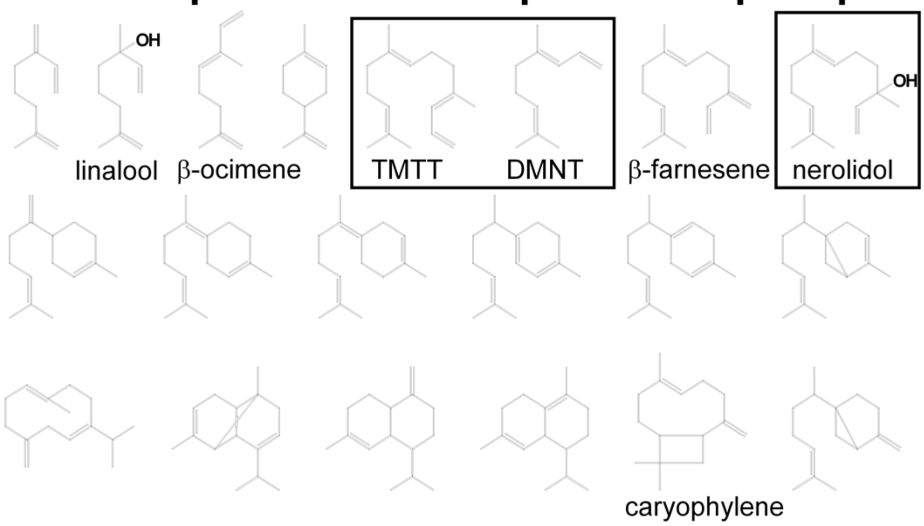


7 monoterpenes (C_{10})
16 sesquiterpenes (C_{15})
2 homoterpenes (C_{11} and C_{16})

Volatile terpenoids induced after feeding on *Zea mays*

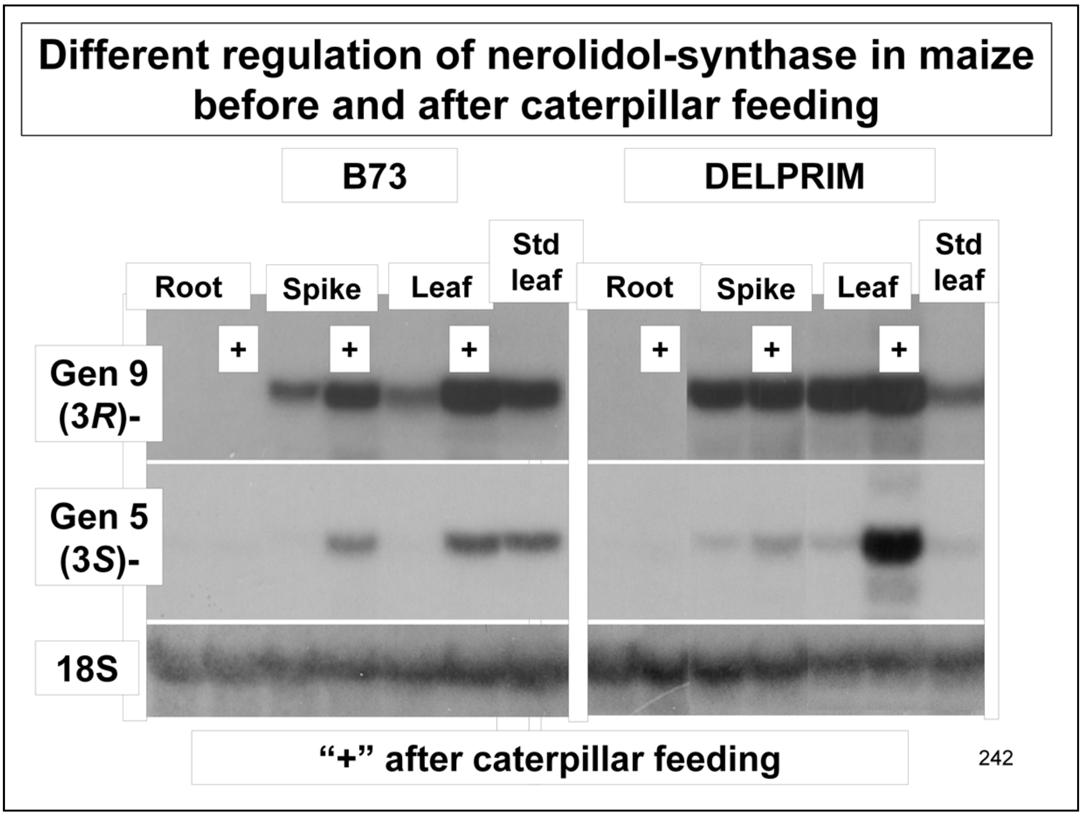
„SOS“ signals of plants – terpenes – from mevalonate

monoterpenes homoterpenes sesquiterpenes



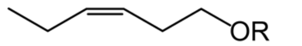
DMNT: (3*E*)-4,8-dimethyl-1,3,7-nonatriene

TMTT: (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene


Different regulation of nerolidol-synthase in maize before and after caterpillar feeding
B73
DELPRIM
Root
Spike
Leaf
Std leaf
Root
Spike
Leaf
Std leaf
+
+
+
+
+
+
Gen 9 (3R)-
Gen 5 (3S)-
18S
"+" after caterpillar feeding
242

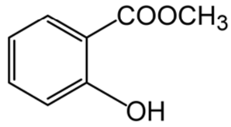
Other „SOS“ signals of plants

From degradation of fatty acids (lipoxygenase)
(*Z*)-hex-3-en-1-ol,
(*Z*)-hex-3-en-1-yl acetate,
(*Z*)-hex-3-en-1-yl butyrate

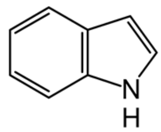


R = H, $COCH_3$, $COCH_2CH_2CH_3$

Aromatic compounds
(from phenylalanine)
methyl salicylate



Nitrogen-containing compounds
(from tryptophane)
indol

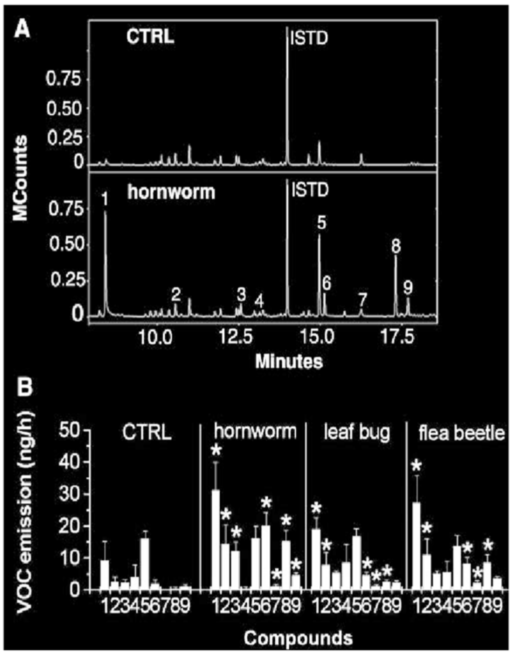


12 h after feeding the profiles of released volatiles changes

From degradation of fatty acids
1 Z-3-hexen-1-ol, **3** Z-3-hexen-1-yl acetate, **6** Z-3-hexen-1-yl butyrate

Terpenoids
2 *E*-β-ocimene, **4** linalool, **5** terpineol, **8** *Z*-α-bergamotene, **9** *E*-β-farnesene

Aromatic compounds
7 methyl-salicylát



Feeding of different insects on tobacco induced similar profiles of volatiles

Kessler and Baldwin, Science **2001**

What happens upon plant damage?

- How does a plant know, that its part is damaged?
- How is the information transferred to other plant parts?
- How does an undamaged plant know about a damage of a neighbour?

Signal for a systemic response has to:

- arise quickly in the damaged spot by means of known induction factors
- be transported to different plant tissues
- elicit defence response
- be present in concentrations corresponding to values found in damaged plants

Mechanism of induced response

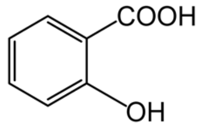
Jasmonate pathway: jasmonic acid

- Salicylate pathway: salicylic acid

- antagonistic pathways

Cause of response – herbivore's mouth secretion (saliva or labial gland secretion?)
– pathogen

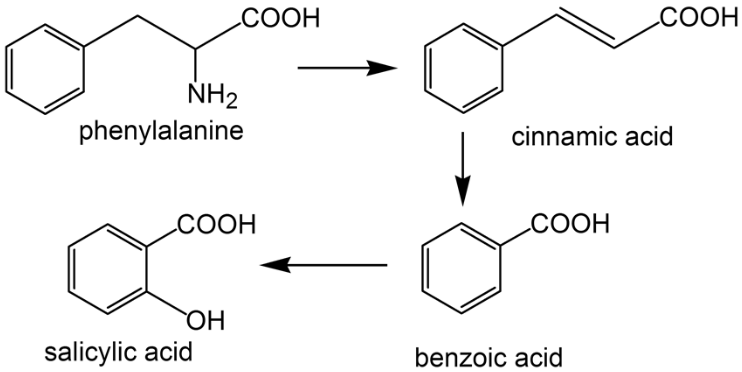
Salicylic acid



- response to pathogen, oldest signal known, best studied
- gene expression both locally at wounded spot and in other tissues (systemic acquired resistance, „SAR“)
- production of phytoalexins

Biosynthesis of salicylic acid

- shikimic pathway *via* phenylalanine

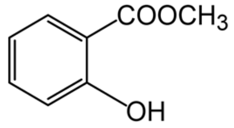


Salicylic acid

- **translocated** from wounded spot to other tissues,
- but also **synthesised systemically** (⇒ SA may not be primar signal)
- induces resistance to pathogens, not to insect feeding

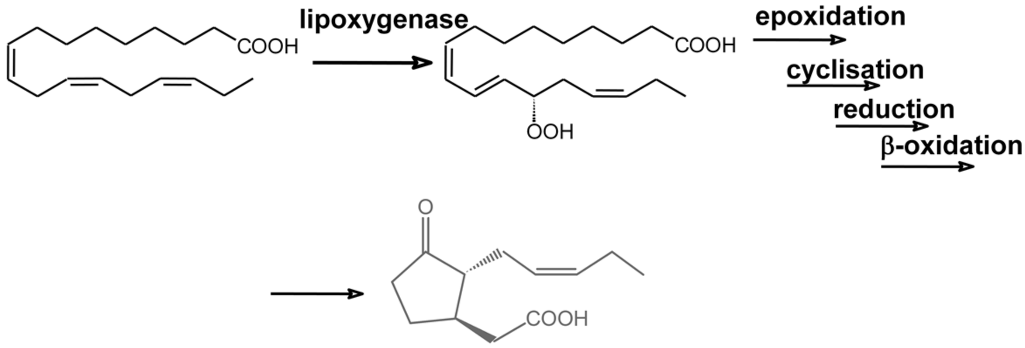
Methyl salicylate

- volatile, detected increased concentrations around a damaged plant
- belongs to „SOS“ signals, communication with neighbouring plants
- it is perceived in undamaged plant and transformed to SA

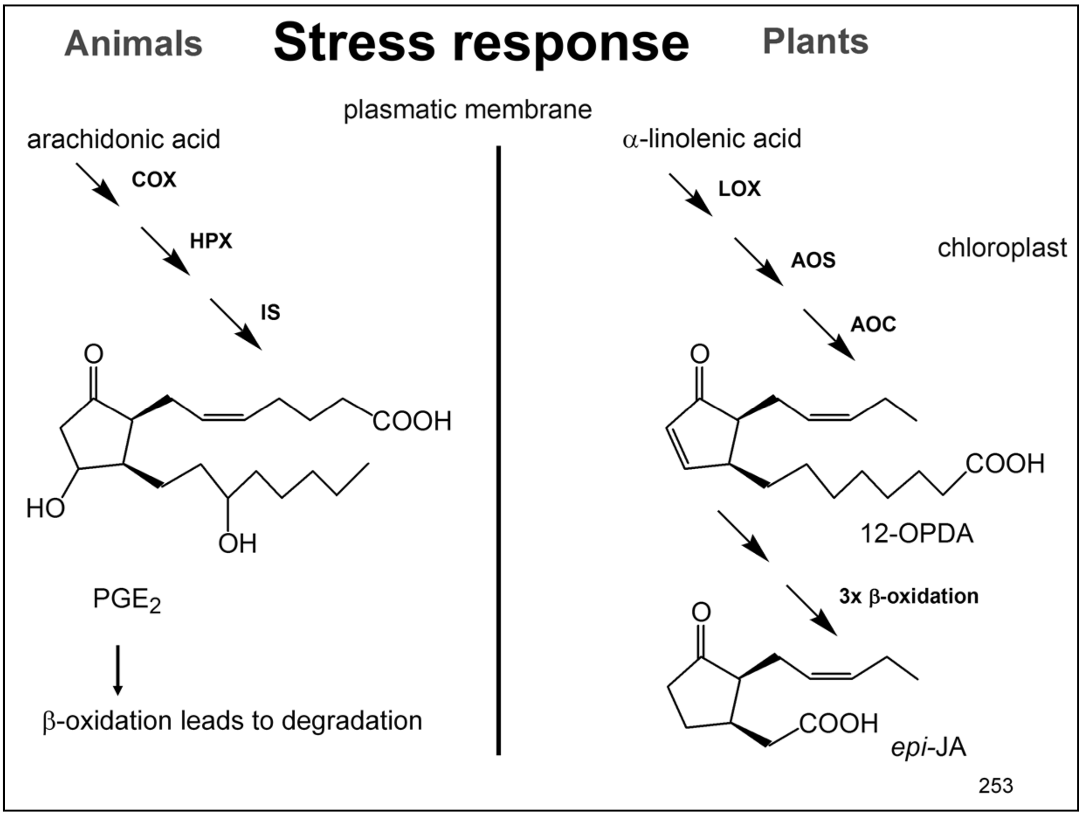


Induction of volatile „SOS“ signals

activation of octadecanoic cascade, formation of JA, gene activation, transcription and synthesis of enzymes producing terpenoids



jasmonic acid (JA)



LOX lipoxygenase

Arachidonic acid (**AA**, sometimes ARA) is a polyunsaturated omega-6 fatty acid 20:4(ω-6).

Both pathways are inhibited by salicylic acid and its esters

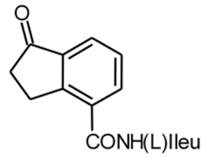
Induction of volatile „SOS“ signals

other elicitors:

fungi and their toxins
parts of cell walls
oligosaccharins

polypeptides (systemin)
methyl salicylate
abiotic factors

synthetic JA analogs:

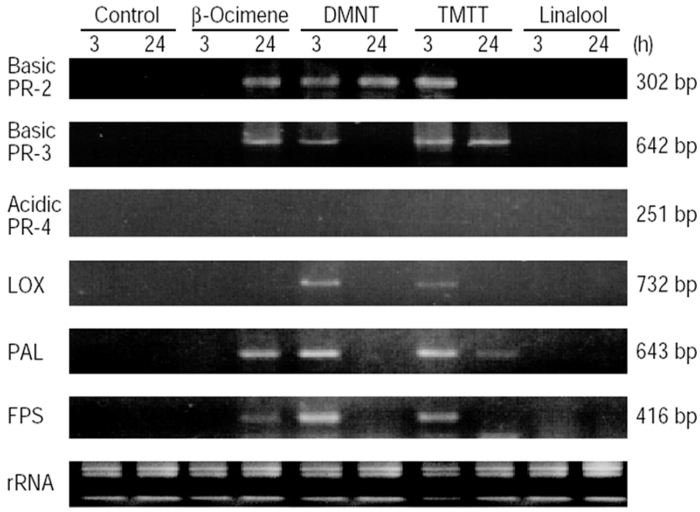


Boland *et al.*

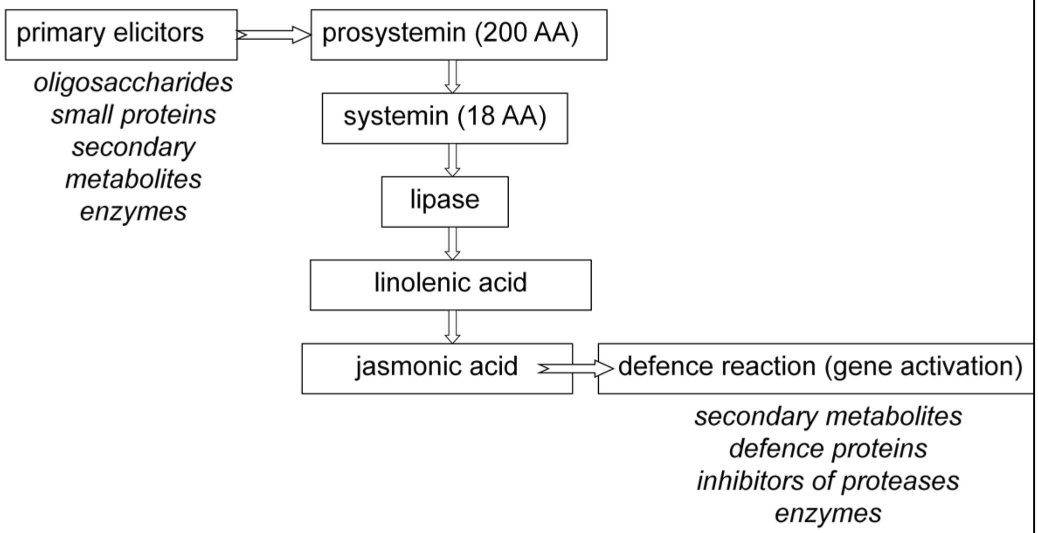
- 5-membered ketone is an important structural element in JA and its analogs
- Production of volatiles and their release are connected to light phase (photosynthesis)

Emitted terpenic compounds influence expression of defence genes

(*Phaseolus lunatus* cv. Sieva and aphid *Teranychus urticae*)



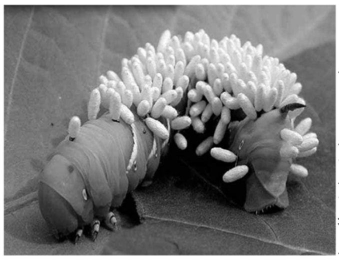
Signalling cascade in plants after herbivore feeding



Who is the reciever of induced volatiles?

- Neighbouring plants (“Talking Trees”)
- Herbivores
- 3rd trophic level: predators and parasitoids

www.usda.gov



http://aggie-horticulture.tamu.edu

Plants interact with environment

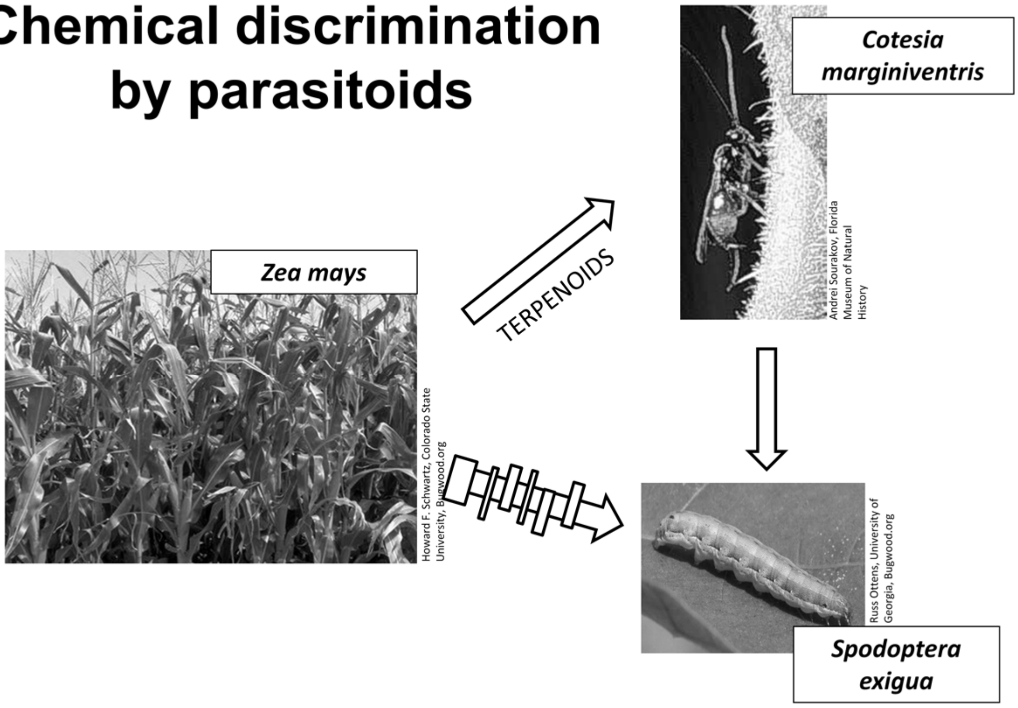
ethylene
Me-JA
Me-salicylate
aldehydes
terpenes

after insect feeding or fungi production of **volatiles** and endogene secondary metabolites:(phenylpropanoids, terpenes, alkaloids)

in neighbouring plants synthesis of **secondary metabolites** without damage

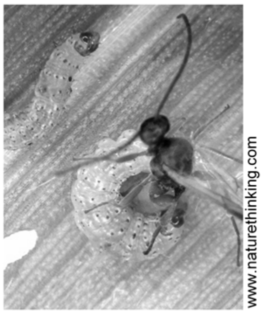
Systemic acquired resistance

Chemical discrimination by parasitoids



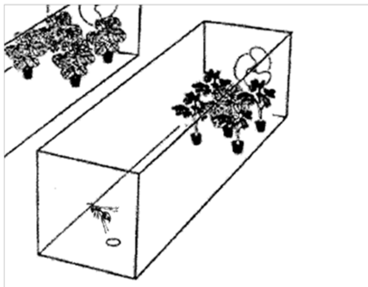
Turlings *et al.* **1990**

Wind tunnel bioassays

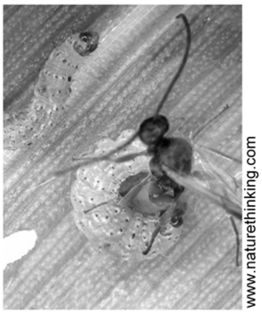


* Two-Choice tests with females *C. marginiventris*

Test 1	Artificial damage	Herbivore damage
Test 2	Artificial damage	Artificial + regurgitant
Test 3	Herbivore damage	Artificial + regurgitant
Test 4	Artificial damage	Undamaged + regurgitant



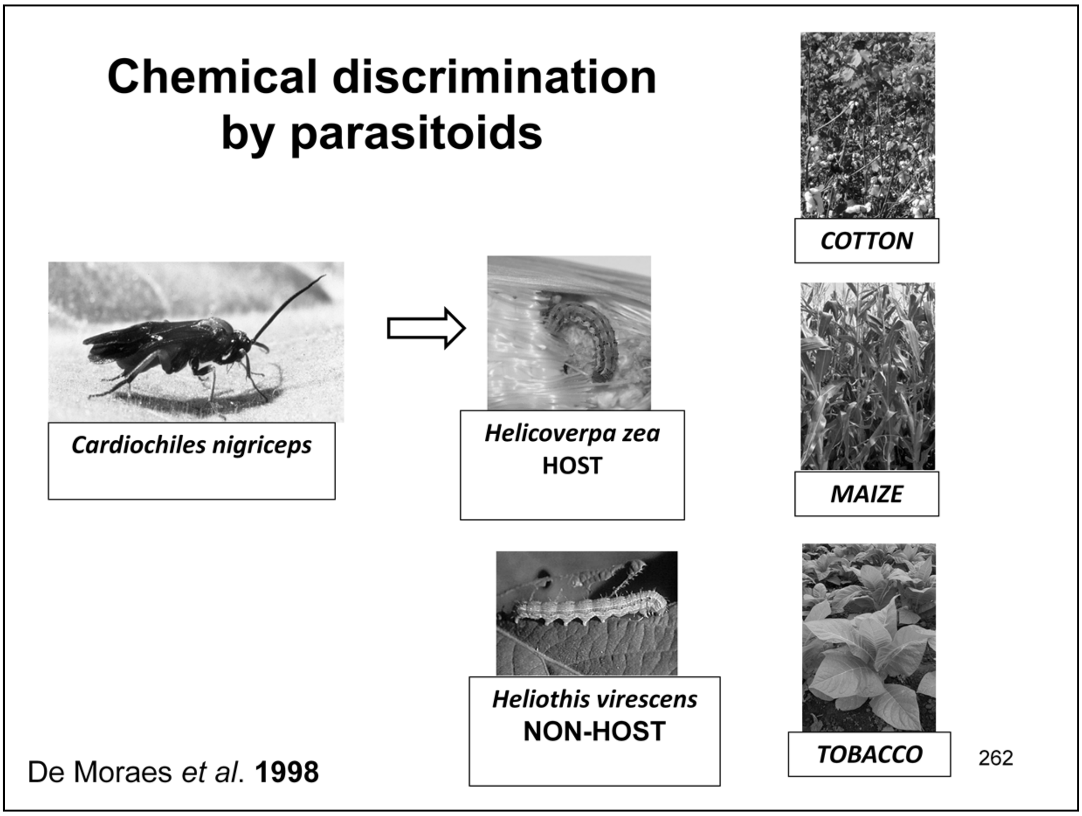
Turlings *et al.* **1990**



Conclusion:

- Terpenes are a reliable signal for parasitoids, because they are associated with herbivore feeding on plants

Turlings *et al.* **1990**



In response to insect herbivory, plants synthesize and emit blends of volatile compounds from their damaged and undamaged tissues, which act as important host-location cues for parasitic insects. Here we use chemical and behavioural assays to show that these plant emissions can transmit herbivore-specific information that is detectable by parasitic wasps (parasitoids). Tobacco, cotton and maize plants each produce distinct volatile blends in response to damage by two closely related herbivore species, *Heliothis virescens* and *Helicoverpa zea*. The specialist parasitic wasp *Cardiochiles nigriceps* exploits these differences to distinguish infestation by its host, *H. virescens*, from that by *H.zea*. The production by phylogenetically diverse plant species and the exploitation by parasitoids of highly specific chemical signals, keyed to individual herbivore species, indicates that the interaction between plants and the natural enemies of the herbivores that attack them is more sophisticated than previously realized.

Field tests: Tobacco

Cardiochiles nigriceps

Tobacco

- Damaged *vs*. undamaged plants with host and non-host herbivore
- Caterpillars and damaged leaves were removed
- *C. nigriceps* wasps distinguished between odours of undamaged plants, plants after feeding by host and non-host herbivore

De Moraes *et al*. **1998**

De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. Nature 393:570-573.

Analyses of volatiles: Tobacco

Cardiochiles nigriceps

Tobacco

- Qualitative or quantitative differences in HIPV?
- 8 main components induced by the complex herbivore-plant, mainly ocimene and DMNT
- **Difference in profiles** of volatiles released by each complex
- **Conclusion:** Quantitative difference: increased emission of volatiles in complex host herbivore-plant

De Moraes *et al*. **1998**

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herbivore-induced plant volatiles (HIPVs)

Field tests and analysis of volatiles: Cotton

Cardiochiles nigriceps

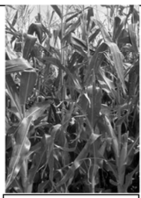
BAVLNA

- Wasps preferred damaged plants
- No difference in overall amounts of volatiles after feeding of host and non-host insects
- **Qualitative** differences found in volatile composition of complexes plant-herbivore

De Moraes *et al*. **1998**

General conclusions

Cardiochiles nigriceps



MAIZE

In **maize,** profiles of volatiles don't differ after feeding of both insect species.

- Induction of volatiles in response to insect feeding is a general phenomenon in plants (3 different plant families)
- Structure of produced volatiles and their profiles may differ in different plants and herbivore species
- Sophisticated system of chemical interactions between herbivore, plant, and parasitoid

De Moraes *et al*. **1998**

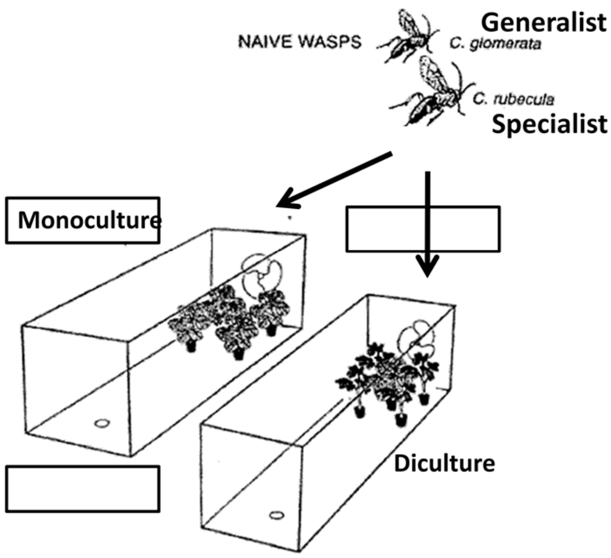
Chemically complex environment

Is foraging efficiency diminished in dicultures?

Brussel sprouts infested with *Pieris rapae* (host)

Potatoes (non-host plant) induced by Colorado potato beetles

Wasps placed in wind tunnel and foraging observed



Perfecto and Vet **2003**

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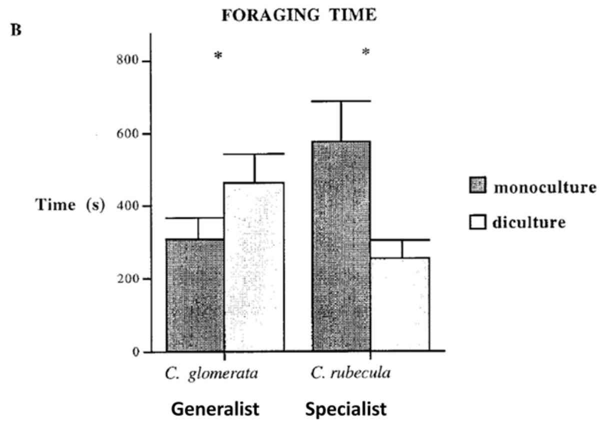
Perfecto, I., and L. E. M. Vet. 2003. Effect of a nonhost plant on the location behavior of two parasitoids: the tritrophic system of *Cotesia* spp. (Hymenoptera: Braconidae), *Pieris rapae* (Lepidoptera: Pieridae), and *Brassica oleraceae.* Environmental Entomology 32:167-174.

Chemically complex environment

Generalist:
Foraging time was higher in a diculture for the generalist wasp

Specialist:
Reversed pattern for specialist wasp



Perfecto and Vet **2003**

Conclusions

- Parasitoids and predators have a high capability for discriminating among chemical cues.
- The chemically complex environment can potentially affect parasitoid/predator foraging behavior.

Possible applications

Hypothesis:

- Increased emission of a specific compound in context of natural environment of damaged plants may attract more natural enemies.

Experiment:

- Application of individual „SOS“ compounds to damaged plants
- Placing of herbivore’s eggs on the leaf underside
- Determination of the degree of predation (Hemiptera)

Kessler and Baldwin **2001**

Kessler, A., and I. T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141-2144.

Results:

- After 24 h, higher mortality of eggs after application of tested „SOS“ compounds to control plants
- After 1 week, the degree of predation increased 5 – 7.5x

Kessler and Baldwin **2001**

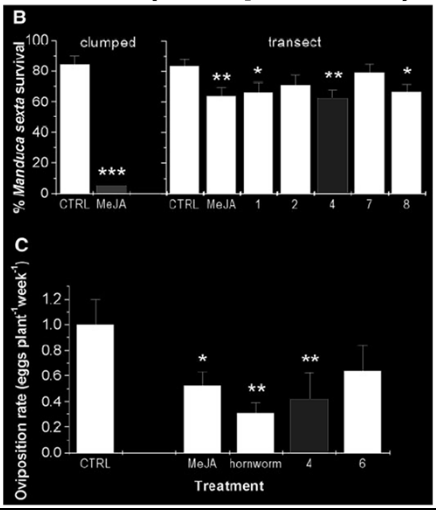
Effects of induced compounds:

B. increase of parasitation of *Manduca sexta* eggs (tobacco hornworm) on tobacco leaves

C. reduced egglaying by *Manduca sexta* females on plants treated with e.g. linalool **(compound 4)**

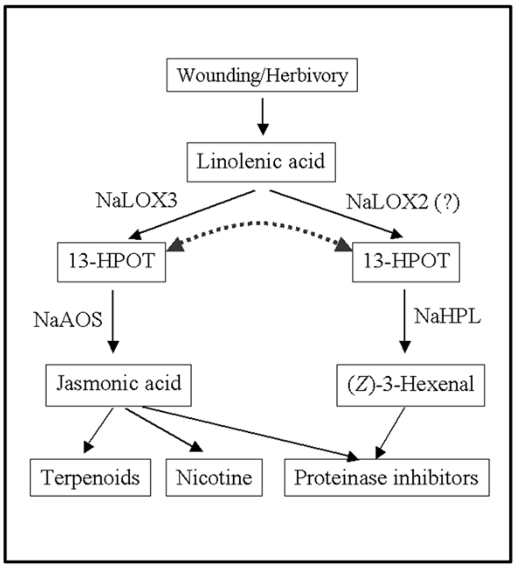
Combination of both effects results in 90% reduction in *M. sexta* population density.

⇒ possible use in plant protection



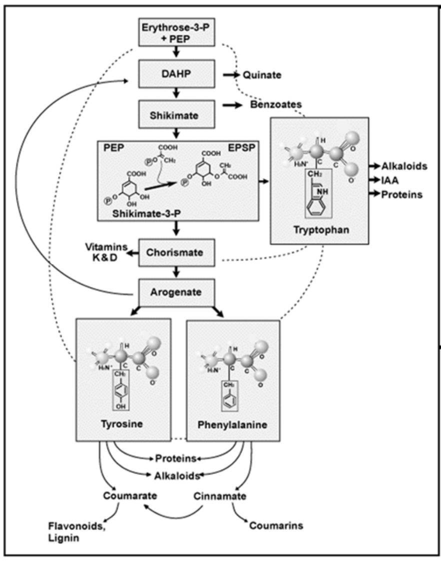
Kessler and Baldwin: *Science* **2001**

Jasmonate Pathway in Wild Tobacco (*Nicotiana attenuata*)



Max Planck Institute for Chemical Ecology

Salicylate Pathway

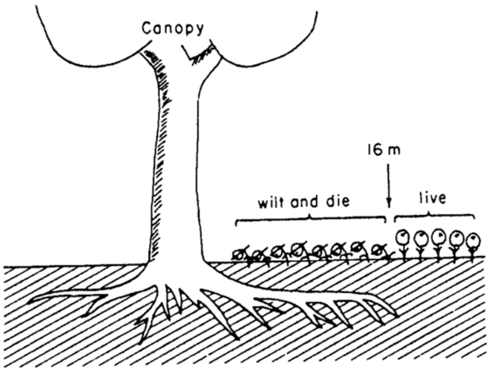


http://plantandsoil.unl.edu

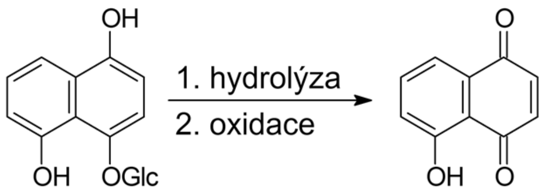
Allelopathy

- chemical interactions between (higher) plants
- chemically mediated fight of plants for nutritives
- allelopathic compounds (toxins) - secondary metabolites, structurally simple – terpenes or aromatics
- allelopathy is common in trees and bushes, plants in desert (lack of water and nutritives)
- exists in all climatic zones

Allelopathy



best known example:
Eastern Black walnut (*Juglans nigra*) and **Common walnut** (*Juglans regia*) – plants under tree wilt and die to the distance of tree roots



juglone

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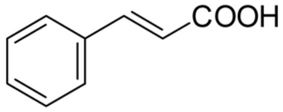
Juglone

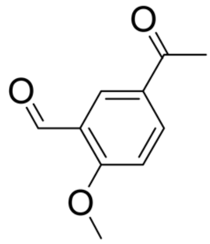
- water-soluble brown pigment
- bound in glycoside
- present in roots, leaves and green peel of nuts
- detectable in depth of 8 m and distance 27 m from tree trunk
- toxic against other plants
- inhibits seed germination (100 % inhibition in letuce at a dose 0,002 %)
- some plants tolerate allelopathic toxins

Allelopathy

Other examples – desert bushes



E-cinnamic acid

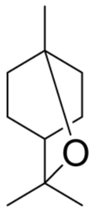


3-acetyl-6-methoxy-
benzaldehyd

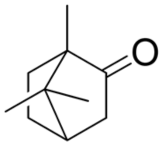
- Toxins are produced in leaves; fallen leaves decompose and release toxins in soil; persistent
- Bush of the genus *Parthenium* (Asteraceae): its toxin (*E*-cinnamic acid) has allelopathic effect to the plant itself.

Allelopathy

- zones of bushes in South California
- dominating *Salvia* and *Artemisia*
- thorny, low bushes, no vegetation in the vicinity despite a suitable climate
- allelopathic toxins - terpenes



1,8-cineol



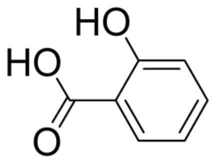
camphor

Periodic natural cycle:

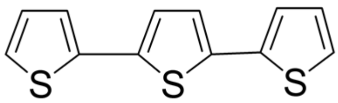
- repeated fires (25 years interval)
- 2 years after the fire, annual plants dominate
- in 3-4 years, thorny bushes start to grow and spread
- in 5-7 years, the bushes dominate, contaminate soil with terpenes and “kill” neighbouring plants

1,8-cineol = eucalyptol

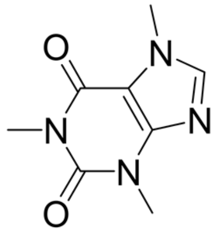
Other compounds produced by plants, inhibiting seed germination or plant growth



salicylic acid
oak (*Quercus*)



α-terthienyl
African marigold (*Tagetes*)

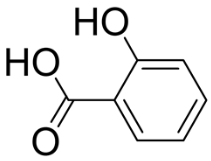


caffeine

- autotoxic effect
- probably autoregulation of plant density
- natural changing of vegetations at a locality
- sustenance of biodiversity

Allelopathic toxins can be a model for synthetic herbicides.

Other compounds produced by plants, inhibiting seed germination or plant growth



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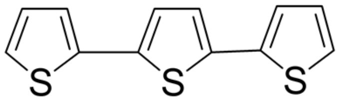

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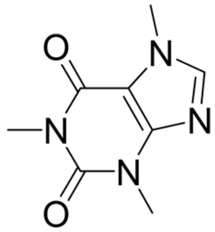


caffeine

- autotoxic effect
- probably autoregulation of plant density
- natural changing of vegetations at a locality
- sustenance of biodiversity

Allelopathic toxins can be a model for synthetic herbicides.

Synthetic herbicides

- The mechanism of effect of **allelopatic compounds** has not yet been studied in detail. Thus, they are not investigated as possible natural templates for herbicides.
- Effect of synthetic herbicides – often manipulation with **fytohormones** and their synthetic analogs.
- Classification of herbicides:

  general

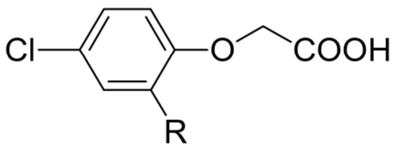
  selective
- Other classification: **contact**
  systemic

Structural types

- **inorganic** – sodium chlorate (**Travex**); general herbicide
- **organic** – main groups:
- ***Chlorinated carboxylic acids*** – for monocotyledons (grasses); t.e.x. trichloacetic acid (**TCA**)

Structural types

- ***phenoxyalkancarboxylic acids***
  herbicides derived from **plant growth regulators**

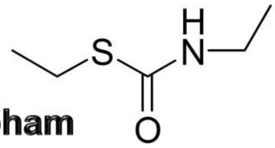


R = CH_3, 2-methyl-4-chlorophenoxyacetic acid (**MCPA**)
R = Cl, 2,4-dichlorophenoxyacetic acid (**2,4-DCPA**)

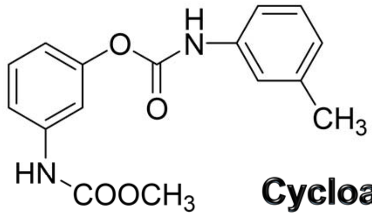
Stimulate growth in low concentration (mimicking auxine), herbicidal effect in higher concentration.

auxine = indolyl-3-acetic acid

- ***Derivatives of carbonic acid*** - carbamates and thiocarbamates – for monocotyledons, use as pre-emergent herbicides (before seed germination)

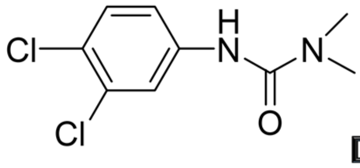


Phenmedipham



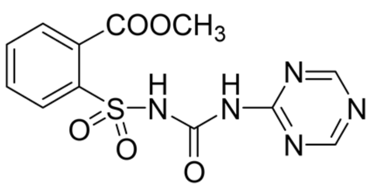
Cycloate

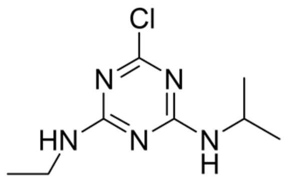
- ***Derivatives of urea*** - inhibit photosynthesis



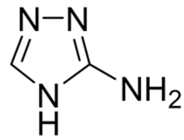
Diuron

- ***heterocyclic compounds*** - triazines, triazols, diazines; inhibit photosynthesis



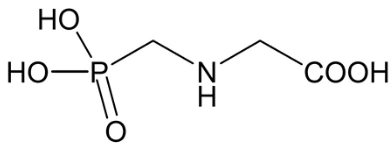


Atrazin



Amitrol

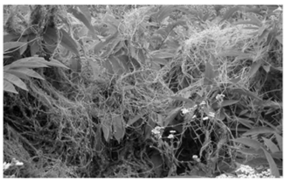
- ***Other structural types*** - anilides, nitriles, phosphonates, phenols

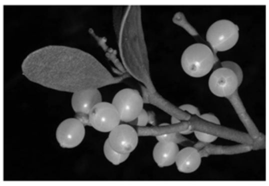


N-fosfonemethyl-glycin
Glyphosate, **Roundup**

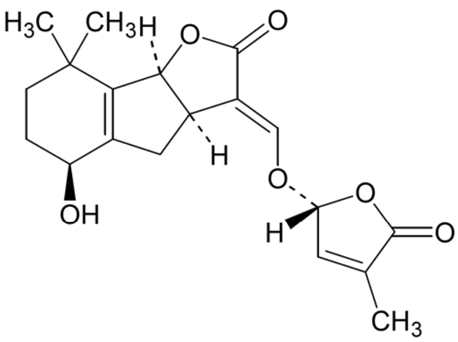
Interaction host-parasite in higher plants

- Parasites in higher plants - mistletoe, dodder (on branches), *Striga* (on roots).
- Seed of a parasitic plant must
  1) germinate near the host,
  2) connect to the host tissue. Parasite responds to root exudates of the host plant.

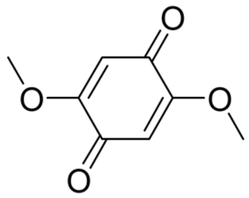




• *Striga* – parasite on sorghum roots (African cereal grown for sweet syrup)



strigol, signal ***1)***
stimulates seed germination of parasitic plant



dimethoxybenzoquinone, signal ***2)***

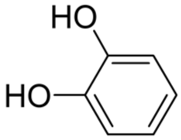
Phytoalexins and phytotoxins

- interaction between plants and microorganisms (earlier between **higher** and **lower** plants)
- wild plant species are usually naturally resistant to diseases caused by microorganisms

Classification of factors causing plant resistance to diseases

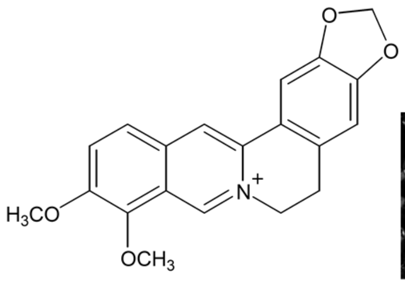
- ***Before microorganism attack:***
- **prohibitins** – inhibit multiplication of microorganism
- **inhibitins** - toxic to microorganism

- ***After microorganism attack:***
- **post-inhibitins** – formed from precursors present in plant constitutively
- **phytoalexins** - synthesised *de novo* by gene expression or by activation of latent enzymatic system



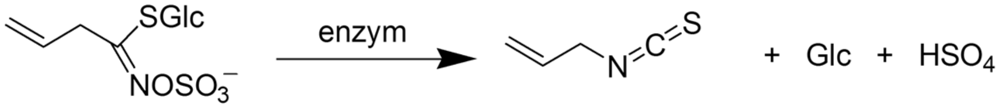
catechol, **prohibitin**
from resistant onion varieties

- structural base of anthocyanins present in many plants and having fungicidal effect
- isoflavonoids and flavonoids are efficient fungicides; they occur e.g. in plants of the genus *Lupinus*



berberin
Mahonia aquifolium roots

- **post-inhibitins** are often glycosides, the toxin may be released by hydrolysis, or hydroquinones, oxidised enzymatically to toxic quinones



sinigrine
(glucosinolate)

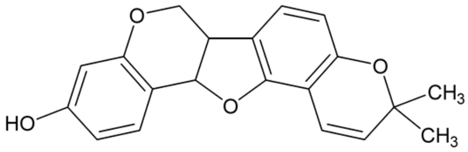
allylisothiocyanate

allylisothiocyanate is highly toxic to pathogenic fungi

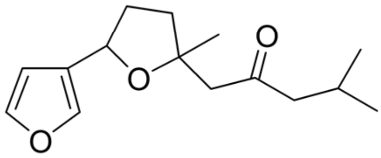
After microorganism attack

- **phytoalexins** – most important and best studied phase of plant defence from microorganisms
- **phytos = plant,   alexos = heal the disease**
- **Phytoalexins are produced *de novo* to defend from disease; response of a plant to micro-organism attack.**
- **Difference between phytoalexins and plant toxins:**
- **toxins** are **constitutive** (produced in plant all the time)
- **phytoalexins** are **induced** (response to pathogen)

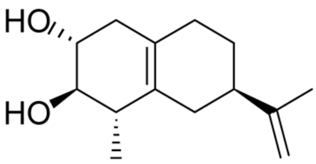
Examples of phytoalexins



phaseollin, first phytoalexin identified; common bean (*Phaseollus vulgaris*)

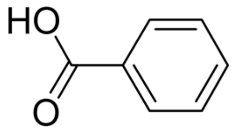


ipomeamaron (sweet potatoes)

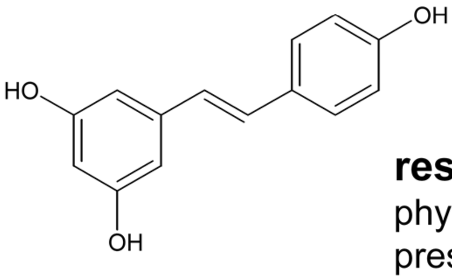


rishitin
(potatoes, *Solanum tuberosum*)

Examples of phytoalexins



benzoic acid,
defence from decay of apples;
preservative in food industry



resveratrol,
phytoalexin of several plant species,
present in red wine

- **Phytoalexins** are to smaller extent formed in response to bacteria or virus attack or to abiotic stress (UV light, heat shock, wound, high concentration of salts in soil)

- **Phytoalexins can be natural models for synthetic fungicides.**

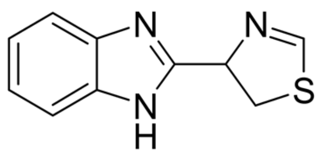
Synthetic fungicides

Classification (effect):
- contact
- systemic

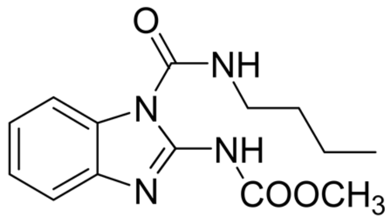
Classification (structure):
- inorganic
- organic

- ***Inorganic fungicides*** – compounds containing sulfur, copper, quicksilver, zink, tin, or baryum

- ***Organic fungicides***
- dithiocarbamates, chlorinated aromatic compounds, anilin derivatives, aromatic nitrocompounds (**contact**)
- benzimidazoles, pyrimidines, piperazines, derivatives of morfoline or triazol (**systemic**)



Benomyl



Thiabendazol

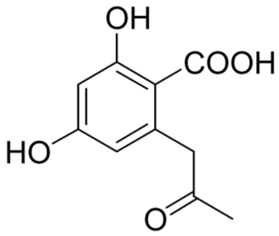
Natural benzoxazinones were isolated from some grass species.

Phytotoxins / pathotoxins

- After pathogen attack, some microorganisms produce secondary compounds that are toxic to the plant (symptoms of a plant disease).
- **Pathotoxins** are microbial metabolites causing pathological changes (symptoms) in the host plant.

Pathotoxins - types of compounds

- low-molecular (aromatics) – influence plant growth (plant wilt)
- high-molecular (peptides, proteins) – cause plant necrosis, decay of tissues

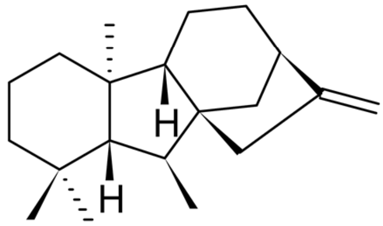


toxin from pathogenic fungus *Ceratocystis*, causing elm wilt (transmitted by bark beetles – genus *Scolytus*)

Some pathotoxins influence plant hormonal system, the wilt is caused by unnatural hormone levels (*Gibberella*).

Gibberellins

- Pathotoxins that influence plant hormonal system, the wilt is caused by unnatural hormone levels.
- Later a big group of diterpenes with *ent*-gibberellane skeleton was discovered in plants and their hormonal function explained.



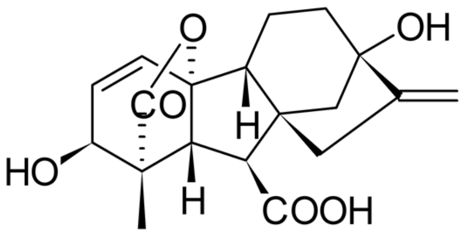
***ent*-gibberellane skeleton**

Nowadays about 90 compounds of this type and effect known (19 or 20 carbon atoms)

Gibberellins are formed in all plant organs. The highest concentrations were detected in places of active growth.

Gibberellins

- Discovered in fifties
- Known as pathotoxins from *Gibberella* (on rice) - the "foolish seedling" disease in rice
- extreme stem elongation that weakens the plant
- first isolated in 1935 from fungal strains (*Gibberella fujikuroi*)



Gibberellic acid, gibberellin A_3

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Structure of **A3** determined in 30ties (20th century)

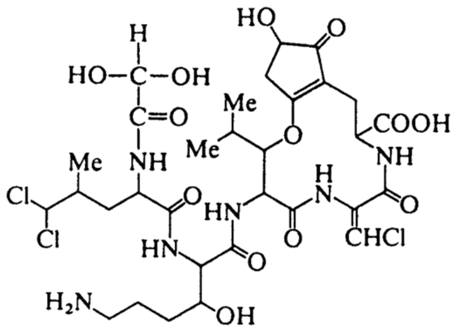
In 50ties, gibberellins found in plant tissues

Gibberellins are stable compounds

No synthetic analogs are in use

Victorin – the most effective pathotoxin known

- disease of oat (*Cochliobolus victoriae*)
- **victorin** is effective at dilution $1:10^6$
- tissues affected by fungus decay (decompose) - cells are fragmented and the tissue looses mechanical hardness (decayed fruits)



- Pathotoxins of some microorganisms have fungicidal effect on other mikroorganisms (e.g. fungus *Epichloe typhina* growing on grasses. Its pathotoxins (sesquiterpenes) „force out“ other fungi parasiting on the grass.

- **Some pathotoxins can be natural model for synthetic fungicides or remedies.**

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Bioactive Plant Products

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Number of pages 308

First edition

ISBN 978-80-213-2571-5