Development of digital learning materials for renewable pharmaceutical practice-oriented skills in English and Hungarian. Preparing university lecturers for educational challenges of the 21st century.

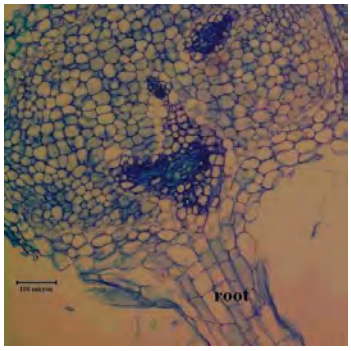
Pharmacognosy 1

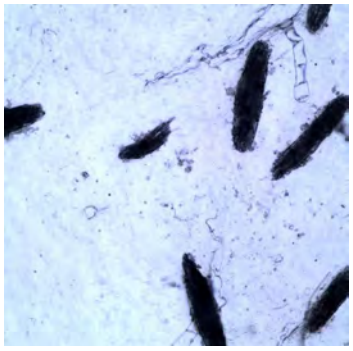
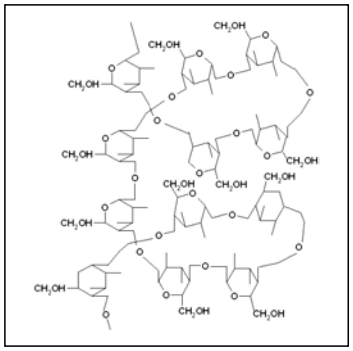
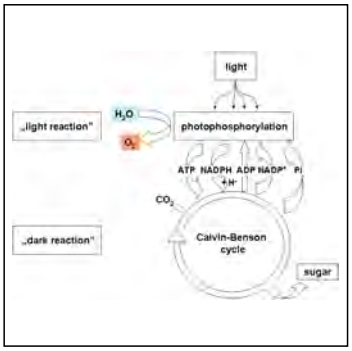
Dr. Ágnes Farkas, Dr. Györgyi Horváth, Prof. Dr. Péter Molnár







NEW SZÉCHENYI PLAN

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Content

Chapter 1
Scope of Pharmacognosy; Scientific and common name of medicinal plants and drugs; Basic ethnobotany

1.1 Scope of Pharmacognosy

Pharmacognosy encompasses the structural and chemical characterization of medicinal plants and drugs, as well as the metabolic pathways leading to the synthesis of pharmacologically active compounds. Various chapters of this teaching supplement will emphasize the significance of natural substances in pharmaceutical research, and introduce different ways of applying plant drugs. A separate chapter will discuss phytotherapy, including the effect and use of drugs, herbal extracts and isolated compounds. A brief introduction of homeopathy will also be provided. Some chapters will provide information on the collection and cultivation of medicinal plants, including breeding strategies and the application of biotechnology.

1.2 Scientific and common name of medicinal plants and drugs

Nomenclature of drugs

The scientific (Latin) name of medicinal plants is based on the binomial nomenclature introduced by the Swedish medical doctor and botanist Linnaeus. The name of the genus (e.g *Rosa*) is followed by the name of the species (e.g. *canina*), the two members being combined into the binomial name *Rosa canina*. The common (English) name of the same species is ‘dog rose’.

In Pharmacognosy the term **drug** can be briefly defined as the dried part or extract of a medicinal plant that is used for medical purposes. The scientific (Latin) **nomenclature of drugs** is based on the scientific names of the source plants. The first part of the drug name is the Latin name of the source plant (genus and/or species) in genitive, while the second part of the drug name is the Latin name of the plant organ that is used for healing purposes. E.g. the false fruits of dog rose are called *Rosae pseudofructus*. In this case only the genus name of ‘roses’ is given (genitive of *Rosa*: *Rosae*), because two distinct rose species can serve as the source plant: *Rosa canina* and *R. pendulina*. The English name of the drug consists of the common name of the plant and the plant part used in English (e.g. rose hip).

Other examples for the nomenclature of drugs can be found below, proceeding from drugs derived from below-ground to above-ground plant parts, and finishing with extracts or simple products of herbs. Finally, a few drugs of animal origin are also included in this chapter.

radix: root

e.g. *Bardanae radix* (burdock root) ← *Arctium lappa* (greater burdock)

Therapeutic uses: diuretic; externally against eczema, wounds, hair loss, dandruff

Figure 1.1
Bardanae radix (burdock root)

e.g. *Ginseng radix* (ginseng root) ← *Panax ginseng*

Therapeutic use: adaptogenic, roborating

Figure 1.2
Ginseng radix (ginseng root)

rhizoma: rhizome

e.g. *Calami rhizoma* (calamus rhizome) ← *Acorus calamus* (calamus/sweet flag)

Protected! Therapeutic use: appetizer

Figure 1.3
Calami rhizoma (calamus rhizome)

bulbus: bulb

e.g. *Allii sativi bulbus* ← *Allium sativum* (garlic)

Therapeutic use: reduces high blood pressure and blood cholesterol level, against atherosclerosis, antibacterial, antifungal

herba: aerial parts of the plant

e.g. *Bursae pastoris herba* (shepherd's purse herb) ← *Capsella bursa-pastoris*

Therapeutic use: haemostyptic (stops bleeding)

Figure 1.4
Bursae pastoris herba (shepherd's purse herb)

folium: leaf

e.g. *Farfarae folium* (coltsfoot leaf) ← *Tussilago farfara* (coltsfoot)

Therapeutic use: expectorant, antitussive, anti-inflammatory

Figure 1.5
Farfarae folium (coltsfoot leaf)

flos: flower

e.g. *Carthami flos* ← *Carthamus tinctorius* (safflower)

seeds: rich in oil;

food colorant – replaces true saffron

Figure 1.6
Carthami flos (safflower)

stigma: stigma

Croci stigma (crocus stigma) ← *Crocus sativus* (saffron crocus)

Therapeutic use: carotenoids as anti-tumor agents

Figure 1.7
Croci stigma (crocus stigma)

anthodium: inflorescence

e.g. *Chamomillae (Matricariae) anthodium* (german chamomile inflorescence) ← *Matricaria recutita* (german chamomile)

Therapeutic use: anti-inflammatory, antispasmodic, immune stimulating

Figure 1.8
Chamomillae anthodium (german chamomile inflorescence) –
Ph. Eur. 6.: *Matricariae flos*

fructus: fruit

e.g. *Anisi fructus* (aniseed) ← *Pimpinella anisum* (anise)

Therapeutic use: expectorant, spasmolytic, carminative

Figure 1.9
Anisi fructus (aniseed)

e.g. *Anisi stellati fructus* ← *Illicium verum* (star anise)

Therapeutic use: expectorant, spasmolytic, carminative

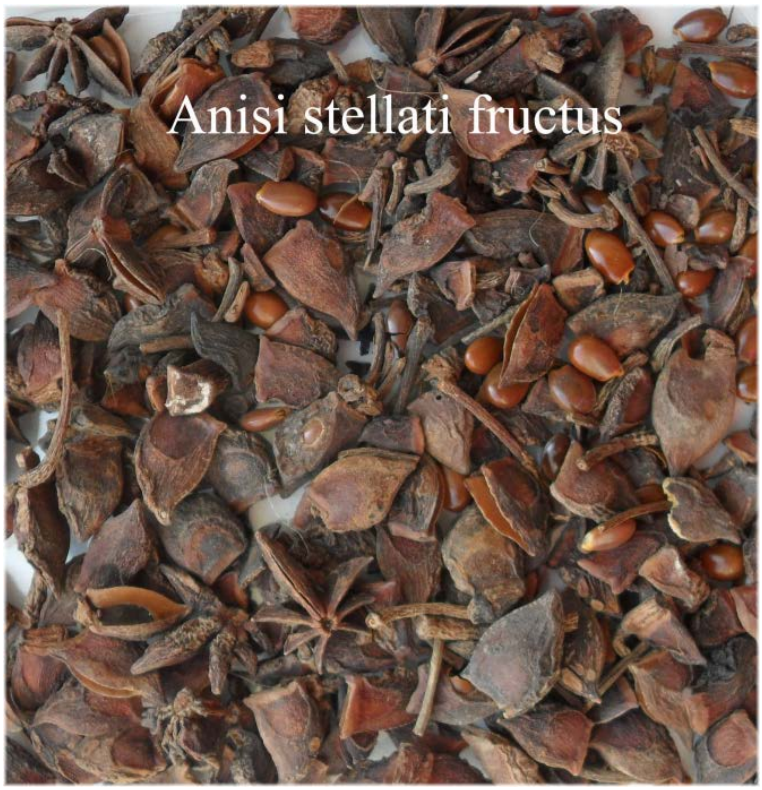


Figure 1.10
Anisi stellati fructus (star anise)

e.g. *Capsici fructus* (pepper fruit) ← *Capsicum annuum* (pepper)

Therapeutic use: against rheuma and hair loss

Figure 1.11
Capsici fructus (pepper fruit)

caput: head

e.g. *Papaveris somniferi caput* (poppy head) ← *Papaver somniferum* (poppy)

Industrial medicinal plant, source of various alkaloids (e.g. morphine: pain killer)

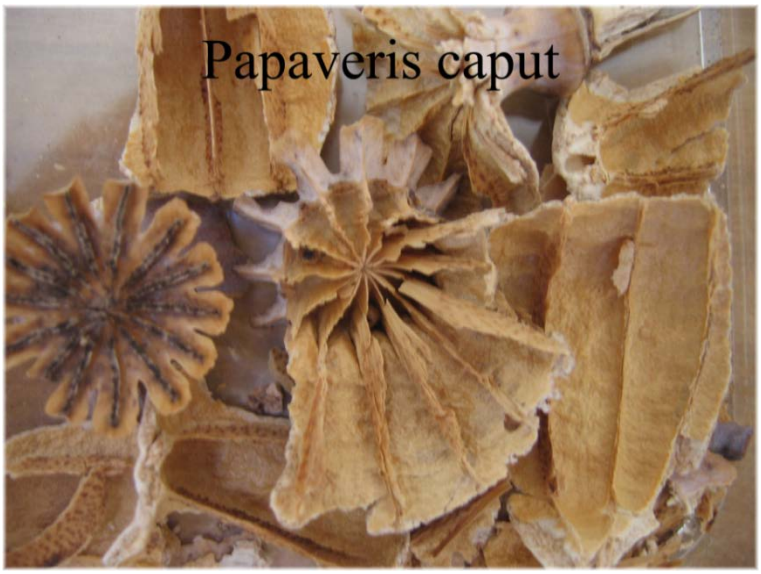


Figure 1.12
Papaveris somniferi caput (poppy head)

pseudofructus: false fruit

e.g. *Cynosbati / Rosae pseudofructus*

(rosehip) ← *Rosa canina* (dog rose), *R. pendulina* (alpine rose)

Source of vitamin C; carotenoids, flavonoids

Figure 1.13
Rosae pseudofructus cum seminibus (rosehip with “seeds”)

Figure 1.14
Rosae pseudofructus sine seminibus (rosehip without “seeds”)

bacca / galbulus: “cone berry”

e.g. *Juniperi bacca* (juniper berry) ← *Juniperus communis* (common juniper)

Therapeutic use: diuretic, carminative, appetizer

Figure 1.15
Juniperi bacca (juniper berry) – Ph. Eur. 6.: *Juniperi pseudofructus*

semen: seed

e.g. *Foenugraeci semen* (fenugreek seed) ← *Trigonella foenum-graecum* (fenugreek): pleasant odour – coumarin;

Therapeutic use: aids digestion, lowers blood-sugar and –cholesterol levels

Figure 1.16
Foenugraeci semen (fenugreek seed)

e.g. *Lini semen* (flax seed) ← *Linum usitatissimum* (flax)

Therapeutic use: laxative due to its mucilage content.

Figure 1.17
Lini semen (flax seed)

stipes: peduncle

e.g. *Cerasi stipes* (cherry peduncle) ← *Cerasus avium* (sweet cherry)

Therapeutic use: diuretic, lowers blood pressure

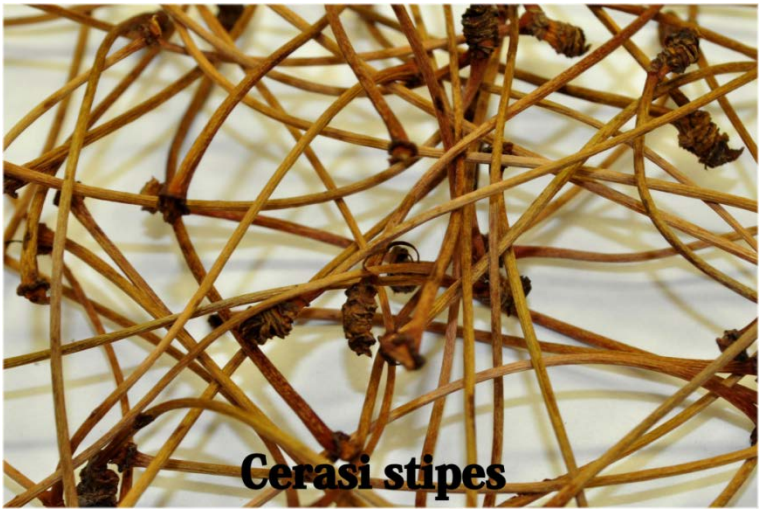


Figure 1.18
Cerasi stipes (cherry peduncle)

summitas: upper twigs with leaves, flowers/fruits

e.g. *Crataegi summitas* ← *Crataegus oxyacantha, C. monogyna* (hawthorn sp.)

Therapeutic use: beneficial for heart and blood vessels, anti-hypertensive

Figure 1.19
Crataegi summitas, Ph. Eur. 6.: *Crataegi folium cum flore* – Hawthorn leaf and flower

cortex: bark

e.g. *Frangulae cortex* (frangula bark) ← *Frangula alnus* (alder buckthorn)

Therapeutic use: laxative

Figure 1.20
Frangulae cortex (frangula bark)

e.g. *Quercus cortex* (oak bark) ← *Quercus robur, Q. petraea* (pedunculate and sessile oak) Therapeutic use: against diaorrhea

Figure 1.21
Quercus cortex (oak bark)

amylum: starch

e.g. *Maydis amylum* (maize/corn starch) ← *Zea mays* (maize/corn)

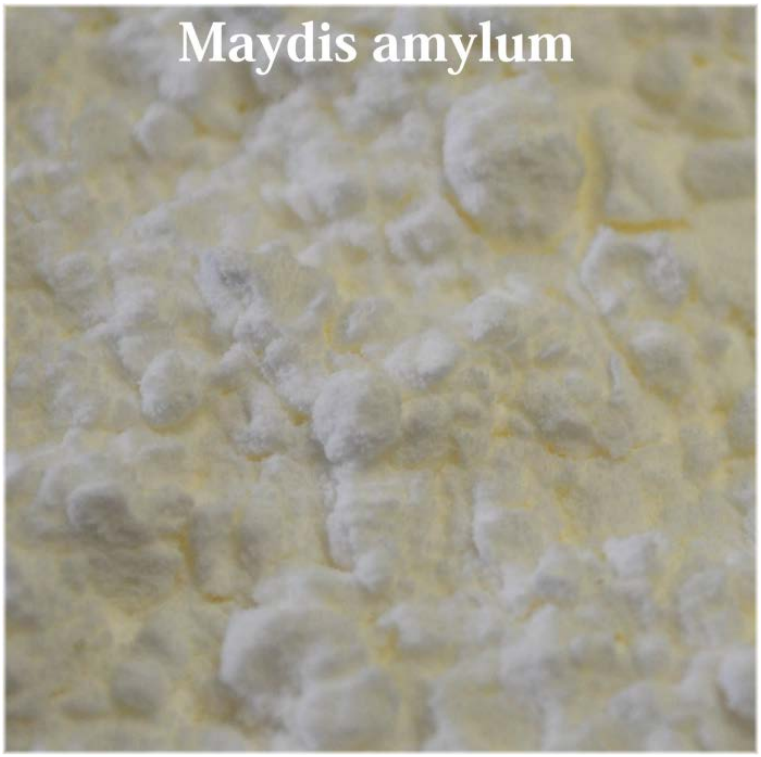


Figure 1.22
Maydis amylum (maize starch)

aetheroleum: essential oil

e.g. *Menthae piperitae aetheroleum* (peppermint oil) ← *Mentha piperita* (peppermint)

Figure 1.23
Mentha piperita (peppermint)

oleum: oil

e.g. *Helianthi oleum* (sunflower oil) ← *Helianthus annuus* (sunflower)

Used in ointments, oily injections.

Figure 1.24
Helianthus annuus (sunflower)

lignum: wood

e.g. *Juniperi lignum* (juniper wood) ← *Juniperus communis* (common juniper)

Source of tar.

Figure 1.25
Juniperus communis (common juniper)

pix: tar

e.g. *Juniperi pix* (juniper tar)

Therapeutic use: in dermatology; treating of psoriasis.

resina: resin

e.g. *Pini resina* (pine resin) ← *Pinus* sp. (pine species)

Used in ointments and plasters.

Figure 1.26
Pinus sylvestris (Scots pine)

Drugs of animal origin

- *Cantharis* ← *Lytta vesicatoria* (Spanish fly)

Therapeutic use: strong diuretic, in veterinary medicine

Figure 1.27
Lytta vesicatoria (Spanish fly) (Pharmacy Museum, Krakow)

- *Blatta orientalis*

Figure 1.28
Jars for storing *Blatta orientalis* (Pharmacy Museum, Krakow)

- *Mel* (honey)
- *Hirudo* (leech)
- *Cera* (wax) – e.g. bee wax: in ointments
- *Cetaceum* (waxy substance ← whales): substituted by jojoba (*Simmondsia chinensis)*

Figure 1.29
Wooden jar for storing *Cetaceum* (Pharmacy Museum, Krakow)

1.3 Basic Ethnobotany

Introduction

Medicinal plants, used in folk therapy by unique healing methods and special healers, play an important part in the everyday life of people living in the isolated regions of the world which are only partially or not provided by official medical or veterinary service. In 3rd world countries basic healthcare is still provided by traditional healers for 70-90% of the population. E.g. in South Africa, 70-80% of the total population (27 million) is healed by traditional medicine, involving 350,000 traditional healers, who apply 3,000 different plants.

Worldwide: 25,000-50,000 higher plants are applied in traditional medicine.

USA and Europe: ca. 120 official effective compounds of plant origin – discovery of 74% derived from traditional medicine – directly or indirectly

Ethno sciences

Ethno sciences (*ethno* meaning “people” or “cultural group”) include ethnomedicine, ethnobotany, ethnopharmacology, medical anthropology, ethno psychiatry, economic botany, archeo(ethno)botany and ethno-veterinary.

Ethnomedicine investigates the traditional knowledge of tribal societies regarding the origin, symptoms and treatments of diseases.

Ethnobotany is the study of the relationship between people and plants. It deals with traditional plant usage, including food and fodder plants, medicinal plants, fibers and

dyeing plants etc. An ethnobotanist may study how people collect wild foods for a meal and fodder, use herbs to treat illness, dye clothes, or apply them in a variety of ways, e.g. as children's toys, handicrafts, tools, furniture and timbers.

Ethnopharmacology is an interdisciplinary science, focusing on the biologically active substances in plants that are traditionally applied in various cultures. Ethnopharmacology encompasses botany, pharmacology, chemistry, as well as pharmacognosy, ethnography, anthropology and archaeology.

Leading journals of the field include

Economic Botany, an interdisciplinary journal focusing on the past, present and potential uses of plants by people. The reports include data on the traditional plant use of a particular area, often compared e.g. with the data of neighbouring countries and cultures to highlight the differences and similarities in the fieldworks.

(http://www.springer.com/life+sciences/plant+sciences/journal/12231)

Journal of Ethnopharmacology publishes reports on biological and pharmacological effects of plants, fungi and animals based on their traditional uses described during fieldworks (http://www.journals.elsevier.com/journal-of-ethnopharmacology).

Journal of Ethnobiology and Ethnomedicine is an online journal available at http://www.ethnobiomed.com. This journal publishes papers on the traditional use of plants, animals and fungi of various regions, mostly compared with data recorded in other countries.

Research methods

Ethno-pharmacobotanical surveys study the **oral tradition** as the most common source in this field. The first step is to choose a new region or village which has not been investigated ethnobotanically earlier. This step is based mostly on the analysis of **written sources**, including historical, geographical, botanical and medical references. The next step is planning the field work involving the acquisition of tools and other necessities (e.g. dictaphone, camera, plant identification keys). During field work, various types of interviews are conducted, handwritten notes are taken, local vocabulary and herbaria are prepared, photos are taken about the plants and informants, and records of the interviews with dictaphone are transcribed. Among the described data, the vernacular plant name, time and method of collection, used plant part, preparation form (e.g. tea, syrup, tincture, vinegar, gargle, rinse, bath, cataplasm, cream or liniment), way of administration and treated diseases (completed by beliefs and peculiar magico-mythological role) can be listed. Plant taxa should be identified as species with plant's identification books of the selected region. In addition, voucher specimens should be also deposited at the institute which co-ordinates the study.

The source of the medical knowledge (studied, heard or read data) is also recorded, and the elements of inherited knowledge should be separated from data originating from any written sources. This step plays a significant role in further analysis of the described data, which means the comparison of these elements with official sources (e.g. pharmacopoeias, scientific literature and references). In the case of describing a new plant species, a new drug or original way of application, phytochemical and/or pharmacological studies should be planned to affirm or disprove the traditional use.

Data types and analyses

The ancient knowledge using plants, animals and fungi can be used in the healing practice of human and veterinary medicine, too. Traditional uses of the listed materials involve both rational and irrational elements. **Rational elements** include using plants for food, fodder and medical purposes, keeping away parasites or as fibers and dyes. These data encompass exact methods of the collection and preparation of specific plants in local remedies. Based on these data the preparations should be replicable in laboratory analyses. **Irrational elements** include the magical use of natural resources accompanied by magic chants, casting spells and pow-wows. These data commonly involve e.g. peculiar numbers, places, dates and specific processes connected to the local uses of the applied materials. These elements are of pivotal importance rather in the ethnographical sciences.

There are 3 components in each healing practice:

(1) the plant, animal or mineral that is used for healing
(2) words that have to be said (chanted) according to the people's believes
(3) action that has to be performed simultaneously (e.g. taking water from the creek)

One of the research topics at the Department of Pharmacognosy, University of Pécs, is the study of the ethnobotanical data in various regions of Transylvania, currently part of Romania. Based on several earlier works from the 16-17th century, new ethnobotanical surveys were carried out since the 1960s in several regions of the country, inhabited mostly by the ethnic groups of Széklers and Csángós.

A few examples of how medicinal plants are used by the Csángó population in the Úz valley (Transylvania, Romania) are summarized below. E.g. the leaves of stinging nettle (*Urtica dioica*) have to be taken from 3 separate nettle plants bearing 5, 7, 9 leaves respectively. They are used against snake bite scrubbed into the injured body parts.

Figure 1.30
Urtica dioica (stinging nettle)

Another example is willow gentian (*Gentiana asclepiadea*), called "candle root herb", because the yellow roots (used for healing) reminded people of candles prepared from

beeswax. It is used in the treatment of jaundice based on colour analogy, which means that the colour of the used plant parts is associated with the colour of the disease or its symptoms (in this case: yellowish colour of the skin).

Figure 1.31
Gentiana asclepiadea (willow gentian)

In veterinary medicine, a well-known example is inducing a local inflammation by placing the roots (rhizomes) of hellebore (*Helleborus* sp., Figure 1.32) into an incision in the ear or breast of an animal (e.g. cattle, sheep) in order to boost non-specific immune responses. Similar applications of various *Adonis* species were reported from Hungary (*A. vernalis* – pheasant's eye, Figure 1.33), Transylvania: *A. transsylvanica* and Mongolia: *A. mongolica*.

Figure 1.32
Helleborus odorus (fragrant hellebore)

Figure 1.33
Adonis vernalis (pheasant's eye)

Significance of the field

Although new plant species, new drug parts or indications can be described mostly from the tropical areas of Asia, Africa and America, attributed to the rich and undiscovered flora elements, other regions such as the Mediterranean can also be characterized by several unidentified and unnoted ethnobotanical data.

The collection of ethnobotanical data is of primary importance mostly in the isolated areas of the world because the old knowledge of rural people about the traditional use of plants, animals, fungi and minerals decreases dramatically nowadays, due to the transformation of the landscape, the erosion of the traditional knowledge, and the migration of young people to cities and abroad.

Underlining the importance of home treatments and the possible plant sources, ethnobotanical works highlight the necessity of the preservation of disappearing medical practices among the indigenous people with further analyses which can play a significant role in recent phytotherapy.

Chapter 2
Wild, protected and cultivated medicinal plants; medicinal plant breeding; plant biotechnology, gene technology

2.1 Medicinal plant (MP) production and usage in Hungary

Beginning of 1990s

- 20-25,000 t MP export from Hungary
- Western-Europe: 100,000 t used (25% from Hungary)

End of 1990s, 2000-onward

- demand for MP increased
- in accordance with the European Pharmacopoeia (Ph. Eur.) the number of herbal drugs doubled in the Hungarian Pharmacopoeia (Ph. Hg. VIII)
- Western-Europe: 140,000 t MP
- Hungarian export decreased: 3-5,000 t –EU market: ~ 1-3 % from Hungary

2.2 Area for MP cultivation in Hungary

End of 1980s

- 37-42,000 ha area
- 35-40,000 t/yr MP production

EU accession (2004)

- not prepared properly
- area decreased to 24-26,000 ha
- amount of MPs collected from wild plants decreased to half

2.3 Trends of MP collection from natural habitats

Europe:

1200-1300 MP species, 90% of drugs collected from wild plants

Hungary:

- drug production > 30% (8000-10000 t drug/year)
- 60-70% MP (120-130 species) from natural habitats

Significance of MP collection

- Western Europe (e.g. Great-Britain): hardly any collection from wild plants, only for personal purposes
- Spain: half of drug production from natural habitats
- Albany: almost exclusively collected from natural habitats

Species spectrum of MP collected from natural habitats

- Hungary: broad
- Spain: main MP: thyme (Figure 2.1)
- Greece: sage (Figure 2.2)
- Turkey, Italy: oregano (Figure 2.3)

Figure 2.1
Thymus vulgaris (garden thyme)

Figure 2.2
Salvia officinalis (common sage)

Figure 2.3
Origanum vulgare (oregano)

2.4 The most important medicinal plants and/or their drug parts collected from natural habitats in Hungary

Below you can find the most important drugs collected from natural habitats in Hungary, together with their source plants (in order of decreasing amounts).

Figure 2.4
Sambucus nigra (European elderberry)

Figure 2.5
Sambuci fructus (elder berry)

Figure 2.6
Urticae folium (stinging nettle leaf)

Figure 2.7
Equiseti herba (equisetum stem)

Figure 2.8
Matricaria recutita (German chamomile)

Figure 2.9
Matricariae flos (matricaria flower)

Figure 2.10
Aesculus hippocastanum (horse chestnut)

Figure 2.11
Hippocastani semen (horse chestnut seed)

Figure 2.12
Rosa canina (rose hip)

Figure 2.13
Hyperici herba (St. John's wort)

Figure 2.14
Solidago canadensis (Canadian goldenrod)

Figure 2.15
Solidago gigantea (giant goldenrod)

Figure 2.16
Solidaginis herba (goldenrod)

Figure 2.17
Taraxacum officinale (dandelion)

Figure 2.18
Taraxaci radix cum herba (dandelion root with flowering shoot)

Figure 2.19
Millefolii herba (yarrow)

Figure 2.20
Viscum album (common mistletoe)

Figure 2.21
Visci stipes (mistletoe)

Figure 2.22
Crataegus laevigata (woodland hawthorn)

Figure 2.23
Crataegus monogyna (common hawthorn)

Figure 2.24
Crataegi summitas (Ph. Eur.: *Crataegi folium cum flore*, *Crataegi fructus*)
(hawthorn leaf and flower, hawthorn berries)

Figure 2.25
Chelidonium majus (greater celandine)

Figure 2.26
Chelidonii herba (greater celandine flowering shoot)

Figure 2.27
Sambuci flos (elder flower)

Figure 2.28
Tilia cordata (small-leaved lime)

Figure 2.29
Tiliae flos (lime flower)

2.5 Collection and purchase of MPs from natural habitats

Collection: no permission is needed

Trading of MPs: requires permission

permit holder is entitled to:

- buy MPs, drugs, essential oils and oils
- produce drugs
- primary processing of MPs
- store MPs / drugs
- pack drugs

Duties of MP purchaser/trader:

- organise the work of collectors
- take over drugs – fresh or dried
- pre-qualify drugs:
  identity, condition, foreign substance content

primary processing: collector or trader

How to collect MPs properly

(1) Be sure you know the plant

Related plant species: drug parts of different species can be collected together

According to Ph.Eur. the drug *Tiliae flos* (lime flower) can be collected from 3 *Tilia* species: *T. platyphyllos* (large-leaved lime), *T. cordata* (small-leaved lime) and the hybrid *T.* x *vulgaris* (common lime). However, another common lime species, *T. argentea* (silver lime) cannot be the source of lime flower, due to the abundant stellate cover hairs which can cause allergic reactions.

Figure 2.30
Tilia cordata (small-leaved lime)

Figure 2.31
Tiliae argenteae flos (silver lime flower)

The drugs *Crataegi folium cum flore* (hawthorn leaf and flower) and *Crataegi fructus* (hawtorn berries) can be collected from 5 different *Crataegus* species: *C. monogyna* (common hawthorn), *C. laevigata* (woodland hawthorn), *C. pentagyna* (small-flowered black hawthorn), *C. nigra* (Hungarian hawthorn) and *C. azarolus* (azarole).

Figure 2.32
Crataegus laevigata (woodland hawthorn)

Related species have to be distinguished

In the case of yarrow flower, a change has occurred from the previous Hungarian Pharmacopoeia (Ph.Hg. VII.) to the current Ph.Hg. VIII. Earlier, yarrow flower (*Achilleae flos*) could be collected from several different *Achillea* species, including *A. collina, A. pannonica* and *A. asplenifolia.* However, in Ph. Hg. VIII. (following Ph.Eur. 6.) the name of the drug has been changed to *Millefolii flos*, and the source plant is exclusively *A. millefolium* (common yarrow).

Figure 2.33
Achillea millefolium (common yarrow)

The drug St. John's wort (*Hyperici herba*) can be derived from a single species, *Hypericum perforatum*, which has a high level of active compounds. Other *Hypericum* species, like *H. hirsutum*, *H. maculatum* and *H. elegans* are similar in appearance, and these species frequently occur together. However, the latter species have lower level of active compounds and they are protected, therefore they cannot be collected to provide *Hyperici herba*.

Figure 2.34
Hypericum perforatum (St. John's wort)

Field horsetail (*Equisetum arvense*) is the source plant of the drug *Equiseti herba*, a well-known diuretic. Plant parts of the closely related marsh horsetail (*E. palustre*) should be excluded from *Equiseti herba*, due to the presence of the toxic alkaloid palustrine.

The flowers (*Sambuci flos*) of elder (*Sambucus nigra*) are official in the current pharmacopoeia, and also the fruits are valued for their mild laxative effect. However, no plant parts of the related *S. ebulus* are used in official medicine.

Figure 2.35
Sambucus ebulus (dwarf elder)

(2) Be sure you know what is toxic / has strong physiological effect

Medicinal plants containing active compounds with a strong physiological effect should always be collected separately. The most well-known examples include alkaloid-containing plants such as *Atropa belladonna* (deadly nightshade) and *Hyoscyamus niger* (henbane). These plants should not be mixed with other, harmless plant (part)s. The person who is collecting such plants should take some precautions: wear gloves, not touch their eyes and mouth, wash their hands afterwards.

Figure 2.36
Atropa belladonna (deadly nightshade)

Figure 2.37
Flower of *Atropa belladonna* (deadly nightshade)

Figure 2.38
Fruits of *Atropa belladonna* (deadly nightshade)

Figure 2.39
Hyoscyamus niger (henbane)

(3) Make sure to collect the appropriate part of the plant

Only the plant parts that provide the appropriate drug should be collected, in accordance with the prescriptions of pharmacopoeias or national standards. Also, attention should be paid to choosing the most suitable period for collection, i.e. when the level of the active compounds in the given plant part is optimal.

When collecting the **aerial parts** (*herba*), the stem often contains less (or no) active compounds compared to the leaves and flowers, thus the maximum proportion of stems is determined in *herba* drugs. E.g. in *Hyperici herba* the length of the stem cannot be more than 40 cm. The proportion of thick, woody plant parts is also limited, e.g. in

Marrubii herba (white horehound flowering shoot) the thickness of stem parts should be ≤ 5mm.

Figure 2.40
Marrubium vulgare (white horehound)

Figure 2.41
Marrubii herba

In the case of **flowers** or **inflorescences**, the length of the peduncle is prescribed. E.g. in matricaria flower (*Matricariae flos*) the maximum peduncle length is 5 cm.

With **roots** and **rhizomes** a certain amount of aerial stem is often permitted. E.g. in restharrow root (*Ononidis radix*) the stem parts can reach a maximum of 3%.

Figure 2.42
Ononidis radix (restharrow root)

The colour of **fruits** and **pseudofruits** should be appropriate, corresponding to their ripeness and maximal content of active substances. E.g. with rosehips (*Rosae pseudofructus*), pink or black pseudofruits should be rejected; with juniper berries (*Juniperi pseudofructus*) only the waxy, blue pseudofruits of the 2nd year can be accepted, while green, unripe cones of the 1st year should be rejected.

(4) Be sure to know when to collect

Various plant parts should be collected during the period of **technological ripeness**, which coincides with maximum levels of active compounds in the given plant organ. There is increasing evidence that the amount and composition of effective substances varies in different seasons and also in different plant parts of the same species (or cultivar). However, with plant species that have not been thoroughly investigated, we have to follow general rules for when to collect what:

- **Below-ground organs (radix, rhizoma):** dormancy period
- **Cortex:** after sap flow started
- **Gemma:** before leafing
- **Folium:** fully developed, but still fresh
- **Flos:** open flowers – with or without calyx
- **Herba:** at the beginning of bloom

With some plants, there are more specific regulations. E.g. *Althaeae folium*, *Hyperici herba* and *Millefolii herba* should be collected when the plant is in full bloom; whereas *Tiliae flos*, *Matricariae flos* and *Solidaginis herba* should be collected at the beginning of bloom;

Figure 2.43
Solidago gigantea (giant goldenrod)

Leaves, flowers and fruits should not be collected when covered with dew or rain. Aromatic plants are preferably collected in dry, sunny weather.

(5) Make sure you know the habitats

In case of plants that have similar morphological features, proper species identification can be aided by being familiar with the plant's habitat preference. E.g. *Tussilago farfara* (coltsfoot) is a pioneer plant, living along roadsides and disturbed places; whereas *Petasites hybridus* (butterbur), whose leaves superficially resemble those of coltsfoot, prefers forests, and occurs typically along creeks.

Figure 2.44
Inflorescence of *Tussilago farfara* (coltsfoot)

Figure 2.45
Leaves of *Tussilago farfara* (coltsfoot)

Figure 2.46
Petasites hybridus (butterbur)

The increasing pollution of various habitats has to be taken into consideration, as well. Plants should not be collected along roads with heavy traffic, due to contamination with dust or heavy metals. There are certain plant species that are particularly prone to accumulate heavy metals: e.g. *Hypericum* (St. John's wort), *Urtica* (nettle) and *Chelidonium* (celandine). In the vicinity of arable lands, MPs may be contaminated with pesticides.

Another important factor is if the **habitat** is **under protection** or not. If the area belongs to a national park or a nature protection area, a specific permit should be issued by the nature protection authority prior to collecting any MPs. E.g. the berries of common juniper (*Juniperus communis*) can be collected with permission of the Kiskunság National Park (Hungary).

(6) Make sure you know how to collect

Various plant parts should be collected with suitable methods and appropriate care, in order to ensure **optimal drug quality** and **preserve the ecological balance** of the ecosystem, which in turn will guarantee continuous availability of drug sources in the future.

The necessary plant parts should be detached properly, without destructing the plant. E.g. lime flowers should be collected without harming the trees (not necessary to cut or break whole twigs); small-size species that provide herb drugs (e.g. *Viola, Centaurium*) should be collected without damaging the below-ground organs.

Using **proper tools** such as scissors, clippers, knives, chamomile combs or cranberry combs, can improve drug quality.

Flowers, which are sensitive to injuries, should be placed into baskets or boxes; juicy fruits can be collected in buckets; herbs, seeds and roots in sacs.

Underground organs (e.g. roots and rhizomes) must be freed from soil. Most frequently they are washed and dried afterwards, in some cases they are peeled. Large organs should be sliced to facilitate drying.

2.6 MPs – becoming endangered

Direct effects

Direct effects like cutting down a forest, establishing new arable lands or draining swamps may lead to the reduction of the MP' habitat and stand. The decrease in the number of individuals may continue up to the point when the species eventually disappears.

Reduction of forest ecosystems has led to a significant decrease in the numbers of *Dryopteris filix-mas* (male fern), *Primula* (primrose) spp., *Adonis vernalis* (pheasant's eye), and as a consequence these species are no longer among MPs that can be collected freely.

Draining of wildwater/swamp ecosystems without preceding ecological studies is the reason for *Acorus calamus* (sweet flag) and *Menyanthes trifoliata* populations becoming reduced in Hungary.

Figure 2.47
Menyanthes trifoliata (bogbean)

Indirect effects

In case of indirect effects, the occurrence and biomass production of a plant species is modified through changes of the whole environment.

Indirect effects include air **pollution and soil contamination** resulting from industrial activities and heavy traffic. Exhaust gases pollute the air, while heavy metals can accumulate in the soil.

Other harmful effects are due to the intensive management of arable fields, often applying pesticides on a large scale.

Ecologically less tolerant species such as *Arnica montana* (mountain arnica) and *Vaccinium* spp. (including cranberry and blueberry) are particularly endangered by the above effects, and they might become rare in heavily polluted areas.

Figure 2.48
Vaccinium myrtillus (blueberry/bilberry)

Figure 2.49
Vaccinium vitis-idaea (cowberry/lingonberry)

2.7 Protection of MPs

Degree of the endangered status

Several factors may influence the degreee of the endangered status:

- ecological conditions, plant associations of a given area – same species: different status at different areas
- life form of the plant species (growing slowly – more endangered)
- plant parts utilized (root, reproductive organs – more endangered)
- time and method of collection (optimal timing, non-destructive methods – spares the plant stands)
- similar species – positive if endangered MP species can be substituted with another species (with bigger area), negative if there is a danger of mixing up the endangered plant species with a similarly looking species
- collected for other purposes – if the MP is used also for food, industrial or ornamental purposes, it will be more threatened
- can be cultivated or not

Endangered and protected species

Hungary

- ca. 500 protected plant species – 160 MPs
- 47 highly protected plant species – 19 MPs

1996: law LIII on nature protection (Hungary)

As a general rule, it is not allowed to collect protected species. They can be collected only with a **permit** issued by the appropriate nature protection authority. These permits allow the collection of MPs only for a limited period and amount of drugs.

It is prohibited to collect highly protected species.

Trade of endangered species and their products – international agreements

CITES: Convention on International Trade of Endangered Species of Wild Fauna and Flora

Supplements: species listed at various levels of protection / prohibition of collection

Some examples from these lists:

- *Panax quinquefolius*
- *Aloe ferox*
- *Orchidaceae*

Figure 2.50
Aloe ferox (cape aloe)

EU: European Cooperative Program for Plant Genetic Resources (ECP/GR), Working Group on Medicinal and Aromatic Plants (MAP WG)

- Criteria for selecting 10 model species:
  - species/genus is medically important
  - known active compound
  - species/genus – significant biodiversity
  - protected or endangered
  - can be maintained partly vegetatively, partly generatively
  - the highest possible number of member states should show interest
- 14 countries: suggested 137 species to be protected
- A priority list was established, comprising 10 plant taxa that were supported by the highest numbers of votes:
  - *Gentiana lutea*
  - *Melissa officinalis*
  - *Carum carvi*
  - *Artemisia* sp.
  - *Mentha* sp.
  - *Hypericum* sp.
  - *Achillea* sp.
  - *Salvia* sp.
  - *Thymus* sp.
  - *Origanum* sp.

Figure 2.51
Gentiana lutea (great yellow gentian)

- Main objectives of the MAP WG:
  develop conservation strategies in Europe, including the following steps:
  - make an inventory of MAP genetic resources – survey native populations of specified plant taxa (habitat data are recorded, sampling, collecting herbarium specimens)
  - ex situ and in situ conservation
  - characterization and evaluation of the genetic and chemical variability of specified plant taxa
  - documentation
  - distribution of collected data among partners

Methods for protecting MPs

Static protection

Static protection: “in situ”, “ex situ” protection: protecting the species OR the area

“Ex situ” maintenance of wild medicinal plants in gene banks:

- reservation of genetic material
- genetic reserve
  - highly productive cultivars can be selected
  - wide range of chemotaxonomic selection

Challenges:

- Ca. 200 MP species – more than 1000 chemical varieties
- Most species are "wild": no data on their reproductive biology.
- Little information on long-term storage of MAPs.

Gene bank – traditional method

(1) Basis collections:
   - only long-term storage / maintenance
   - storage under -10°C
   - 5% seed moisture content

(2) Active collections:
   - maintenance, research, seed exchange – mid-term storage
   - +4°C
   - 5-7% seed moisture content

Gene bank: seeds have to be treated, germinated

Gene bank – modern method

"In vitro" meristem- and shoot-cultures:

- increasing role
- several species (e.g *Mentha piperita*, *Lavandula intermedia*) can be maintained only vegetatively

"Ex situ" reservation

- chemotaxonomic gardens, living collections

Dynamic protection

Dynamic protection includes domestication ("on farm") – protection AND increasing production

Protection of MPs in Hungary

- 1980s: 4-5 million Ft/year – financial support
  - Research Institute of Medicinal Plants, Budakalász
  - Agrobotanical Research Institute, Tápiószele
- 1990s: 10-15 million Ft
  - further 6 institutions joined the program
- 2000s: no financial support from the state – reservation programs declined
- 2009-2010: 3-3,5 million Ft/year – support renewed

2.8 Main Medicinal and Aromatic Plants (MAPs) cultivated in Hungary

This section provides the list of cultivated MAPs and their drug parts that have the greatest significance in Hungary.

Figure 2.52
Sinapis alba (white mustard)

Figure 2.53
Sinapis albae fructus

Figure 2.54
Papaver somniferum (poppy)

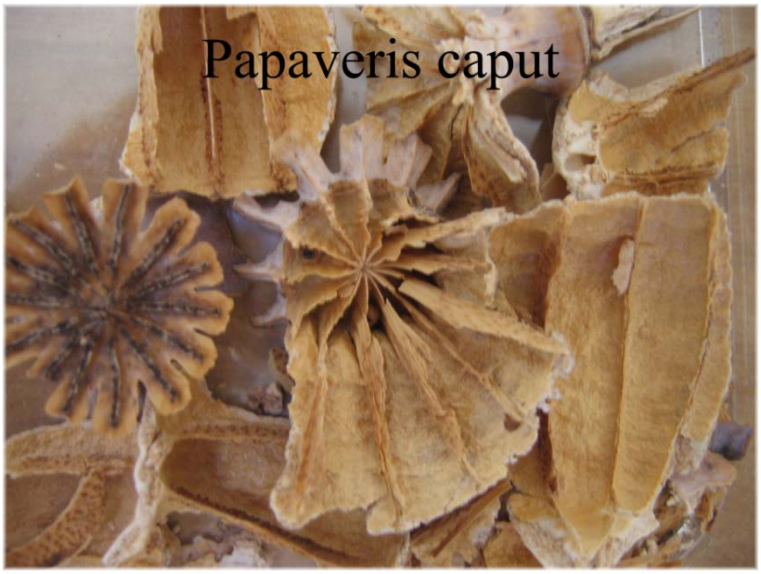


Figure 2.55
Papaveris caput (poppy head)

Figure 2.56
Carum carvi (caraway)

Figure 2.57
Carvi fructus (caraway fruit)

Figure 2.58
Coriandri fructus (coriander fruit)

Figure 2.59
Foeniculum vulgare (fennel)

Figure 2.60
Inflorescence of *Foeniculum vulgare* (fennel)

Figure 2.61
Foeniculi fructus (fennel fruit)

Figure 2.62
Silybum marianum (milk thistle)

Figure 2.63
Silybi mariani fructus (milk thistle fruit)

Figure 2.64
Anethum graveolens (dill)

Figure 2.65
Anethi fructus (dill fruit)

Figure 2.66
Pimpinella anisum (anise)

Figure 2.67
Anisi fructus (aniseed)

Figure 2.68
Melissa officinalis (lemon balm)

Figure 2.69
Melissae folium (melissa leaf)

Matricariae recutita (German chamomile) – see Figure 1.8, 2.8 and 2.9

Figure 2.70
Valerianae radix (valerian root)

Figure 2.71
Majorana hortensis (marjoram)

Figure 2.72
Majoranae herba (marjoram flowering shoot)

Figure 2.73
Ocimum basilicum (sweet basil)

Figure 2.74
Basilici herba (basil herb)

Figure 2.75
Satureja hortensis (summer savory)

Figure 2.76
Saturejae herba (savory flowering shoot)

2.9 Domestication of MPs

Reasons for domestication

- Certain MPs are used more widely, for new therapeutic purposes, new products etc., and the increased demand cannot be ensured by natural ecosystems (e.g. *Hypericum, Solidago, Secale cornutum*).
- Standard quality of drugs can be ensured only in case of known, constant composition of active compounds.
- Pesticide or heavy metal contamination of wild sources can result in lower quality drugs. In cultivation, pesticide usage is documented, controlled (e.g. *Chelidonium, Urtica*).

Figure 2.77
Chelidonium majus (greater celandine)

- Certain MPs become rare, protected (e.g. *Arnica, Veronica*).

- Some exotic MPs that are traditionally imported, can be grown in Hungary, as well. Selecting suitable genotypes and the development of local cultivation technology is necessary (e.g. *Rosmarinus*).

Figure 2.78
Rosmarinus officinalis (rosemary)

- Number of MP collectors decreases.
- Areas from where MPs can be collected are becoming scarce.

Domestication process

The domestication process of any plant species is a long-term task, amounting to 10-15 years. It requires the following steps.

(1) Selection of biological material

Wild populations are heterogeneous, considering both morphological and phytochemical features. The selection of optimal genotypes is needed. Data should be collected on the genetic, reproductive- and production biology of the given taxon. Genetically inherent features should be distinguished from modifications caused by the environment.

(2) Optimising the ecological conditions:

Phenotypic characters determined by the genetic background can be realized only in case of optimising environmental conditions and agrotechnology.

Species with wide ecological tolerance can adapt more easily to the artificial system.

The environmental conditions of natural habitats can serve as starting point, but they are not exclusively valid. E.g. German chamomile (*Matricaria recutita*) typically occurs on saline soils in nature. The plant's ability to accumulate salt provides a competitive advantage at its natural habitat, where other, less salt-tolerant species cannot survive. However, in cultivation it turned out that chamomile can perform much better and is able to provide higher quality drug when grown on nutrient-rich soil.

The following conditions should be optimised:

(a) Optimal soil properties (pH, aeration, lime and humus content, thickness of humus layer)
(b) Water requirement – can affect yield and active compound content, fruit ripening etc.; plants with high water requirement should be irrigated
(c) Temperature
(d) Light or shade (e.g. forest understorey plants require shading in cultivation)

(3) Agrotechnology

Reproduction

Experiments should be carried out to determine which is the most suitable plant part for reproduction (e.g. seeds or stolons).

Problems: seeds of wild species: low and changeable germination ability.

Plant protection and weed suppressing technology

should be established according to the principles of environment and nature protection.

Species living in their original ecosystems are balanced, however, in populations removed from their native habitat earlier unknown pests may appear and should be dealt with.

Optimal nutrition levels should be achieved.

Water should be supplemented if necessary for optimal biomass production, and accumulation of active compounds.

Optimising harvest

The optimal developmental stage for harvesting should be selected, in accordance with the maxima of dry matter content and active compounds.

Plant organ to be harvested and frequency of harvest should be determined, and proper tools should be applied.

Primary processing (postharvest technologies)

Drying, purification, cutting

2.10 MP Breeding

Biological background

Cultivar or population with known, determined genetic features:

- required phytochemical features
- required yield to be produced economically

Hungarian Cultivar List:

2007: 40 MAP species with 70 cultivars

Trends in MP breeding

Increasing production

Not total biomass, but drug part production should be increased.

- *Valeriana officinalis*: aim of breeding: increase root production, optimise root form
- *Mentha* spp., *Ocimum basilicum, Melissa officinalis*: increase shoot mass; improve stem/leaf proportion
- *Hypericum perforatum, Chrysanthemum parthenium*: quality of inflorescence should be improved

Figure 2.79
Valeriana officinalis (valerian)

Figure 2.80
Chrysanthemum parthenium (feverfew)

Improving regenerating ability

Improved regenerating ability has major significance in MPs, whose aerial parts are harvested. It facilitates more harvests per year and an increase in profit. Examples include *Thymus vulgaris* and *Majorana hortensis*.

Increasing the mass of vegetative reproductive organs

It is important in species where vegetative reproduction is dominant over sexual reproduction. Well-known examples are *Mentha piperita* (peppermint), which reproduces with stolons; and

Artemisia dracunculus (tarragon), frequently spreading with sprouts.

Figure 2.81
Mentha piperita (peppermint)

Figure 2.82
Artemisia dracunculus (tarragon)

Changing phytochemical properties

The level of **active compounds** should be increased, and/or the ratio of components should be changed. E.g. in *Hyssopus officinalis, Lavandula* spp. and *Ocimum basilicum* the essential oil content should be increased; in *Hypericum perforatum* and *Silybum marianum* the level of phenolic compounds; while in *Cucurbita pepo* and *Oenothera erythrosepala* the oil content of seeds should be raised by breeding efforts.

Ratio of components is modified

E.g. carvone should be the main essential oil component in caraway (*Carum carvi* oil), while chamazulene should be dominant in chamomile oil.

The level of toxic compounds should be lowered

Examples include common sage (*Salvia officinalis*), the essential oil of which should contain low levels of the toxic beta-thujone; and comfrey (*Symphytum officinale*), where the level of pyrrolizidine alkaloids should be kept as low as possible.

There are two opposing trends in poppy (*Papaver somniferum*) breeding: in Hungary the main goal is to achieve high alkaloid (and morphine) content for pharmaceutical utilisation; while in Western Europe poppy cultivars with lower morphine level have been developed, which are more suitable for food purposes.

Enhancing resistance

Against biotic factors

E.g. *Mentha* cultivars that are resistant against the fungus *Verticillium*, which causes wilt disease, were bred in the USA.

Against abiotic factors

Winter-hardy cultivars of rosemary (*Rosmarinus officinalis*) and fennel (*Foeniculum vulgare*) were developed in Hungary and Germany, respectively

Enhancing organoleptic features – drug quality

Organoleptic features of drugs, such as their colour and odour can largely contribute to customer preferences. E.g. pot marigolds (*Calendula officinalis*) with bright orange petals (in ligulate flowers of the inflorescence) represent better quality than the ones with pale yellow petals. Similarly, marjoram (*Majorana hortensis*) should preferably have a strong spicy odour.

Enhancing agrotechnology

- Influence growth rate, competitive ability (e.g. earliness, ability to suppress weeds, suitability for harvest with machines)
- Enhance reproductive biological features (e.g. non-dehiscent capsule which facilitates harvesting seeds; all seedlings germinating at the same time)
- Modify plant morphological features (e.g. seed size, root form, leaf size)

Methods of breeding

Selection breeding

- based on selection of individuals, clones etc.
- e.g. India: *Cinchona* spp. – selecting individuals with high levels of alkaloids
- most simple and cheap
- successful for most MPs – majority of wild populations: great genetic variability
- does not require species-specific genetic knowledge
- disadvantage: we can stabilize only the variability that is present in the given population
- efficiency can be increased by environmental pressure: under controlled conditions: individuals or lines that are able to produce high level of active compounds even under unfavourable conditions should be selected – this refers to strong genetic background (e.g. poppy cultivars 'Monaco', 'Blue Gemona')

Breeding via crosses

- unites the favourable traits of 2 genotypes
- e.g. *Carum carvi* – 1-, 2-year ecotypes;
  Mentha spicata – chemical varieties
- traditional crossing (pollination) or *in vitro* fusions, followed by selection according to our purposes
- interspecific crosses are rather rare:
  e.g. 'Blue Danube' poppy (*P. orientale x P. somniferum*)
- requires deeper genetic knowledge (flower biology, fertilisation, heritability etc.)

Polyploids

- rarely applied in MP breeding
- the naturally occurring forms of some MP genera form polyploid series:
  Achillea spp., *Mentha* spp., *Valeriana* spp.
- however, an increase in chromosome numbers is rarely accompanied by an increase in the amount of the drug part or active compounds
- there are a few examples when tetraploid cultivars were successfully introduced (e.g. chamomile – *Matricaria recutita*, dill – *Anethum graveolens*)

Mutation breeding

- performed with gamma-rays or chemical mutagens
- rare in MP breeding
- results are by chance,
- applied to alter a specific gene, or if no success is expected from other methods
- e.g. *Mentha piperita* – *Verticillium* resistance was developed with this method

In vitro breeding techniques

- hybridisation, protoplast fusion, haploid cultures, embryogenesis
- few MPs – basic research
  (*Papaver somniferum, Datura stramonium, Nicotiana tabacum*)
- practical results (cultivars): only few examples (with strong industrial background) (e.g. *Salvia sclarea*)

Figure 2.83
Datura stramonium (thornapple)

Figure 2.84
Nicotiana tabacum (tobacco)

Figure 2.85
Salvia sclarea (clary sage)

2.11 Plant biotechnology

Classic biotechnology

Living organisms (e.g. microorganism) or their parts (e.g. enzymes) are used to synthesize a product (industrial technologies), e.g. in case of microbial fermentation.

New PLANT biotechnology

Modification of the genetic program in plants, plant cells or cell organelles; development of individuals (cells or plants) with new features; the technological usage of their newly evolved abilities.

It can be utilised in plant breeding, plant protection, producing propagation materials.

Biotechnology of reproduction

Modification of sexual or asexual (vegetative) reproduction in *in vitro* cultures of plant cells, tissues or organs.

Somatic cell genetics

Modification of the genetic program of the plant cell indirectly, via cell-level manipulation, in cell- or protoplast-cultures

Gene technology

Modification of the genetic program of the plant cell or cell organelle, with molecular genetic methods

GMOs (genetically modified organisms)/ GM plants/ transgenic plants/ transformant plants:

a foreign gene (transgene) is inserted into the nucleus (genome) with molecular methods of gene technology; the gene will be integrated, functioning and new features will be transmitted to next generations

Gene technology involves the direct molecular modification of the genetic material. It applies various methods of molecular biology, cell genetics and tissue cultures.

The main steps of producing GMOs are as follows:

(a) isolation and multiplication of the gene(s) with the required character(s)
(b) gene inserted into a vector; gene transmitted to recipient cell, gene is integrated within the genome; new gene is functioning
(c) regeneration of transgenic plant from the genetically modified cells

1st generation GM plants were developed with the following goals:

- for agricultural purposes
- to protect the environment
- to make plants resistant against pests
- reduce the use of chemicals (e.g. pesticides)

2nd generation GM plants

- plants with better inner qualities
  (e.g. larger amount of essential fatty acids, less trans fatty acids)
- changes influencing plant metabolism
- development of drought-, cold-resistant cultivars

3rd generation GM plants

- produce organic molecules
- produce pharmaceutically active compounds

Examples for successfully applying biotechnological methods in management of MPs include:

- the large-scale, rapid cloning of *Dioscorea* species that are valuable sources of the steroid sapogenin diosgenin (Chaturvedi et al. 2007)
- conservation of the endangered medicinal plant *Acorus calamus* through plant tissue culture (Bhagat 2011)

Chapter 3
Tea drugs, primary processing of medicinal plants, plant extracts; Qualification and phytochemical evaluation of medicinal plants; Industrial medicinal plants

3.1 Tea drugs, primary processing of medicinal plants, plant extracts

Primary processing of plant drugs

Primary processing of plant drugs includes the following steps:

(1) Harvest
- at the optimal time ("technological ripeness")
- method of harvest or collection should be appropriate

(2) Transfer – quick, proper transfer of crude drug to the place of drying

(3) Assessment of drugs before further processing:

species (source plant) identification, should be healthy, with characteristic sensory characters, including no foreign matter

(4) Storage before processing: toxic and non-toxic drugs separated; drugs with strong smell – separately;

(5) Pre-processing before drying:
- purification (by hand or sieve, washing, peeling)
- cutting, slicing
- removing leaves and flowers from stem
- fermentation

(6) Drying – removing water without harming the drug
There are several factors that influence the speed and efficiency of drying, such as:

- temperature of drying air (usually < 60°C)
- moisture content of air
- speed and direction of air flow
- duration of drying
- method of drying:
  - natural (open-air)
  - artificial

Figure 3.1
Equipment for natural drying

Artificial drying:

with drying equipment

(a) cold air (8-12 days, ventilation)
(b) warm air (4-12 hours) – most frequent
(c) hot air (2-5 min, 200-1000°C) – mainly industrial drugs
(d) lyophilisation

1 kg dry drug can be obtained from:
- flos: 6-7 kg crude drug
- folium or herba: 5-6 kg
- (pseudo)fructus, radix, cortex: 2-3 kg
- semen: 1.2 - 2 kg

(7) Purification and comminution of drugs
- conveyer belt (by hand, 1-10 m/min)
- cutters
- trituration: dried aerial parts: removing valuable leaf- and flower-parts from the stem
- millers – finer comminution
- sieve equipments: fractionation, removing dust (chamomile flowers – removing peduncles)
- seed drugs – removing particles with different size

Grade of comminution

no comminution: small flowers, inflorescences

scissus: coarsely cut – **sieve size 1** (6.3mm): flowers

conscissus: comminuted – **sieve size 2** (4.0mm): leaves, herb drugs

semiconscissus: finely comminuted – **sieve size 3** (2.0mm): root, rhizome, bark, woody parts, leathery leaves, fruits, seeds

sieve size 4, 5 – powdered substances – suitable for industrial processing (tablet, capsule)

sieve size 6 (0.32 mm): fine powder – tea bags

Herbal tea drugs

- tea – aqueous extract
- "monotea" – single tea drug
- "species" – mixture of 2 or more tea drugs
- tea drug with strong physiological effect (e.g. *Chelidonii herba*): under medical supervision, with exact dosage
- mild tea drugs – not dangerous: prevention, treatment of chronic/acute diseases
- ! continuous consumption – long-term usage not always beneficial (e.g. tea drug with high tannin content – might cause liver damage in case of prolonged use)

Herbal teas should not be mixed up with the tea prepared from black or green tea leaves (*Theae folium*, source plant: *Camellia sinensis*, syn. *Thea sinensis*). Herbal teas can be prepared by means of infusion or decoction (see below) from various plant parts of MAPs.

Below you can find the definition of herbal teas **according to Ph.Eur.**: **"Herbal teas – Plantae ad ptisanam"**

> "Herbal teas consist exclusively of one or more herbal drugs intended for oral aqueous preparations by means of decoction, infusion or maceration. The preparation is prepared immediately before use.
>
> Herbal teas are usually supplied in bulk form or in sachets.
>
> The herbal drugs used comply with the appropriate individual European Pharmacopoeia monographs or in their absence to the general monograph on *Herbal drugs* (*1433*).
>
> Recommendations on the microbiological quality of herbal teas (*5.1.4. – Category 4*) take into account the prescribed preparation method (use of boiling or non-boiling water)."

Preparing herbal teas – ways of extraction

Liquid extracts = Extracta fluida

Infusum (infusion): pour boiling water on the drug and leave for 10-15 min with the lid on (or: after soaking, 20 min in steam), filter

Decoctum (decoction): boil for 10-15, or 40 min in steam after soaking, filter

Digestio: soak for longer time (4-8 h) at 30-70°C (e.g. *Frangulae cortex*, *Uvae ursi folium*)

Maceratio (maceration): soak for longer time (8-12 h) at room temperature (e.g. *Althaeae radix*, *Rosae pseudofructus*)

Figure 3.2
Uvae ursi folium (bearberry leaf)

Figure 3.3
Althaeae radix (marshmallow root)

Other liquid extracts

Special maceration: in vinegar, wine, oil – e.g. with garlic, St.John's wort, arnica

Solutio (solution): special technology (e.g. *Aqua aromatica*)

Sirupus (sugary solution): *Sirupus aurantii, S. rubi idaei, S. laxans*

Tinctura (tincture, alcoholic extract): usually soaked in 40-70% ethanol, or with percolation

- homeopathy: **mother tinctures**
- **gutta (drops):** usually alcoholic extracts
- **medical wines:** tincture mixed with white or red wine

Soft and dry extracts

Extracta (soft and dry extracts):

- **soft extract:** semi-solid
- **dry extract:** solid

usually with evaporation of the solvent used for extraction

- extractum fluidum (dry matter: 15-50%)
- extractum subspissum (dry matter: 50-70%)
- extractum spissum (dry matter: 70-85%)
- extractum siccum (dry matter: ~ 94%)

Other extracts

Supercritical fluid extraction (SCFE): usually with CO_2

Essential oil extraction: water steam distillation, cold pressing

Fatty oil extraction: with solvent, pressing

Hydrophilic substances: lyophilisation following aqueous extraction, spray drying, vacuum drying (instant powders, granules)

Freshly pressed juices (e.g. cabbage, beetroot) pasteurized, lyophilized

Pulpa - laxative agents (e.g. *Tamarindi pulpa*)

Other formulas with pharmaceutical technological methods (cyclodextrin powder, granulation, tablet, dragee, lozenge, capsule, ointment)

Figure 3.4
Tamarindi pulpa (tamarind)

3.2 Qualification and phytochemical evaluation of medicinal plants

The quality control of MPs is regulated by the following:

- GAP – Good Agricultural Practice
- GMP – Good Manufacturing Practice
- ISO – International Organisation for Standardisation, 9002:1994 standard
- Ph.Hg.VIII.
- Ph.Eur., DAB (Deutsches Arzneibuch)
- MSZ (Magyar Szabvány = Hungarian Standards)

GAP: Good Agricultural Practice

- Quality – plants with known genetic background
  - Collection: chemically well-defined taxon
  - Cultivation: controlled propagation material (cultivar)
- Cultivation site – ecological conditions – optimal production – controlled technology
  Collection: preserving the natural environment, sustaining biodiversity
  - Cultivation: species or cultivars – ecological requirements – adaptation to cultivation area
  - Minimal biological, chemical or physical contamination
- Post-harvest procedures
  - maintaining or improving the quality of the product
  - primary processing, packaging, storage

Quality control / Assessment

(1) Identification of collected or harvested plant material, basic quality characters
(2) Quality assessment of the drug as end product:
organoleptic, phytochemical analysis
(3) Monitoring changes during storage and packaging of drugs

Quality control (2 and 3), issuing retail trade permissions:

- earlier: Research Institute of Medicinal Plants (RIMP) (Hungarian: GyNKI)
- today: accredited quality control laboratories

Ph.Hg.VIII. – Ph. Eur.: general notices

"Plant drugs – Plantae medicinales" regulates:

- production
- identification
- methods of examination
- detection of pesticides

GMP: Good Manufacturing Practice

MPs, herbal preparations

- standardization of effective substances (prescribing the amount of total effective compounds or the amount of a specific compound)
- organic solvent (used for extraction) residues cannot be present or have to be under the prescribed limit

- no heavy metals or pesticide residues are allowed
- microbiological quality is prescribed
- quality of packaging material, method of packaging have to comply with prescriptions

Ph. Eur.

- ca. 200 plant drugs
- 30 oleum
- 30 aetheroleum
- 20 tincturae
- 10 extracta

Individual monograph structure

Latin and English name of the drug and the source plant(s)

Identification

- macroscopic examination
- microscopic examination
- identification reactions
- TLC (thin layer chromatography), GC (gas chromatography) or spectroscopic identification

Purity examinations

- organoleptic examination
- foreign matter: other plant parts, foreign contamination (≤ 2% m/m)
- loss on drying (drying at 105°C – weight loss)
- total ash
- ash insoluble in hydrochloric acid
- pesticide residue examination (bio product: 0.01mg/kg)
- microbiological purity
- heavy metals (Pb, Cd, Hg, Fe, As) < limit

Phytochemical assessment

- determining the extract content (aqueous or alcoholic extract)
- swelling index – mucilage-containing drugs (swelling of 1g drug in water (ml), after 5 h, at room temperature)
- bitterness value – the highest dilution, from which 5ml still tastes bitter
- tannin content
- haemolytic index – saponin-containing drugs (the dilution still causing total haemolysis)
- alkaloid content
- essential oil content
- other active compounds (e.g. total flavonoid content) – quantitative evaluation usually with a spectroscopic analysis

3.3 Industrial medicinal plants

In the previous decades numerous developments of industrial medicinal plants took place in Hungary. Leading researchers of several projects worked at the **Research Institute of Medicinal Plants (**RIMP, Budakalász, Hungary). Some examples can be found below.

Papaver somniferum (poppy)

Main aspects of breeding:

- increase yield (seed production),
- opium poppy – pharmaceutical industry: increase the concentration of morphine or other active compounds (alkaloids)
- food poppy – decrease alkaloid content
- **János Kabay:** elaborated the technique of extracting alkaloids from poppy straw

Figure 3.5
Capsule of *Papaver somniferum* (poppy)

Mentha piperita (peppermint)

- developing production technology
- withdrawing *Mentha rubra* from breeding – essential oil composition was not suitable

Ricinus communis (castor oil plant)

- cultivars with smooth capsule and low stem were developed – to facilitate harvest of seeds, which are rich in oil

Figure 3.6
Ricinus communis (castor oil plant)

Secale cornutum (ergot) ← *Claviceps purpurea*

- developing production technology
- Miklós Békésy: artificial infection of rye
- later: automation, breeding for active compounds (ergot alkaloids), production of propagation materials

Figure 3.7
Secale cornutum (ergot)

Digitalis purpurea (purple foxglove) and *Digitalis lanata* (woolly foxglove)

- phytochemical research (sources of cardenolide-type cardiac glycosides) and breeding

Figure 3.8
Inflorescence of *Digitalis purpurea* (purple foxglove)

Figure 3.9
Digitalis lanata (woolly foxglove)

Figure 3.10
Inflorescence of *Digitalis lanata* (woolly foxglove)

Domestication of MPs growing in natural habitats

- *Acorus calamus* (sweet flag, calamus)
- *Rhamnus frangula* (alder buckthorn)
- *Matricaria recutita* (German chamomile)
- *Gypsophyla paniculata* (baby's breath)
- *Menyanthes trifoliata* (bogbean)

Figure 3.11
Gypsophyla paniculata (baby's breath)

Cultivation of foreign MPs

- *Hyoscyamus muticus* (Egyptian henbane)
- *Datura metel* (devil's trumpet)
- *Ammi majus* (bishop's flower), *Ammi visnaga* (toothpickweed)

Figure 3.12
Flower of *Datura metel* (devil's trumpet)

Figure 3.13
Fruit of *Datura metel* (devil's trumpet)

Elaborating agrotechnology of plants with essential oil

- *Mentha* x *piperita* (peppermint)
- *Mentha spicata* convar. *crispa* (spearmint)
- *Lavandula* sp. (lavender)
- *Salvia* sp. (sage)
- *Thymus vulgaris* (garden thyme)
- *Anethum graveolens* (dill)
- *Carum carvi* (caraway)
- *Foeniculum vulgare* (fennel)

- *Pimpinella anisum* (aniseed)
- *Coriandrum sativum* (coriander)
- *Levisticum officinale* (lovage)
- *Angelica archangelica* (garden angelica)

Figure 3.14
Lavandula angustifolia (English lavender)

Figure 3.15
Levisticum officinale (lovage)

Figure 3.16
Angelica archangelica (garden angelica)

MPs containing rutoside - Gubányi Emil

- *Fagopyrum esculentum* (buckwheat)
- *Fagopyrum tataricum* (tartary buckwheat) – tolerates drying better, almost no decrease in rutin content
- *Sophora japonica* flower bud – new source with higher rutin content

Figure 3.17
Fagopyrum esculentum (buckwheat)

Plant steroid research

plant steroids can serve as sources for producing sex hormones, hormones of the adrenal glands by the pharmaceutical industry

- *Dioscorea* sp. – couldn't be cultivated in Hungary
- *Solanum* sp. (*S. aviculare* / *S. laciniatum*) – cultivation successful

Apocynaceae family

- *Catharanthus roseus* – cancer therapy
- *Vinca minor* – enhancing cerebral blood circulation

Figure 3.18
Vinca minor (lesser periwinkle)

Current sources of medications

- *Cucurbita pepo* (squash, pumpkin) → *Cucurbitae semen* → treatment of benign prostate hypertrophy (BPH)

Figure 3.19
Cucurbita pepo (pumpkin)

- *Silybum marianum* → *Silybi mariani fructus* → hepatoprotective dragee with silimarine

Figure 3.20
Silybum marianum (milk thistle)

- *Claviceps purpurea* → *Secale cornutum* → ergot alkaloids (e.g. ergometrine used to prevent bleeding after childbirth; ergotamine used for treatment of acute migraine attacks)
- *Valeriana officinalis* → *Valerianae radix* → valepotriates used as anxiolytic, in treatment of insomnia

Chapter 4
Natural substances in pharmaceutical research

Pharmacognosy itself is a multidisciplinary field within pharmaceutical sciences. It involves the knowlegde of drugs derived from plants, animals and fungi, used for medicinal purposes. Pharmacognosy requires the following knowledge:

- **biological** (morphology, anatomy, genetics)
- **biochemical** (biosynthesis and metabolism of compounds)
- **chemical** (structure and physico-chemical feature of metabolites, etc.)
- **analytical** (investigations, qualification-quantification determination)
- **therapeutical** (medicinal use, side effects, etc.)

4.1 Usage of Medicinal Plants

Medicinal plants can be used for a variety of purposes. The most relevant uses include therapeutical and pharmaceutical applications. Aromatherapy uses essential oils produced by plants. Food industry uses medicinal plants as flavouring materials (e.g. spices, natural aromas) or colouring materials. Many plants containing flavonoids have antioxidant effects, therefore they can be additives. Cosmetic and household industries use essential oils or their compounds isolated from approx. 1400 species. Medicinal plants have other industrial applications, for instance in dye-, textile- and chemical industry.

4.2 Some definitions

Plants produce chemically different compounds. We have to distinguish these materials according to the aim of their utilization.

Content materials: they are biosynthetized by the plants (during universal and special metabolism) and are characterized with different chemical structures.

Active constituents: they are specific content materials that are biologically active substances. They are responsible for the pharmacological effects of the drug.

e.g. *Digitalis*-glycosides are the active constituents in the genus *Digitalis*. Chamomile (*Matricaria recutia*) contains three groups of active compounds: essential oil, flavonoids and mucilage.

Characteristic constituent (marker-compound, principal-compound): it is also a kind of content material, but is used for analytical purposes, quantitative measurements and product standardization. It is not necessarily an active constituent, but the plant can produce it in great amounts.

Accompanying constituents: they are content materials, which help or prevent the effect of active constituents, and they may cause side-effects.

Ballast constituent: it must be removed during the extraction procedure, because it may disturb the correct quantitative measurements. Tannins, fatty oils and to a lesser degree chlorophyll are the most frequent ballast contituents, but the aim of the extraction always has to be taken into consideration. E.g. “Determination of tannins in herbal

drugs" is an official method in the European Pharmacopoeia, therefore, in plants containing tannins, these are active compounds, not ballast constituents.

4.3 Therapeutical utilization of medicinal plants

(1) Mono-component products

They contain pure constituents, e.g. herbal medicines.

Characteristics:

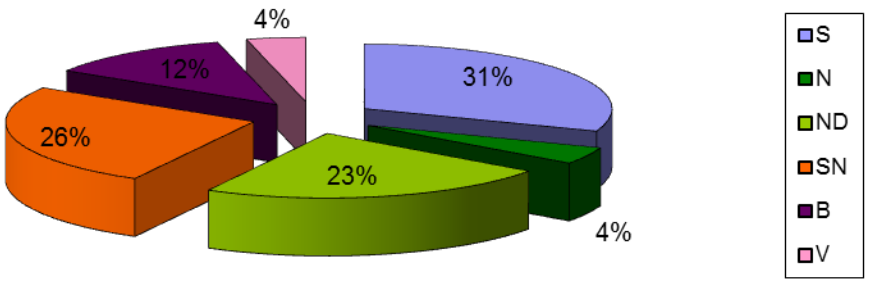
- High activity
- Accompanying constituents do not influence the effect
- Easy analysis
- Exact dosage

(2) Multi-component products

They contain more than one constituent, e.g. teas, tinctures, extracts, oils, syrups, etc. Herbal products and dietary supplements are multi-component products.

Characteristics:

- They have complex effect, because there is synergism between components.
- There are no pure constituents: is not possible to isolate or the isolation is not economical
- Active constituent is not known



S: synthetic, **N:** natural constituent, **ND:** derivative of natural constituent, **SN:** synthetic developed based on natural constituent, **B:** constituent (peptid, protein) produced by biological (mammal) organism or cell culture, **V:** vaccine

Figure 4.1
Distribution by origin of medicines licensed between 1981-2006
(Source: Csupor D.: Fitoterápia. JATEPress, Szeged, 2007)

4.4 Types of pharmaceutical utilization of natural constituents

There are three types of pharmaceutical utilization of natural constituents:

(1) Application of natural constituents in direct form
(2) Application of derivatives of natural constituents
(3) Model- or marker-compounds of synthetic medicines – “leader molecules”

(1) Application of natural constituents in direct form

Taxol is a good example when a natural substance becomes a medicine. **Taxol (= paclitaxel)** was isolated from the bark of pacific yew (*Taxus brevifolia*) in 1971. Chemically it is a diterpene-alkaloid. Today it can be used in medicines for treating lung-, ovarian- or breast cancer. Paclitaxel stabilizes microtubules and as a result, interferes with the normal breakdown of microtubules during cell division.

Figure 4.2
Taxus baccata (common yew)

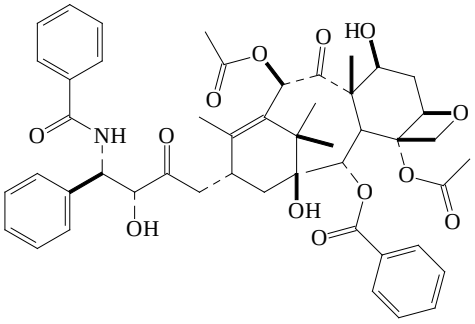


Figure 4.3
Chemical structure of taxol A

(2) Application of derivatives of natural constituents

Camptotheca acuminata (camptotheca, happy tree), a plant native to southern China and Tibet, is the source of **camptothecin (CPT)**, a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I. It was discovered in 1966 by Wall and Wani in systematic screening of natural products for anticancer drugs. It was isolated from the bark and stem of *Camptotheca acuminata*, which has been used in cancer therapy in Traditional Chinese Medicine. CPT showed remarkable anticancer activity in preliminary clinical trials but also low solubility and (high) adverse drug reaction. Because of these disadvantages synthetic and medicinal chemists have developed numerous syntheses of camptothecin and various derivatives to increase the benefits of the chemical, with good results. Two CPT analogues have been approved and are used in cancer chemotherapy today, **topotecan** (in lung and ovarian cancer) and **irinotecan** (in colon cancer) (Wall et al. 1966, Efferth et al. 2007, Akimoto and Calvo 2008).

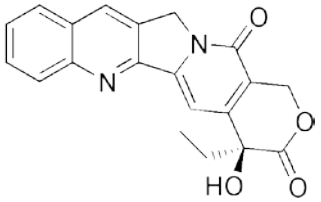


Figure 4.4
Chemical structure of camptothecin

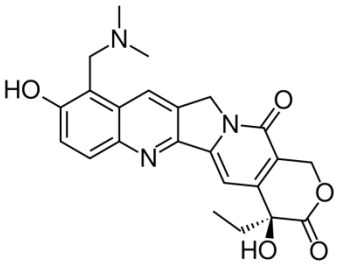


Figure 4.5
Chemical structure of topotecan

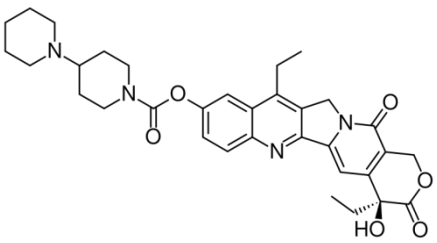


Figure 4.6
Chemical structure of irinotecan

(3) Model- or marker-compounds of synthetic medicines – "leader molecules"

Galega officinalis (goat's rue) has been known since the Middle Ages for relieving the symptoms of diabetes mellitus. Upon analysis, it turned out to contain compounds related to guanidine, a substance that decreases blood sugar by mechanisms including a decrease in insulin resistance. However, the compounds detected in goat's rue were too toxic for human use. Georges Tanret identified an alkaloid from this plant, **galegine**, that was less toxic, and this was evaluated in unsuccessful clinical trials in patients with diabetes in the 1920s and 1930s. Other related compounds were being investigated clinically at this time, including biguanide derivatives. This work led ultimately to the discovery of **metformin**, currently recommended in international guidelines for diabetes management as the first choice for antidiabetic pharmacotherapy alongside diet and exercise (Witters 2001, Bailey et al. 2007, Nathan et al. 2009).

Figure 4.7
Inflorescence of *Galega officinalis* (goat's rue)

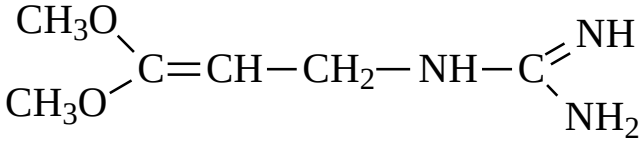


Figure 4.8
Chemical structure of galegine

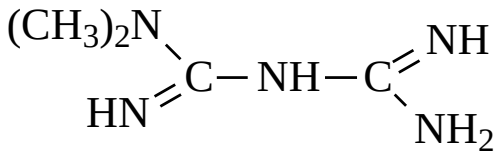


Figure 4.9
Chemical structure of metformin

4.5 Industrial production of natural drugs

There are five different techniques to produce pharmacologically active compounds from natural drugs.

(1) Isolation
(2) Total synthesis
(3) Semi-synthesis from natural compound
(4) Biotechnology
(5) Combination of biotechnology and chemical methods

(1) Isolation

Quinine (quinoline-alkaloid) was isolated from the bark of *Cinchona pubescens* Vahl. (Rubiaceae) in 1817. *Cinchonae cortex* is an official drug in the Ph. Eur. The quinine-content of the bark is 3-14%. Total production of the world: 5000 – 10 000 tons of bark, which corresponds to 300 – 500 tons of quinine/year. Its synthesis is also solved: in 1944. R. Woodward and W. Doering developed total synthesis, while in 2001. G. Stork achieved stereoselective synthesis.

Figure 4.10
Cinchonae cortex (cinchona bark)

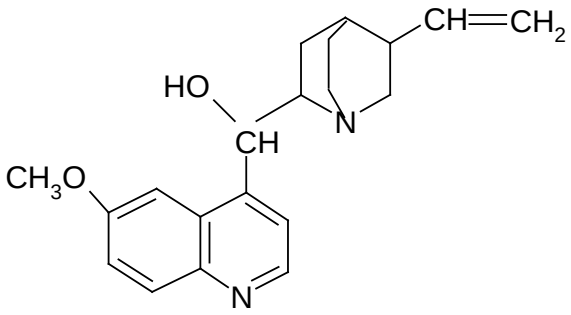


Figure 4.11
Chemical structure of quinine

Digitalis glycosides: *Digitalis purpurea* (common or purple foxglove) and *Digitalis lanata* (woolly foxglove, in Hungary this plant is protected, but it can be cultivated) produce Digitalis glycosides, e.g. **digoxin**. Digoxin is used in medicine for treatment of cardiac insufficiency (atrial fibrillation). In 1930, the structure of sugar part was discovered. The structure identification was finished in 1952. The leaves contain 0.05-0.1% cardiac glycosides. Other relevant compounds are digitoxin and lanatoside C.

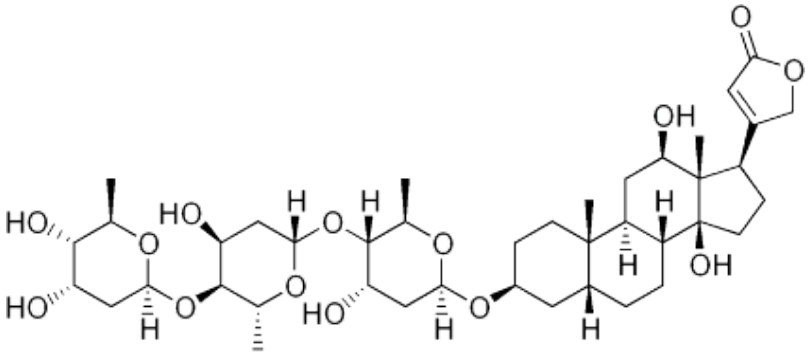
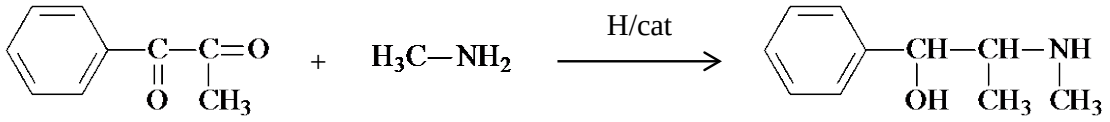


Figure 4.12
Chemical structure of digoxin

(2) Total synthesis

Ephedrae herba (*Ephedra distachya* L., Ephedraceae) produces ephedrine, commonly used as a decongestant and stimulant. The source plant is protected in Hungary, therefore it cannot be collected. Figure 4.13 shows the synthetic production of ephedrine.



phenyl methyl diketone methyl amine ephedrin

Figure 4.13
Synthetic production of ephedrine

(3) Semi-synthesis from natural compound

Opium poppy (*Papaver somniferum* L., Papaveraceae) produces opium, which contains morphine 10-14%, codeine 1%, tebaine 0.2-0.5% and other alkaloids. Codeine is a cough-suppressant. This compound can be produced from morphine or tebaine (see below).

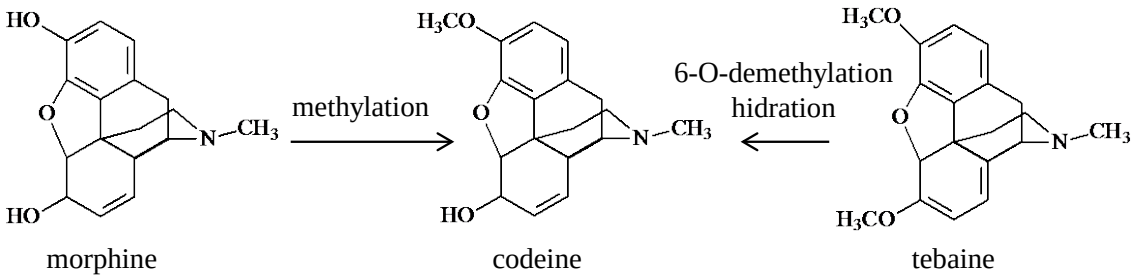


Figure 4.14
Synthetic pathways of codeine

"Birth" of morphine

- 1805: Sertürner (Friderich Wilhelm Adam Sertürner), German pharmacist – isolation of morphine
- origin of the name "morphine" – Morpheus, the God of sleeping
- 1816: reported the chemical and pharmacological properties of morphine (self-studies)
- 1848: Laurent – determination of molecular formula
- 1925: determination of graphic formula (structure)
- 1820: industrial production of opium alkaloids from opium (Merck), use of morphine instead of opium

János Kabay (1896 – 1936)

A Hungarian chemist, János Kabay, invented and internationally patented a method (in 1931) to extract morphine from poppy straw. Poppy straw was the cheapest raw material for isolation of morphine. In opium poppy the alkaloids are bound to meconic acid. The method is to extract from the crushed plant with diluted sulfuric acid, which is a stronger acid than meconic acid, but not so strong to react with alkaloid molecules. The extraction is performed in many steps (one amount of crushed plant is at least six to ten times extracted, so practically every alkaloid goes into the solution). From the solution obtained at the last extraction step, the alkaloids are precipitated by either ammonium hydroxide or sodium carbonate. The last step is purifying and separating morphine from other opium alkaloids.

(4) Biotechnology

Ergotamine is an important alkaloid, which is found in a sclerotium produced by a fungus (*Claviceps purpurea* (Fries) Tulasne, Clavicipitaceae). A sclerotium is a compact mass of hardened fungal mycelium, which develops on rye or wheat spike. Today ergotamine can be produced with biotechnological methods, in liquid cultures at 24°C, for 6-10 days (1-6 g/L peptide alkaloids). Ergotamine is a vasoconstrictor (in case of giving birth or migraine).

Miklós Békésy (1903-1980) got Kossuth-prize for his achievement of industrial cultivation of *Secale cornutum* (ergot) in Hungary.

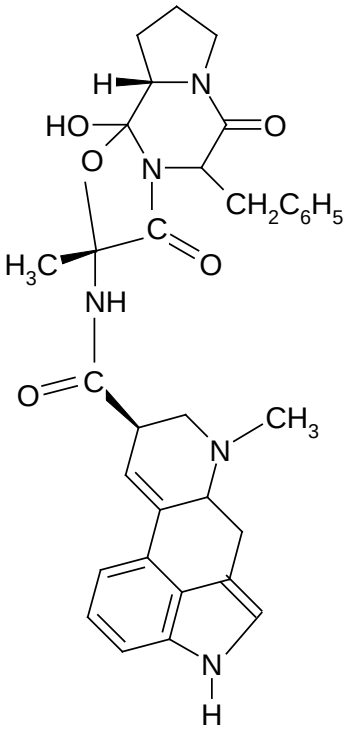


Figure 4.15
Chemical structure of ergotamine

Advantages of biotechnology are: stereospecific pathways, low energy intensity, environmental technology

Disadvantages of biotechnology are: prolonged research work, species selection, optimization, expensive maintenance of cultures

(5) Combination of biotechnology and chemical methods

Ergometrin is also an important alkaloid, which is found in the sclerotium produced by another fungus *Claviceps paspali* (Clavicipitaceae). Ergometrin is a myometrium (muscle of uterus) vasoconstrictor. In liquid cultures paspalic acid can be synthesized (during 8-10 days, 8-10 g/L), and it can be converted into ergometrine with chemical synthesis (see below).

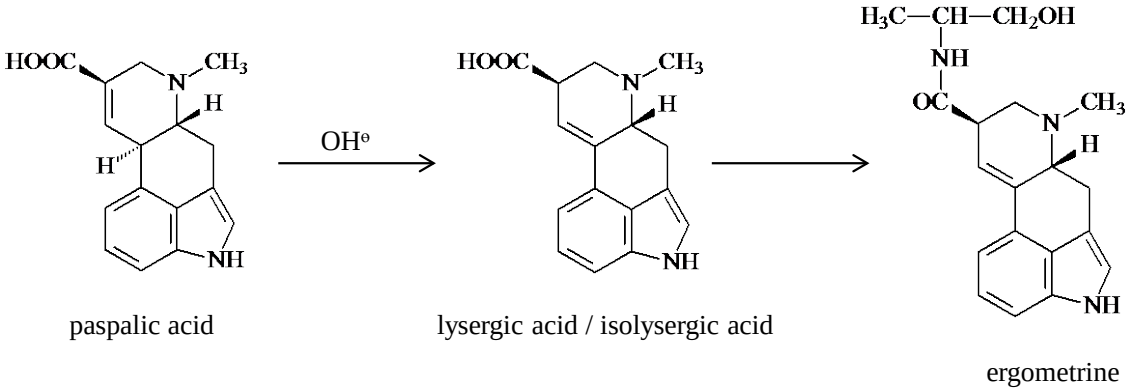


Figure 4.16
Synthesis of ergometrine

4.6 Modifying natural substances

Substances of natural origin are often modified, for a variety of purposes, such as modification or intensification of the effect, or improvement of physico-chemical properties. Modification can be achieved with diverse methods (Figure 4.17).

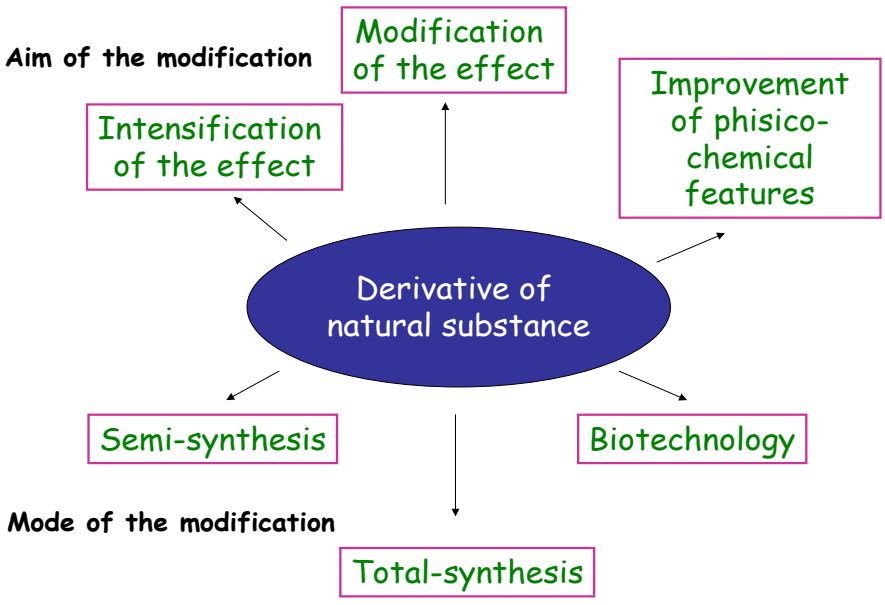


Figure 4.17
Aims and modes of modifying natural subtances

Aim of the modification: intensification of the effect

Papaverine (Figure 4.18) was isolated from opium in 1848. Its pharmacological effect was described in 1916. The etoxy analogue of papaverine, **etaverine** (Figure 4.19), was prepared in 1931, but its resorption was not appropriate. Therefore another derivative, 3,4-dihydroetaverine (=drotaverine), was produced in the Chinoin Pharmaceutical Industry in 1961. Today this compound can be found as an active ingredient in the medicine called No-Spa®. In this case the intensification of the effect of papaverine was the aim. Perparine (= etaverine) is an antispasmodic, smooth muscle relaxant, but it is more effective (3 times) than papaverine.

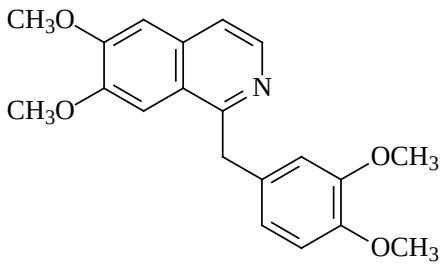


Figure 4.18
papaverine (benzyl-isoquinoline alkaloid)

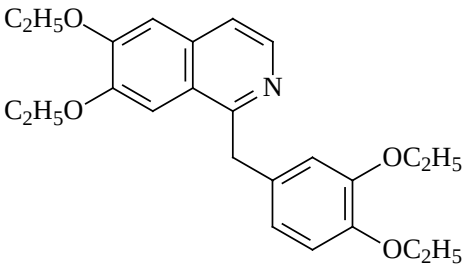


Figure 4.19
etaverine (= perparine)

Aim: modification of the effect

Lesser periwinkle (*Vinca minor*) contains an indole alkaloid called **vincamine** (Figure 4.20). It was the active compound of Devincan® medicine, which was an antihypertensive product discovered by Kálmán Szász in 1957. The effect of vincamine was modified, and **vinpocetine** (apovincaminic acid ethyl ester, Figure 4.21) was produced. Today vinpocetine is the active ingredient of the medicine called Cavinton®. This product is prescribed for patients with age-related memory impairment. Vinpocetine has celebral blood-flow enhancing effect, being more effective (10 times) than vincamine.

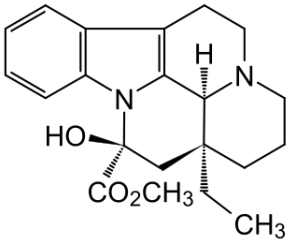


Figure 4.20
vincamine

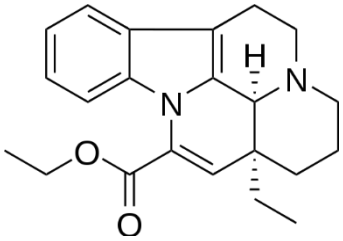


Figure 4.21
vinpocetine

Aim: improvement of physico-chemical features

In a previous section we have seen that although **camptothecin**, isolated from *Camptotheca acuminata*, showed remarkable anticancer activity, its low solubility and adverse drug reactions prevented widespread clinical use. Various derivatives have been synthesized to increase the benefits of this compound, and finally two analogues, **topotecan** and **irinotecan** have been approved and are used in cancer chemotherapy today.

4.7 Significance of natural compounds for pharmaceutical industries

The natural compounds produced by plants, fungi or bacteria show unique chemical diversity. During isolation procedures, which are often time-consuming, unexpected new structures can be found. These structures become principal or model components for synthetic chemistry. In most cases the isolated compounds show some kind of biological activity in different *in vitro* and *in vivo* model systems.

Chapter 5
Phytotherapy; (traditional) herbal medicines and dietary supplements

5.1 Definition of Phytotherapy

Phytotherapy is the study of the use of extracts from natural origin as medicines or health-promoting agents. We can distinguish traditional and "modern", evidence-based phytotherapy.

Traditional phytotherapy is often used as synonym for herbalism and regarded as "alternative medicine" by much of Western medicine, although effects of many substances found in plants are supported by scientific evidence.

Modern phytotherapy can be considered the scientific study on the effects and clinical use of herbal medicines. **Evidence-based phyotherapy** relies on various levels of scientific evidences, such as *in vitro* tests, pre-clinical studies (*in vivo* model systems) and clinical studies. Evidence-based phytotherapy applies herbal products, whose efficacy and safety have been demonstrated with appropriate scientific methods.

5.2 Phytotherapy or Ethnomedicine

It is important to distinguish between phytotherapy and ethnomedicine. In some cases, ethnomedicinal data support the same (or similar) use of a plant, as in evidence-based phytotherapy. However, as we will see from the examples below, this is frequently not the case.

- Medicinal plants are used for the same purposes in phytotherapy and ethnomedicine:
  - *Hypericum perforatum* (St. John's wort) – mild antidepressant
- Both phytotherapy and ethnomedicine – but for different purposes:
  - *Agrimonia eupatoria* (agrimony): ethnomedicine: TBC, phytotherapy: gall-bladder disease
- Used only in the ethnomedicine:
  - *Rumex crispus* (curled dock) – astringent, laxative
- Related plant is used in phytomedicine:
  - ethnomedicine: *Inula britannica* (British yellowhead), phytotherapy.: *I. helenium* (elecampane)
- Used in ethnomedicine, but there is no clinical evidence:
  - *Achillea crithmifolia*

Figure 5.1
Agrimonia eupatoria (agrimony)

Figure 5.2
Inflorescence of *Agrimonia eupatoria* (agrimony)

Today people often (re)turn to various forms of alternative medicine, because they lose their confidence in allopathic medication, which often treats the symptoms of a disease, without putting an end to its cause, and neglects the individual. Frequently, people are afraid of the numerous side-effects and interactions synthetic drugs may produce. Thus, compliance ("cooperation") between doctors and patients remains weak in many cases. Phytotherapy, being one of the most popular branches of alternative medicine, is accepted and promoted by some medical doctors, but rejected by several others. Severe problems may arise if the patient does not inform the doctor about applying natural remedies together with (or instead of) the prescribed drugs.

Several people prefer natural remedies to synthetic drugs, because they think that anything that is natural is completely harmless and can be applied safely. However, medicinal plants and their products may also have **side effects**, e.g. nausea, vomiting, diarrhoea. It is well known that *Asteraceae* species or cinnamon bark oil can cause allergic skin reactions. Furanocoumarins may cause photodermatitis. Peppermint, hyssop and sage essential oils can produce epileptic attack. Plants belonging to *Solanaceae* family, such as thornapple, devil's trumpet and henbane, can cause hallucinations due to their alkaloid content.

5.3 Interactions

Besides, **interactions** are common with natural products, as well. Natural products can interact with drugs and with other natural products by the same pharmacokinetic and pharmacodynamic mechanisms as drugs. Examples include application of a laxative drug (e.g. *Frangulae cortex*) and a drug against diarrhoea (e.g. *Quercus pubescens*) together. Dietary fibers prevent the absorption of active compounds of other medicines. Interactions between contraceptives (anti-baby pills) and herbal medicines containig *Hypericum perforatum* (St. John's wort) are also well-documented.

Interaction types

- **synergism:** interaction of drugs so that the total effect is greater than the sum of the individual effects (e.g. anti-inflammatory components in chamomile strengthen each other's effects)
- **antagonism:** the involvement of multiple agents reduces the overall effect, because they act in opposite ways (e.g. *Rheum* – rhubarb: antraquinones vs. tannins)

Figure 5.3
Rheum rhabarbarum (rhubarb)

5.4 Composition and therapeutical value of phytotherapeutics

Determination of the composition of phytotherapeutics

The aim: the synergism of the active components should be optimal (the best).

- Quantity of components is important: if they are responsible for pharmacological effect
- Problem: there is no data or few information about:
  - the active component(s) of the drug
  - interaction between the components

Accompanying constituents of the drug can influence the pharmacological effect. Accompanying constituents are content materials, they help or prevent the effect of active constituents, but they may cause side effects, as e.g. saponins. They are produced in 70-75% of plants (in different quantities, typically in the range 0.1-25%). Their absorption is not effective in the bowels, but they can influence the absorption of other substances (drugs).

Types of Phytotherapeutics

(1) Preparations that can be prepared in pharmacies or as home remedies:
tea, infusion, decoctum, extract, tincture, ointment, etc.
(2) Refined main component-concentrate:
e.g. *Legalon 70 pills* ← *Silybi mariani fructus* (milk-thistle fruit), standardized for silibine, a hepatoprotectant
(3) Standardised for plant-extract
(4) Contain only pure component

Determination of therapeutical value of phytotherapeutics

The following values should be measured experimentally:

- **Efficacy** (Effective Dose – ED_{50})
- **Safety** (Lethal Dose – LD_{50})

Permanent quality should be maintained by applying GAP, GMP and GLP.

Besides, the pharmacological effect should be determined in the course (progress) of an illness. Recommendations for therapeutical use should be formed, taking into consideration possible **side effects** and **contraindications**, as well.

Correct Application, Dosage of Phytotherapeutics – Some examples

- Antraquinones: they have laxative effect; may destroy mucous membrane in the bowels; can cause K-ion deficit; cause convulsion (spasm) in the bowels.
- Some plants are not used internally: e.g. drugs containing pyrrolizidine alkaloids, such as comfrey root (*Symphyti radix)*
- Use drugs in the required amount: e.g. *Valerianae radix*: 2-3x 1-2 ml tincture diluted with water; 2-3 g drug/daily
- Botanical identification is very relevant, for instance, the poisonous *Conii fructus* can easily be confused with *Anisi fructus*, which can be safely applied in phytotherapy

Figure 5.4
Symphytum officinale (comfrey)

Phytotherapeuticum forte vs. mite

Herbal drugs applied in phytotherapy may have a pronounced pharmacological effect (**phyotherapeuticum forte**), with severe adverse effects. This group includes drugs containing alkaloids and cardiac glycosides, the majority of which cannot be used as self-remedy (as a tea drug). The other group contains drugs which have less marked physiological effect, and can usually be safely applied (**phytotherapeuticum mite**).

Examples for plants containing **alkaloids** (may cause hallucination or have toxic effect):

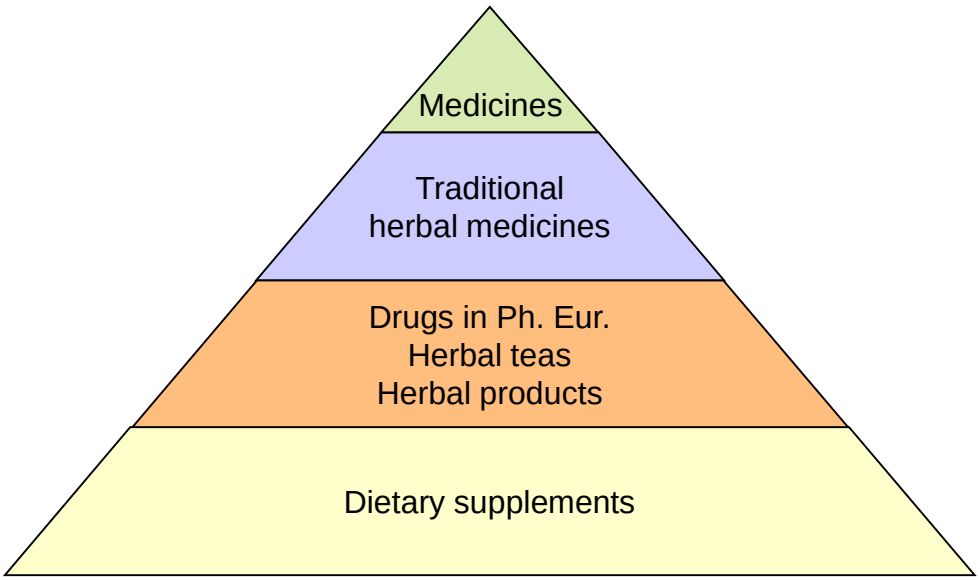
- *Papaver somniferum* (opium, morphine, semi-synthetic heroin)
- *Erythroxylum coca*, *E. novogranatense* (*Cocae folium*, cocaine)
- *Cannabis sativa* ssp. *indica* (marijuana, hashish – resin of the female flowers, THC)
- *Atropa belladonna* (atropine: mixture of D- and L-hyoscyamine)
- *Hyoscyamus niger* (atropine, scopolamine)
- *Datura* sp. (atropine, scopolamine)

Figure 5.5
Cannabis sativa (hemp)

5.5 Properties of herbal medicines used in phytotherapy

- They contain one or more herbal component(s), vitamins, inorganic salts, microelements, refined materials (e.g. fatty acids, flavonoids).
- They contain many chemical components, therefore, composition is known only partially.
- Components responsible for the pharmacological effect are known or partially known or not identified.
- It is more difficult to obtain and maintain permanent quality and efficiency.

There are several factors that can influence the **quality of herbal medicines**. The most important is the starting raw material. Standard quality can be ensured only by cultivated plants or wild plants with known chemical constituents. Agronomists must adhere to the regulations of GAP (Good Agricultural Practice) and GMP (Good Manufacturing Practice), which can guarantee optimal quality of raw plant materials.



The size of every level of the pyramid represents the number of the products. Hence, there are more dietary supplements than medicines or traditional herbal medicines.

Figure 5.6
Hierarchy of herbal products traded in Hungary

Responsible authorities

GYEMSZI (National Institute of Quality- and Organizational Development in Healthcare and Medicines (previous name: **OGYI –** National Institute of Pharmacy) is responsible for the authorization and inspection of medicines, traditional herbal medicines and other herbal products. Another Hungarian Institute, **OÉTI** (National Institute for Food and Nutrition Science) deals with the category of dietary supplements, but it only registers these products. OÉTI does not examine the quality of the products by analytical techniques. In the case of dietary supplements, only the manufacturers are responsible for the quality of their products. Unfortunately, there are many adulterated products in this category on the market.

Herbal medicine

- It contains one or more herbal material(s) or extract(s) or their combination.
- "Well-established use" category
- National Institute of Pharmacy authorizes them.
- This type of application may be submitted "if the applicant can demonstrate that the active substances of the medicinal product have been in well-established medicinal use within the (European) Community for at least ten years, with recognised efficacy and an acceptable level of safety".

Traditional herbal medicinal product (= THMPD)

- This category is regulated by Decree of the Minister of Health No. 52/2005 (XI. 18.) and Directive 2004/24/EC
- They are herbal medicines, which are administered orally, externally, or by inhalation, are intended to be used without supervision by a medical doctor.

- The intended use of a herbal medicine will only be authorised on the basis of its traditional history and/or the recognised pharmacological properties of the herbal ingredient(s).
- Vitamins and minerals may be added to the herbal medicine provided that their use is ancillary to the herbal ingredient(s).
- Attributing a preventive or curative effect to these products is allowed.
- They contain one or more herbal substances, preparations or their combination as active agents.
- They can be distributed in pharmacies.
- Their authorization is possible via abridged registration.
- Need to demonstrate that the herbal medicine has been in use within the EU for at least 30 years or 15 years within the EU, and 30 years outside the EU.

Requalification of herbal products (in Hungary)

Those herbal products, which contain only herbal component(s), can be in the market until their date of expiry, but not later than 1 April, 2013.

Requalification is possible:

- to herbal medicine or traditional herbal medicine (product) or
- to dietary supplement

Herbal tea

Herbal tea is a herbal or plant infusion and usually not made from the leaves of the tea bush (*Camellia sinensis)*. Typically, a herbal tea is simply the combination of boiling water and dried fruits, flowers or herbs. Herbal teas should be prepared and traded in pharmacies. Pharmacists can give appropriate information about the preparation, the application period, the side effects and the possible interactions of herbal teas. In Hungary approx. 81 drugs are used for preparing tea, peppermint (*Mentha piperita*) being among the most popular plants. Today new plants, e.g. *Uncaria* sp., *Euphorbia hirta*, appeared in tea mixtures, but there are no clinical evidences related to these plants.

Dietary supplement

It is a preparation intended to supplement the diet and provide nutrients, such as vitamins, minerals, fiber, fatty acids, or amino acids that may be missing or may not be consumed in sufficient quantities in a person's diet. Some countries define dietary supplements as foods, while in others they are defined as drugs or natural health products.

Supplements containing vitamins or dietary minerals are included as a category of food in the Codex Alimentarius, a collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety. Herbal tea, traditional herbal medicinal products, special foods for the athletes, nutritions, formula and medicinal nutritions do not belong to the category of dietary supplement. We mentioned that there are many adulterated products in this category on the market. For instance, in several cases manufacturers “contaminate” the product with syntethic drugs, but this “active ingredient” is not indicated on the packaging.

Chapter 6
Aromatherapy and homeopathy

6.1 Aromatherapy

Aromatherapy is a form of alternative medicine that uses volatile plant materials, known as essential oils (EOs), and other aromatic compounds for the purpose of altering a person's mind, mood, cognitive function or health. EOs are extracted from plants through steam distillation or expression.

Why is aromatherapy worth considering?

- It has holistic view, therefore aromatherapy can restore the balance of the body-mind-spirit
- It can be applied together with allopathic medicine
- Stress can stand in the background of various diseases, and aromatherapy can reduce stress
- Its therapeutic possibilities: first aid, relaxation, improving general health, boosting the immune system, etc.

Figure 6.1 demonstrates the most important therapeutical systems using plant materials, including aromatherapy. Aromatherapy can be considered as part of phytotherapy, because it uses materials (EOs) originating from plants.

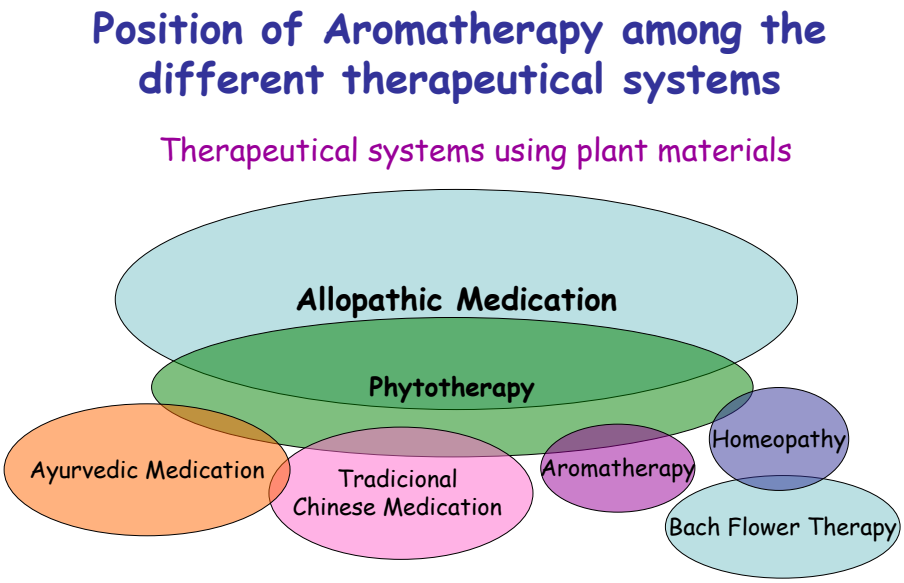


Figure 6.1
Position of aromatherapy among the different therapeutical systems using plant materials

Processing the smell of odorant molecules

Air contains a lot of odorant molecules. These molecules bind to odorant receptors. Olfactory receptor cells are located in the nasal epithelium. After binding, the receptor cells are activated and send electric signals to the olfactory bulb. The signals are relayed

in glomeruli. After it, the mitral cells transmit signals to a higher region of the brain (hypothalamus).

The brief history of aromatherapy

Mesopotamia

The use of medicinal plants in different types of medicines was very important. Herbs such as fennel (*Foeniculum*) or aromatic plants like juniper (*Juniperus*) were frequently applied. People in Mesopotamia were able to isolate different materials from plants, for instance, fatty oils, essential oils and resins. They made perfumes, the most important steps included: maceration of aromatic plants in water, then dissolving essence in fatty oil. Among the essential oils cedar (*Cedrus*), cypress (*Cupressus*), myrtle (*Myrtus*), juniper (*Juniperus*), cinnamon (*Syzygium*) and thyme (*Thymus*) were the most popular.

Figure 6.2
Cedrus deodara (Himalayan cedar)

Figure 6.3
Cupressus sempervirens (Mediterranean cypress)

Figure 6.4
Myrtus communis (common myrtle)

Egypt

The application of odours was very common among the pharaoh and the clergy. The following essential oils were used: cedar, cypress, olibanum (*Boswellia*), juniper, myrrh (*Commiphora*), rose. They used these materials for preparing ointments. Olibanum was used as incense. People knew the antibacterial properties of EOs, balsams and resins,

because these materials were used in embalming process. Balsam is a resin dissolved in volatile oil. People distinguished the embalming materials of the rich (myrrh, cedar) and the poor (cinnamon, sandalwood, thyme). Medicinal and cosmetic applications of the EOs were also popular.

The Greek

These people adopted the use of the essential oils from the Egyptians. They considered the odours as the gift of the Gods. Medicinal application of the EOs and plants should be highlighted at this time. Hippocrates (460 BC - 370 BC) described the therapeutic effects of oils. There was a famous story in the Greek history. During the black death (plague) in Athens, the Greek burnt medicinal plants containing EOs (e.g. lavander, hyssop and rosemary) on the streets, and the evaporated fume stopped the spread of this serious disease.

Romans

These people used aromatic essences during massage and in Roman bath but it was considered as luxury. In rich families there was a servant called "cosmetae", who provided bath and massage for mothers. Roman perfumers were called "aromatarii" and they started the trade of EOs, fragrances and perfumes.

Middle-East (Arabian, Persian) cultures

They possessed a highly developed bath-culture and medical system. They played a crucial role in the trade of EOs. Essences spread from the Middle-East to Europe (Spain, South France, Greece). Avicenna was a Persian doctor, who applied water-distillation for the first time. Many terracotta distillation equipments were discovered by archeologists during excavations.

In the Middle Ages

Following the demise of the Roman Empire, the use of aromatics declined in Europe. Medicinal plants were found only in cloister gardens.

From the Renaissance to the 20th century

Different perfumes were made, because people did not like having a bath, they were stinking and dirty. In World War II, the Australian soldiers had tea tree EO in their bag, because this oil has antiseptic and regenerative properties, therefore soldiers could treat their wounds and injuries. In the 20th century: artificial odours became more important than natural materials. The word "aromatherapy" was first coined in 1928 by **Rene-Maurice Gattefosse (1881-1950).** He published his work in 1964. He was a French chemist working in the family perfumery business. One day he worked in his laboratory, and burnt his hand. He plunged his hands into a nearby flask containing lavender oil. He realised that the burn healed quickly with little scarring, and therefore he concluded that the oil must have had an antiseptic and curative effect. He published several scientific papers and books in connection with the topic of aromatherapy. Another famous person, **Robert Tisserand**, should be highlighted, who continued Gattefosse's work. He published the following books: Essential Oil Safety – A Guide for Health Care Professionals, The Art of Aromatherapy.

Properties of EOs

EOs are volatile materials, they contain mono-, sesquiterpenes and phenylpropane derivatives. They have characteristic smell, and they can be isolated by water-steam distillation or expression from different parts of the plants. They are hydrophobic materials, therefore they can not be dissolved in water, but some components dissolve into water phase (during preparation of herbal teas or aromatic water). They contain several constituents (5-300 volatile components). In every EO one main component can be detected, e.g. thymol in the thyme EO (*Thymi aetheroleum*). According to the composition there are significant differences between species or individuals. A **chemotype** (sometimes **chemovar**) is a chemically distinct entity within a plant species, with differences in the composition of the secondary metabolites. A chemotype (ct.) comprises plants producing EO with different composition than other representatives of the same species. A good example of a plant with many polymorphic chemotypes is thyme (*Thymus vulgaris*). While largely indistinguishable in appearance, specimens of *T. vulgaris* may be assigned to one of seven different chemotypes, depending on whether the dominant component of the EO is thymol, carvacrol, linalool, geraniol, α-terpineol, or eucalyptol. Such chemotypes may be indicated as *Thymus vulgaris* ct. thymol, or *Thymus vulgaris* ct. geraniol (sweet thyme), etc. Such an indication has no taxonomic standing. The thymol chemotype causes more allergic reactions than the linalool-ct. Environmental factors (solar radiation, soil, etc.) influence the composition of the EO.

EOs must not used in undiluted form, only in alcoholic or oily solutions, in ointments (absorption through the skin). Oral administration is available in France and England by medical practitioners, but great care must be exercised in this case, because EOs are potentially toxic materials. The use of EOs should not be recommended in infants, babies and little children (e.g. *Menthae piperitae aetheroleum*: until 5-7 year-old – local application of menthol to their nostrils → apnoea and collapse of lung, cardiac arrest; e.g. *Hyssopi aetheroleum* – epileptic attack)

In the case of the following EOs caution should be recommended:

Peppermint: it should not be recommended for infants, babies and little children (until 7 years); danger of apnoea and collapse of lung, cardiac arrest may occur.

Thyme: it should not be recommended for little children (under 3 years), the main component of the oil, thymol is a dermal and mucous membrane irritant.

Oregano: skin and mucous membrane irritant (because of thymol), should not be used in children under 2 years of age.

Rosemary: it contains camphor, which readily causes epileptiform convulsions, should be used with caution (in oral dose).

Juniper: should not be used during pregnancy (risk of abortion) and kidney disease (risk of inflammation).

Camphor: it should not be used in children under 7 years of age (see peppermint), because epileptiform convulsions may occur and it can absorb through the placenta freely.

Bitter orange: it is contraindicated in patients with gastric and duodenal ulcer; the oil is moderately phototoxic - if it is applied to the skin at a concentration over max. use level (1.4%), skin must not be exposed to sunlight or sunbed rays for 12 h.

Melissa: skin sensitisation reaction possible during external application; citral can cause a rise in ocular tension (to avoid oral use in cases of glaucoma).

Lemon, Sweet orange: see bitter orange.

Eucalyptus: see camphor.

Cinnamon bark: moderate dermal irritant, strong dermal sensitiser, moderate mucous membrane irritant; externally max. use level 0.1%!

There are some EOs, which can increase the blood pressure: rosemary, hyssop, cedar, common sage (Dalmatian), thyme.

EOs causing epileptiform convulsions: sweet fennel, hyssop, camphor, common sage (Dalmatian), rosemary

Tropical EOs:

Melaleuca leucodendron (Myrtaceae), Cajeput: evaporation (respiratory infections), aromatic bath, mouth-wash

M. viridiflora, Niaouli: see cajeput

M. alternifolia, Tea tree: bactericide, antiseptic

Myrtus communis, Myrtle: inhalation (respiratory infections), meditation

Tropical EOs – perfume industry:

Aniba rosaeodora (Brazilian rosewood)

Cananga odorata (Annonaceae): "Ylang-ylang"

Citrus sp. (Rutaceae)

Pharmacological effects of EOs

Effect on respiratory system

EOs have antimicrobial and anti-inflammatory effect. Inhaled EOs pass down the trachea into the bronchi.

Secretolytic effect: increase the production of mucus in the respiratory tract and make the phlegm thinner and less sticky.

Secretomotoric effect: help the cilia – tiny hairs that line the respiratory tract – to transport the phlegm out of the lungs.

- *Thymus* (thyme) sp.: EO main component: thymol, carvacrol
- *Syzygium aromaticum* (clove): eugenol
- *Cinnamomum cassia, C. zeylanicum* (cinnamon): cinnamic aldehyde
- *Matricaria recutita* (chamomile): chamazulene
- *Pimpinella anisum* (aniseed), *Illicium verum* (star anise), *Foeniculum vulgare* (fennel): trans-anethole
- *Eucalyptus* (eucalyptus) sp.: cineole = eucalyptol
- *Pinus* (pine) sp.: α-pinene

Figure 6.5
Eucalyptus sp. (eucalyptus)

Anti-inflammatory, antiseptic, for inhalation, treating oral cavity:

- *Matricaria recutita* (Asteraceae) – German chamomile
- *Salvia officinalis* (Lamiaceae) – common sage
- *Thymus vulgaris* (Lamiaceae) – garden thyme
- *Eucalyptus* sp. (Myrtaceae) –eucalypt
- *Syzygium aromaticum* (Myrtaceae) – clove

Stomachic, appetizing effect

amara-aromatica (aromatic bitter materials): stimulate stomach wall ➔ gastric juice is produced, increase the appetite

- aperitive drinks, spices
  - *Angelica archangelica* (Apiaceae) – garden angelica
  - *Foeniculum vulgare* (Apiaceae) – sweet fennel
  - *Pimpinella anisum* (Apiaceae) – anise (aniseed)
  - *Citrus aurantium* (Rutaceae) – bitter orange
  - *Mentha piperita* (Lamiaceae) – peppermint
  - *Syzygium aromaticum* (Myrtaceae) – clove
  - *Artemisia absinthium* (Asteraceae) – common wormwood
  - *Acorus calamus* (Araceae) – common sweet flag, calamus

Figure 6.6
Aurantii epicarpium et mesocarpium (orange epicarp et mesocarp)

Figure 6.7
Artemisia absinthium (common wormwood)

Effect on the liver and gall-bladder

cholagogue: promotes the discharge of bile from the system

choleretic: increases the volume of bile secreted from the liver

Some plants containing EOs inhibit the production of bile-stones, e.g. *Mentha piperita* (Lamiaceae)

Cholagogue EOs: *Acorus calamus* (Araceae), *Carum carvi* and *Foeniculum vulgare* (Apiaceae), *Lavandula officinalis* (Lamiaceae)

Carminative effect

Prevents formation of gas in the gastrointestinal tract or facilitates the expulsion of gas, thereby combating flatulence.

- *Pimpinella anisum (Apiaceae)* – aniseed
- *Foeniculum vulgare (Apiaceae)* – fennel
- *Carum carvi (Apiaceae)* – caraway
- *Coriandrum sativum (Apiaceae)* – coriander
- *Ocimum basilicum (Lamiaceae)* – sweet basil
- *Mentha piperita (Lamiaceae)* – peppermint
- *Matricaria recutita (Asteraceae)* – German chamomile

Figure 6.8
Ocimum basilicum (sweet basil)

Spasmolytic EOs

- *Matricaria recutita (Asteraceae)* – German chamomile
- *Carum carvi (Apiaceae)* – caraway
- *Foeniculum vulgare (Apiaceae)* – fennel
- *Citrus aurantium (Rutaceae)* – bitter orange
- *Mentha piperita (Lamiaceae)* – peppermint
- *Melissa officinalis (Lamiaceae)* – lemon balm
- *Cinnamomum sp. (Lauraceae)* – cinnamon
- *Achillea sp. (Asteraceae)* – yarrow

Diuretic effect:

EOs can increase the excretion of water from bodies.

- *Levisticum officinale* (Apiaceae) – lovage
- *Petroselinum hortense* (Apiaceae) – parsley
- *Juniperus communis* (Cupressaceae) – juniper

Figure 6.9
Petroselinum crispum (garden parsley)

! Caution – nephritis may occur

! *Juniperi pseudofructus and aetheroleum*: contraindicated in case of pregnancy (risk of abortion).

Supporting blood circulation, refreshing, use in a bath

Following EOs are contraindicated in patients with high blood pressure, heart disease:

- *Rosmarinus officinalis* (Lamiaceae) – rosemary
- *Mentha piperita* (Lamiaceae) – peppermint
- *Pinus* sp. (Abietaceae) – pines
- *Citrus* sp. (Rutaceae) – citrus fruits

Sedative effect

- *Valeriana officinalis* (Valerianaceae) – valerian
- *Lavandula officinalis* (Lamiaceae) – lavender
- Ylang-ylang

In case of headache (for compress)

- *Citrus limon* (Rutaceae) – lemon
- *Citrus aurantium* (Rutaceae) – orange
- *Pinus* sp. (Abietaceae) – pines
- *Lavandula* sp. (Lamiaceae) – lavenders

Figure 6.10
Citrus limon (lemon)

In case of pollen-allergy

Use dry inhalation, drop EO on a paper handkerchief

- *Mentha piperita* (Lamiaceae) – peppermint
- *Matricaria recutita* (Asteraceae) – chamomile

In massage oils

In case of external use EOs must be diluted in base oils (carrier oils). Base oils are fatty oils, e.g. *Amygdalus communis* var. *dulce* – sweet almond (Rosaceae), *Arachis hypogaea* – peanut (Fabaceae), *Persea americana* – avocado (Lauraceae), *Simmondsia chinensis* – jojoba (Buxaceae/Simmondsiaceae), *Olea europaea* – olive (Oleaceae)

Figure 6.11
Persea americana (avocado)

Figure 6.12
Olea europaea (olive tree)

EOs official in Ph. Eur. 5 and 6

- **Anise oil:** *Anisi aetheroleum*
- **Bitter-fennel fruit oil**: *Foeniculi amari fructus aetheroleum*
- **Bitter-orange-flower oil:** *Aurantii amari floris aetheroleum*
- **Cassia oil**: *Cinnamomi cassiae aetheroleum*
- **Cinnamon bark oil, ceylon**: *Cinnamomi zeylanici corticis aetheroleum*
- **Cinnamon leaf oil, ceylon**: *Cinnamomi zeylanici folii aetheroleum*
- **Citronella oil**: *Citronellae aetheroleum*
- **Clary sage oil**: *Salviae sclareae aetheroleum*
- **Clove oil**: *Caryophylli floris aetheroleum*
- **Coriander oil**: *Coriandri aetheroleum*
- **Eucalyptus oil**: *Eucalypti aetheroleum*
- **Juniper oil**: *Iuniperi aetheroleum*
- **Lavender oil**: *Lavandulae aetheroleum*
- **Lemon oil**: *Limonis aetheroleum*
- **Matricaria oil**: *Matricariae aetheroleum*
- **Mint oil, partly dementholised**: *Menthae arvensis aetheroleum partim mentholi privum*
- **Neroli oil**: *Neroli aetheroleum*
- **Nutmeg oil**: *Myristicae fragrantis aetheroleum*
- **Peppermint oil**: *Menthae piperitae aetheroleum*
- **Pine sylvestris oil**: *Pini sylvestris aetheroleum*
- **Rosemary oil**: *Rosmarini aetheroleum*
- **Star anise oil**: *Anisi stellati aetheroleum*
- **Sweet orange oil**: *Aurantii dulcis aetheroleum*
- **Tea tree oil**: *Melaleucae aetheroleum*
- **Thyme oil**: *Thymi aetheroleum*
- **Turpentine oil, Pinus pinaster type**: *Terebinthi aetheroleum ab pinum pinastrum*

Storage, Application of EOs

- EOs are relatively sensitive to the environment → should be stored in a cool place in dark bottles to avoid photo-oxidation.
- Current "code of practice" of aromatherapists recommends the use of EOs for external application only → EOs can cause damage to the sensitive mucous membranes.
- EOs should never be taken internally without medical supervision!
- EOs are wildly use topically both in local and whole body massage.
- Aromatherapy may also be affected by using EOs in aromatic bath.
- Aerial diffusion: for environmental fragrancing or aerial disinfection
- Direct inhalation: for respiratory disinfection, decongestion, expectoration as well as psychological effects
- Before use of an EO, make skin test!

6.2 Homeopathy

Homeopathy is an alternative medicine. It has holistic view, therefore it can restore the balance of the body-mind-spirit. The origin of the word "homeopathy": Greek homoios (like) and pathos (treatment).

Homeopathy is based on the theory that a small dose of what the patient is suffering from will help to cure the condition → is termed: „Like cures like" („Similia similibus curantur"). This is the most important law of this alternative medicine. For example: Allium cepa (homeopathic remedy) – could be prescribed for cases of hay-fever for patients suffering from stinging nose and eyes. Homeopathy uses diluted materials: the more the drug is diluted, the greater its ability to cure. The system of homeopathy was established and ealborated by Samuel Hahnemann (a German physician) (1755-1843). He began his work in the 1790s. Today a homeopathic consultation involves an in-depth discussion, during which patients are asked about their daily routines and general health condition. After consultation and physical examination doctors try to find the best medicine for the particular patient.

Hahnemann conducted self-studies and decided to examine the effect of taking different materials on himself. He collected and published his experiences. He examined the effect of a wide range of plants, animals and mineral extracts. His results were published in „The Organon of Medicine" (first edition: in 1810, final ed.: posthumously in 1843).

Doctors treat patients with acute and chronic diseases. Homoeopathic medicines are made from plants (e.g. chamomile, belladonna), animal products (e.g. snake venom) and minerals (mercury, sulphur). Homeopathic medicines are prepared by diluting and shaking the items – this process may be repeated several times. To date there are few double blind, randomised trials and laboratory experiments on animals that support the efficacy of homeopathic medicines. However, several clinical studies arrived at the conclusion that the homeopathic preparation did not have stronger effect than the placebo.

What is the problem with homeopathy?

Medicines are diluted to the point that no trace of the original substance can be found, therefore it is hard to understand how the therapy works. Side effects are uncommon,

but in the first period of the treatment symptoms can become worse, which is the positive sign of recovery.

Diagnosis

Process of diagnosis and determination of appropriate remedy is long, takes more than 1 hour. Doctors observe how you are dressed, how you walk and talk, if you are over-underweight, etc. During the examination, the following questions are asked from patients: What is the state of health of your family?, Are you employed?, Do you have any difficulty with relationships?, What foods do you like and dislike?, Do you have any allergies?, How does a change in the weather affect you?

Homeopathic remedies

In Hungary approximately 300 homeopathic medicines are on the market. Most of them are derived from plant material. The two main types of remedies are: mono-component products and complex homeopathic remedies. Sublingual application is the most frequent mode of administration.

Types of homeopathic remedies: cream (e.g. Arnica®), toothpaste (e.g. Homeodent®), syrup (e.g. Stodal®), injection (e.g. Traumeel®), pills (e.g. Arnica montana®).

Homoeopathic preparations in the European Pharmacopoeia 5th edition

Homoeopathic preparations - Praeparationes homoeopathicas

Definition

Homoeopathic preparations are prepared from substances, products or preparations called stocks, in accordance with a homoeopathic manufacturing procedure. A homoeopathic preparation is usually designated by the Latin name of the stock, followed by an indication of the degree of dilution.

Raw materials

Raw materials for the production of homoeopathic preparations may be of natural or synthetic origin. A raw material of botanical, zoological or human origin may be used either in the fresh state or in the dried state. Where appropriate, fresh material may be kept deep-frozen. Raw materials of botanical origin comply with the requirements of the monograph on *Herbal drugs for homoeopathic preparations*. Where justified and authorised for transportation or storage purposes, fresh plant material may be kept in ethanol (96 per cent *V/V*) or in alcohol of a suitable concentration, provided the whole material including the storage medium is used for processing. Raw materials comply with any requirements of the relevant monographs of the European Pharmacopoeia.

Vehicles

Vehicles are excipients used for the preparation of certain stocks or for the potentisation process. They may include for example: purified water, alcohol of a suitable concentration, glycerol and lactose. Vehicles comply with any requirements of the relevant monographs of the European Pharmacopoeia.

Stocks

Stocks are substances, products or preparations used as starting materials for the production of homoeopathic preparations. A stock is usually one of the following: a mother tincture or a glycerol macerate, for raw materials of botanical, zoological or human origin, or the substance itself, for raw materials of chemical or mineral origin. Mother tinctures comply with the requirements of the monograph on *Mother tinctures for homoeopathic preparations.* Glycerol macerates are liquid preparations obtained from raw materials of botanical, zoological or human origin by using glycerol or a mixture of glycerol and either alcohol of a suitable concentration or a solution of sodium chloride of a suitable concentration.

Herbal drugs for homoeopathic preparations - Plantae medicinales ad praeparationes homoeopathicas

Definition

Herbal drugs for homoeopathic preparations are mainly whole, fragmented or cut plants, parts of plants including algae, fungi or lichens in an unprocessed state, usually in fresh form but sometimes dried. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs for homoeopathic preparations. Herbal drugs for homoeopathic preparations are precisely defined by the botanical scientific name of the source species according to the binomial system (genus, species, variety and author).

Production

Herbal drugs for homoeopathic preparations are obtained from cultivated or wild plants. Suitable cultivation, harvesting, collection, sorting, drying, fragmentation and storage conditions are essential to guarantee the quality of herbal drugs for homoeopathic preparations. Herbal drugs for homoeopathic preparations are, as far as possible, free from impurities such as soil, dust, dirt and other contaminants such as fungal, insect and other animal contaminants. They do not present signs of decay. If a decontaminating treatment has been used, it is necessary to demonstrate that the constituents of the plant are not affected and that no harmful residues remain. The use of ethylene oxide is prohibited for the decontamination of herbal drugs for homoeopathic preparations. Adequate measures have to be taken in order to ensure that the microbiological quality of homoeopathic preparations containing one or more herbal drugs comply with the recommendations given in the text on *Microbiological quality of pharmaceutical preparations* (*5.1.4*).

Identification

Herbal drugs for homoeopathic preparations are identified using their macroscopic and, where necessary, microscopic descriptions and any further tests that may be required (for example, thin-layer chromatography).

Tests

When a fresh plant is used as a starting material for the manufacture of homoeopathic preparations, the content of foreign matter is as low as possible; if necessary, the maximum content of foreign matter is indicated in the individual monographs. When a dried plant is used as a starting material for the manufacture of homoeopathic

preparations, a test for foreign matter (*2.8.2*) is carried out, unless otherwise prescribed in the individual monographs.

A specific appropriate test may apply to herbal drugs for homoeopathic preparations liable to be falsified. If appropriate, the herbal drugs for homoeopathic preparations comply with other tests, for example, total ash (*2.4.16*) and bitterness value (*2.8.15*). The test for loss on drying (*2.2.32*) is carried out on dried herbal drugs for homoeopathic preparations. A determination of water (*2.2.13*) is carried out on herbal drugs for homoeopathic preparations with a high EO content. The water content of fresh herbal drugs for homoeopathic preparations is determined by an appropriate method. Herbal drugs for homoeopathic preparations comply with the requirements for pesticide residues (*2.8.13*).

The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant. The content of pesticide residues may be determined by the method described in the annex to the general method. The risk of contamination of herbal drugs for homoeopathic preparations by heavy metals must be considered. If an individual monograph does not prescribe limits for heavy metals or specific elements, such limits may be required if justified. Limits for aflatoxins may be required. In some specific circumstances, the risk of radioactive contamination is to be considered.

Assay

Where applicable, herbal drugs for homoeopathic preparations are assayed by an appropriate method.

Storage

Fresh herbal drugs are processed as rapidly as possible after harvesting; they may also be stored deep-frozen or in ethanol (96 per cent *V/V*) or in alcohol of a given concentration. Store dried herbal drugs protected from light.

Potentization

Dilutions and triturations are obtained from stocks by a process of potentisation in accordance with a homoeopathic manufacturing procedure: this means successive dilutions and succussions, or successive appropriate triturations, or a combination of the 2 processes.

The potentisation steps are usually one of the following:

- 1 part of the stock plus 9 parts of the vehicle; they may be designated as “D”, “DH” or “X” (decimal),
- 1 part of the stock plus 99 parts of the vehicle; they may be designated as “C” or “CH” (centesimal).

The number of potentisation steps defines the degree of dilution; for example, “D3”, “3 DH” or “3X” means 3 decimal potentisation steps, and “C3”, “3 CH” or “3C” means 3 centesimal potentisation steps. “LM-” (or “Q-”) potencies are manufactured according to a specific procedure.

In homeopathy 5C, 6C, 9C, 12C, 30C, 200C and D2, D4, D6, D12, D30, D200 dilutions are frequently used.

During potentization solutions are vigorously shaken and tapped on a resilient (flexible) surface. This process is known as *succussion*.

Insoluble solids, such as quartz, are diluted by grinding them with lactose (trituration).

Trituration is also the name of the process for reducing the particle size of a substance by grinding, as by grinding of powders in a mortar with a pestle.

Homeopathic remedies should not be stored near pungent materials, e.g. perfumes, EOs (peppermint) or toothpaste (containing mentol).

Dosage forms

A dosage form of a homoeopathic preparation complies with any relevant dosage form monograph in the European Pharmacopoeia and with the following:

- for the purpose of dosage forms for homoeopathic use “active substances” are considered to be “dilutions or triturations of homoeopathic stocks”,
- these dosage forms are prepared using appropriate excipients,
- the test for uniformity of content is normally not appropriate. However, in certain circumstances, it is required.

Homoeopathic dosage form “pillule”

Pillules for homoeopathic use are solid preparations obtained from sucrose, lactose or other suitable excipients. They may be prepared by impregnation of preformed pillules with a dilution or dilutions of homoeopathic stocks or by progressive addition of these excipients and the addition of a dilution or dilutions of homoeopathic stocks. They are intended for oral or sublingual use.

Homoeopathic dosage form “tablet”

Tablets for homoeopathic use are solid preparations obtained from sucrose, lactose or other suitable excipients according to the monograph on *Tablets*. They may either be prepared by compressing one or more solid active substances with the excipients or by impregnating preformed tablets with a dilution or dilutions of homoeopathic stocks. The preformed tablets for impregnation are obtained from sucrose, lactose or other suitable excipients according to the monograph on *Tablets*. They are intended for oral or sublingual use.

Mother tinctures for homoeopathic preparations - Tincturae maternae ad praeparationes homoeopathicas

Definition

Mother tinctures for homoeopathic preparations are liquid preparations obtained by the solvent action of a suitable vehicle upon raw materials. The raw materials are usually in the fresh form but may be dried. Mother tinctures for homoeopathic preparations may also be obtained from plant juices, with, or without the addition of a vehicle. For some preparations, the matter to be extracted may undergo a preliminary treatment.

Production

Mother tinctures for homoeopathic preparations are prepared by maceration, digestion, infusion, decoction, fermentation or as described in the individual monographs, usually using alcohol of suitable concentration. Mother tinctures for homoeopathic preparations are obtained using a fixed proportion of raw material to solvent, taking the moisture

content of the raw material into account, unless otherwise justified and authorised. If fresh plants are used, suitable procedures are used to ensure freshness. The competent authorities may require that the freshness is demonstrated by means of a suitable test.

Mother tinctures for homoeopathic preparations are usually clear. A slight sediment may form on standing and that is acceptable as long as the composition of the tincture is not changed significantly. The manufacturing process is defined so that it is reproducible.

Production by maceration

Unless otherwise prescribed, reduce the matter to be extracted to pieces of suitable size, mix thoroughly and extract according to the prescribed extraction method with the prescribed extraction solvent. Allow to stand in a closed vessel for the prescribed time. The residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the 2 liquids obtained are combined.

Adjustment of the contents

Adjustment of the content of constituents may be carried out if necessary, either by adding the extraction solvent of suitable concentration or by adding another mother tincture for homoeopathic preparations of the vegetable or animal matter used for the preparation.

Identification

Where applicable, at least 1 chromatographic identification test is carried out.

Tests

The limits in an individual monograph are set to include official methods of production. Specific limits will apply to each defined method of production. *If the test for relative density is carried out, the test for ethanol need not be carried out, and vice versa.*

Relative density. The mother tincture for homoeopathic preparations complies with the limits prescribed in the monograph.

Ethanol. The ethanol content complies with that prescribed in the monograph.

Methanol and 2-propanol: maximum 0.05 per cent *V/V* of methanol and maximum 0.05 per cent *V/V* of 2-propanol, unless otherwise prescribed.

Dry residue. Where applicable, the mother tincture for homoeopathic preparations complies with the limits prescribed in the monograph.

Pesticides. Where applicable, the mother tincture for homoeopathic preparations complies with the test. This requirement is met if the herbal drug has been shown to comply with the test.

Assay

Where applicable, an assay with quantitative limits is performed.

Storage

Protected from light. A maximum storage temperature may be specified.

Labelling

The label states:

- that the product is a mother tincture for homoeopathic preparations (designated as "TM" or "Ø"),
- the name of the raw material using the Latin title of the European Pharmacopoeia monograph where one exists,
- the method of preparation,
- the ethanol content or other solvent content, in per cent *V/V*, in the mother tincture,
- the ratio of raw material to mother tincture,
- where applicable, the storage conditions.

The official monographs of homoeopathic preparations in the European Pharmacopoeia 5th edition

Arsenious trioxide for homoeopathic preparations – Arsenii trioxidum ad praeparationes homoeopathicas

As_2O_3

Content: 99.5 per cent to 100.5 per cent of As_2O_3.

Common stinging nettle for homoeopathic preparations – Urtica dioica ad praeparationes homoeopathicas

Definition: Whole, fresh, flowering plant of *Urtica dioica* L.

Copper for homoeopathic preparations – Cuprum ad praeparationes homoeopathicas

Cu

Content: 99.0 per cent to 101.0 per cent of Cu.

Garlic for homoeopathic preparations – Allium sativum ad praeparationes homoeopathicas

Definition: Fresh bulb of *Allium sativum* L.

Honey bee for homoeopathic preparations – Apis mellifera ad praeparationes homoeopathicas

Definition: Live worker honey bee (*Apis mellifera* L.).

Hypericum for homoeopathic preparations – Hypericum perforatum ad praeparationes homoeopathicas

Definition: Whole, fresh plant of *Hypericum perforatum* L., at the beginning of the flowering period.

Iron for homoeopathic preparations – Ferrum ad praeparationes homoeopathicas

Fe

Definition: Obtained by reduction or sublimation as a fine blackish-grey powder.

Content: 97.5 per cent to 101.0 per cent.

Saffron for homoeopathic preparations – Croci stigma ad praeparationes homoeopathicas

Definition: Dried stigmas of *Crocus sativus* L. usually joined by the base to a short style.

Materia Medica

Materia medica is a Latin medical term for the body of collected knowledge about the therapeutic properties of any substance used for healing (i.e., medicines).

Structure

- Latin name of the remedy
- Origin
- Description
- Results of medicine-examination
- Previous experience, observations
- Clinical suggestions
- Pharmacological data
- Individual symptoms, which help in choosing the suitable remedy

Toxic plants and fungi applied in homoeopathy

- **Aconit** - *Aconitum napellus* (Ranunculaceae) – acute infection (cold, cough), depression (in pregnancy)
- **Agaricus** - *Amanita muscaria* (Amanitaceae) – illnesses of central nervous system (epilepsy)
- **Anhalonium** - *Lophophora williamsii* (Cactaceae) – mental fatigue, hallucination, decomposition of movement
- **Colchicum** - *Colchicum autumnale* (Liliaceae) - arthritis, gout, colonitis
- **Colocynthis** - *Citrullus colocynthis* (Cucurbitaceae) – acute pains - rheumatism; laxative, against gastrointestinal spasms
- **Mezereum** - *Daphne mezereum* (Thymeleaceae) - eczema, psoriasis
- **Nux vomica** - *Strychnos nux-vomica* (Loganiaceae) - irritability, insomnia, gastrointestinal disorders, etc.
- **Rhus toxicodendron** - *Rhus toxicodendron* (Anacardiaceae) – skin- and joint diseases (herpes zoster, herpes, eczema)
- **Tabacum** - *Nicotiana tabacum (*Solanaceae) – severe gastric problems, nausea, travel sickness

Figure 6.13
Flowers of *Aconitum napellus* (aconite)

Figure 6.14
Colchicum autumnale (autumn crocus)

Homeopathic practice in England:

- There are postgraduate trainings: available to physicians through the Faculty of Homoeopathy at the Royal London Homoepathic Hospital
- Following 6-month, full-time course, doctors graduate as members or fellows
- Pharmacists, nurses, dentists may achieve the associate membership

Chapter 7
Drugs of fungus and animal origin

7.1 Drugs of fungus origin

Drug: *Secale cornutum*

Fungus: *Claviceps purpurea* (Clavicipitaceae)

This fungus can produce sclerotium, which is a compact mass of hardened fungal mycelium, develops on rye or wheat spike.

Active compounds: alkaloids, see below.

Usage:

- **ergotamine:** vasoconstrictor (at giving birth, treatment of migraine)
- **ergotoxine:** antihypertensive
- **ergometrine:** myometrium vasocontrictor

Lysergic acid diethylamide (LSD) as an illegal drug, can be made from lysergic acid.

Drug: *Tinder fungus*

Fungus: *Fomes fomentarius* (Polyporaceae)

It grows on old, moulding beech-wood.

Active compounds: special polysaccharides

Usage: Research with mice showed an extract of *Fomes fomentarius* altered immune function and *in vitro* research showed an anticancer effect.

Drug: *Laricis fungus*

Fungus: *Fomes officinalis* (Polyporaceae), Agarikon

It grows on *Larix decidua* (European larch).

Active compounds: cellulose, resin

Usage: in ethnomedicine – asringent, appetizer (it has bitter taste).

Drug: *Ganoderma*

Fungus: *Ganoderma lucidum* (Ganodermataceae)

reishi mushroom

It grows on different trees, hard tinder fungus, cultivated in greenhouse in Japan.

Active compounds: glucan, xilan, oligosaccharides

Usage, effect: anti-tumor, immunomodulatory activities, decreases blood sugar levels, in cancer treatment

Figure 7.1
Ganoderma lucidum (reishi)

Drug: Shii-take

Fungus: *Lentines edodes* (Polyporaceae)

shii-take

It is native to East Asia. It grows on moulding trees. It can be cultivated.

Active compounds: (1-3)- and (1-6)-β-bonded heteroglucans, heterogalactans, lentinan, vitamins, protein, lipids, minerals

Usage: see reishi

Drug: *Schizophyllum*

Fungus: *Schizophyllum commune* (Schizophyllaceae)

It is a very common fungus, but not edible.

Active compounds: (1-3)- and (1-6)-β-bonded heteroglucans

Uses: aspecific immunstimulant, intravenous injection (in cancer therapy)

7.2 Drugs of lichen origin

Drug: *Lichen islandicus* (Ph. Eur. 5.)

Lichen: Iceland moss, *Cetraria islandica* (Parmeliaceae)

It grows abundantly in the mountainous regions of northern countries.

Active compounds: special polysaccharides, galactomannan, cetraric acid

Uses, effect: antibacterial, cough-medicine, respiratory tract infections

Figure 7.2
Lichen islandicus (iceland moss)

Drug: *Lichen quercus*

Lichen: Oak moss, *Evernia prunastri* (Parmeliaceae)

Active compounds: EO, moss acids

Usage, effect: antibacterial, gastrointestinal problems, respiratory tract infections. Its EO is used in perfume industry.

7.3 Drugs of animal origin

Drug: *Blatta orientalis*, cockroach

The killed, dried and chopped animals are used. From the animal an infusion or tincture is prepared. Previously it was used as a strong diuretic in homoeopathy.

Drug: *Cantharis*

Animal: *Lytta vesicatoria*, Spanish fly (Figure 1.27)

This animal is killed by chloroform and dried (drying temperature ≤ 40°C).

Active compound: 0.6-0.9% cantharidin, which is an irritant vesicant (blister-inducing) substance, it has rubefacient effect.

Usage: the powdered drug was used for preparing tincture (Tinctura cantharidis) and ointment (Unguentum cantharidatum veterinarium).

Today in homoeopathy the products are used in case of genito-urinary problems and in dermatology.

Drug: *Cetylis palmitas* (Ph. Eur. 5.)

It is a mixture of C14-C18 esters of lauric, myristic, palmitic and stearic acids (“Cetyl esters wax”).

Animal: *Physeter macrocephalus*, Sperm whale

Today this animal is an endangered species! Therefore cetylis palmitas can substitute the milky-white waxy substance (spermaceti), which is in its head and vertebral column. This special wax is used in ointment (Unguentum Stearini). Today jojoba “oil”, a plant wax, can also substitute the spermaceti.

Drug: *Gelatina*

Gelatin

Purified protein obtained either by partial acid hydrolysis (type A), partial alkaline hydrolysis (type B) or enzymatic hydrolysis of collagen from animals (including fish and poultry); it may also be a mixture of different types.

Usage: in pharmaceutical technology (making capsules, suppositors).

Drug: *Hirudo*

Animal: *Hirudo medicinalis,* European medicinal leech

This water animal produces hirudin, an anti-coagulant material. Hirudin reduces blood coagulation.

Drug: *Jecoris aselli oleum* (Ph. Eur. 5.)

Cod-liver oil

Purified fatty oil obtained from the fresh livers of *Gadus morhua* L. (Atlantic cod) and other species of *Gadidae*, solid substances being removed by cooling and filtering.

Composition: cod-liver oil contains saturated and unsaturated fatty acids, vitamin A-, D-,E

Usage: in the case of lack of vitamins.

Drug: *Mel* (Ph. Eur. 5.)

Honey

Animal: *Apis mellifera*

Composition:

70-80% invert sugar (glucose : fructose 1:1), 1-10% saccharose, water, protein, EO, flavonoid

Usage: in case of respiratory problems: cough, inflammation of the throat. Seasoning material in herbal teas.

Chapter 8
Photosynthesis and related metabolic pathways for the formation of effective substances

Photosynthesis is the basis of the formation of all compounds of plant origin. Autotrophic plants (primary producers) are able to photosynthesize, during which process they convert light energy into chemical energy. In the process they reduce carbon-dioxide to organic compounds like glucose, and a by-product, oxygen is formed.

Although plants can utilize only 0.2% of solar radiation, they can produce ca. 2×10^{11} t organic material/year.

The end-product of photosynthesis are carbohydrates, which can be taken up by consuming organisms (consumers) – serving as sources of chemical energy.

The "by-product" of the photosynthetic process is oxygen, which is released into the environment of photosynthesizing plants.

The formation of oxygen and the reduction of carbon-dioxide takes place via the successive disjunction of electrons.

Overall equation of the photosynthetic process: $6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$

8.1 The scene of photosynthesis

Photosynthesis takes place in the leaf mesophyll, which is delimited by the upper (adaxial) and lower (abaxial) epidermis. The largest part of the leaf mesophyll is filled with the tissue responsible for photosynthesis, called chlorenchyma, which in most plants consists of palisade and spongiose parenchyma. This assimilating ground tissue is composed of chloroplast-containing parenchyma cells (Figure 8.1)

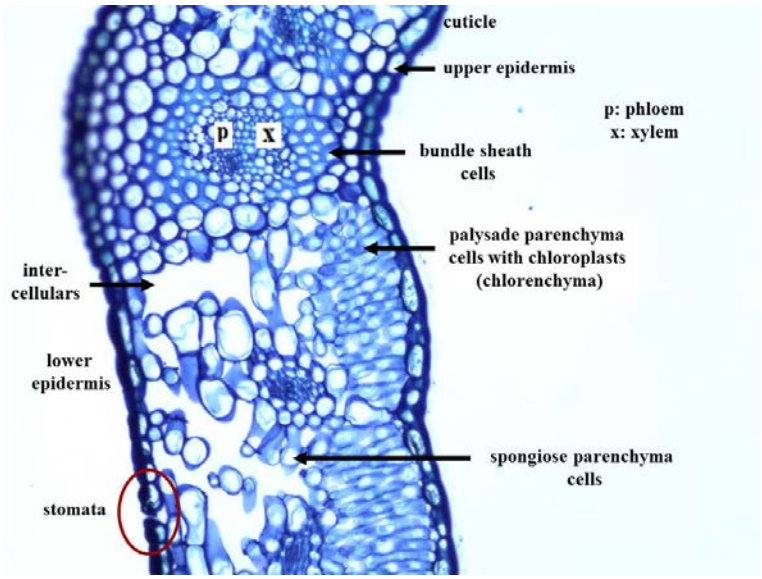


Figure 8.1
A typical dicotyledonous leaf (*Helleborus odorus*)

Chloroplasts are cell organelles surrounded by double membrane layer (Figure 8.2). The invaginations of the inner membrane develop the thylakoid membrane system. The inside of the thylakoid membrane is called the lumen, and outside the thylakoid membrane is the stroma, where the "dark reactions" take place. The thylakoid membrane contains some integral membrane protein complexes that catalyze the light reactions.

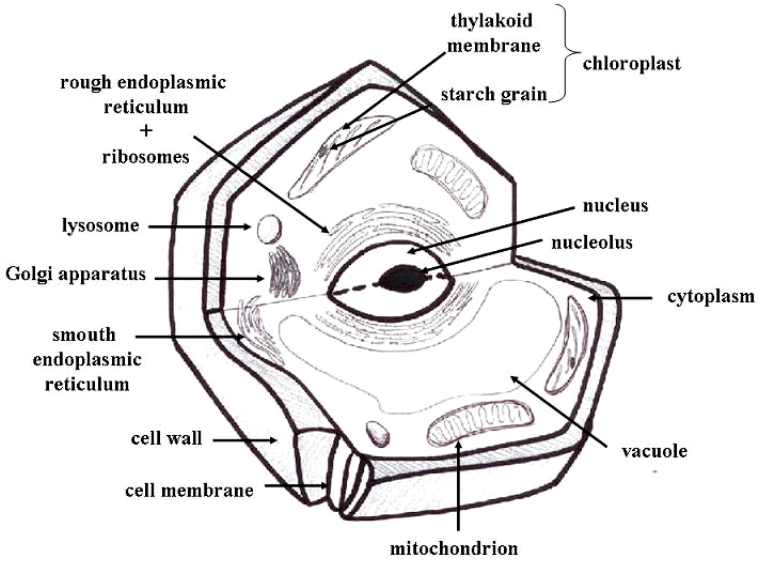


Figure 8.2
The plant cell

8.2 Chemical composition of the chloroplast

- Water - about 80%
- Protein - 60-70% (dry weight)
  - insoluble in water:
    - thylakoid;
  - pigment:
    - chlorophyll-protein complexes
    - chromoproteids (Fe-S-protein, ferredoxine, plastocyanine)
  - ATP-synthase
  - soluble in water:
    - stroma proteins, e.g. RUBISCO
- DNA, RNA
- Nucleotids (ATP, NADP)
- Lipids

8.3 Pigments in the chloroplast

Chlorophylls are pigments that are responsible for the green colour of plants. Chlorophylls absorb light with the highest intensity in the blue portion (400 to 450 nm) of the electromagnetic spectrum, and with less intensity in the red portion (650 to 700 nm). The photosynthetically active radiation (PAR) wavelengths fall between 400 and

700 nm. Chlorophylls belong to the porphyrin pigments, with a magnesium ion at the centre of the chlorin ring (Figure 8.3). The most important chlorophyll pigments are Chlorophyll A, and Chlorophyll B.

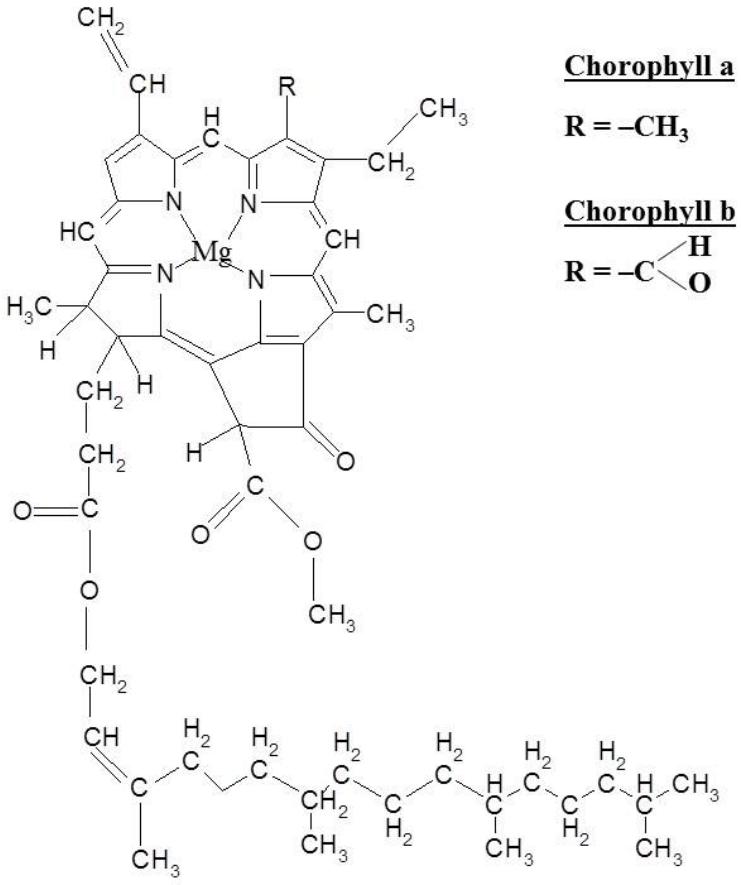


Figure 8.3
The chemical structure of chlorophyll a and b

Another pigment involved in the photosynthetic process is the red-orange β-carotene that is structurally a tetraterpene, having beta-rings at both ends of the molecule (Figure 8.4).

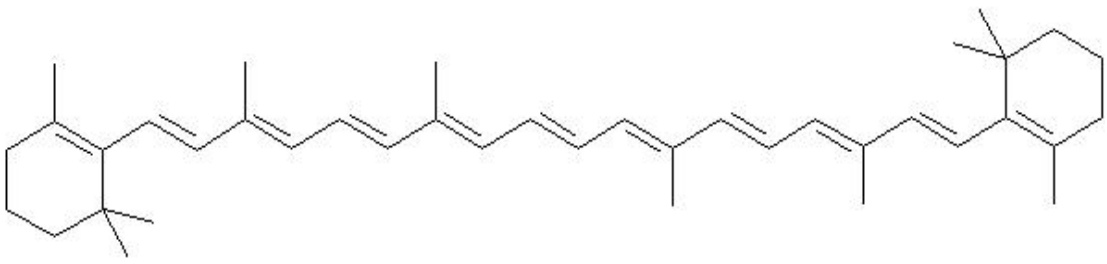


Figure 8.4
The chemical structure of ß-carotene

8.4 Light-dependent reactions of photosynthesis

The light-dependent reactions comprise the first stage of photosynthesis. In this process light energy is converted into chemical energy, which is stored in energy-carrying molecules ATP and NADPH. The **light reactions of photosynthesis** include the following major events:

- reduction of $NADP^+ \rightarrow NADPH$
- phosphorylation of $ADP \rightarrow ATP$
- oxydative cleavage of $H_2O \rightarrow$ formation of O_2

The light-dependent reactions take place on the thylakoid membrane of the chloroplast. There are four major protein complexes in the thylakoid membrane: Photosystem I (PSI), Photosystem II (PSII), Cytochrome b/f complex and ATP synthase (Figure 8.5). These four complexes work together to ultimately create the products ATP and NADPH.

The two photosystems absorb light energy through pigments, particularly the chlorophylls. Chlorophyll *a* plays a central role in this process, since it is located in the reaction centre of each photosystem. The light-dependent reactions begin in PSII. When a Chlorophyll *a* molecule within the reaction centre absorbs a photon, an electron in this pigment attains an excited state (gets to a higher energy level). Since this state is quite unstable, the electron is transferred to another molecule, establishing a chain of redox reactions, which together comprise the electron transport chain. The direction of the electron flow is from PSII to quinone and plastoquinone, then the cytochrome b/f complex, followed by plastocyanin, to PSI. In PSI the electron receives the energy from another photon. The final electron acceptor is $NADP^+$. The first electron donor is water, which splits into hydrogen ions (protons) and oxygen in the Hill-reaction.

The cytochrome b/f complex and the ATP synthase work together to create ATP in a process called photophosphorylation (Figure 8.5). The energy of electrons from PSII is used to pump protons from the stroma to the lumen. The proton gradient developing across the thylakoid membrane will serve as the force to form ATP by the ATP synthase.

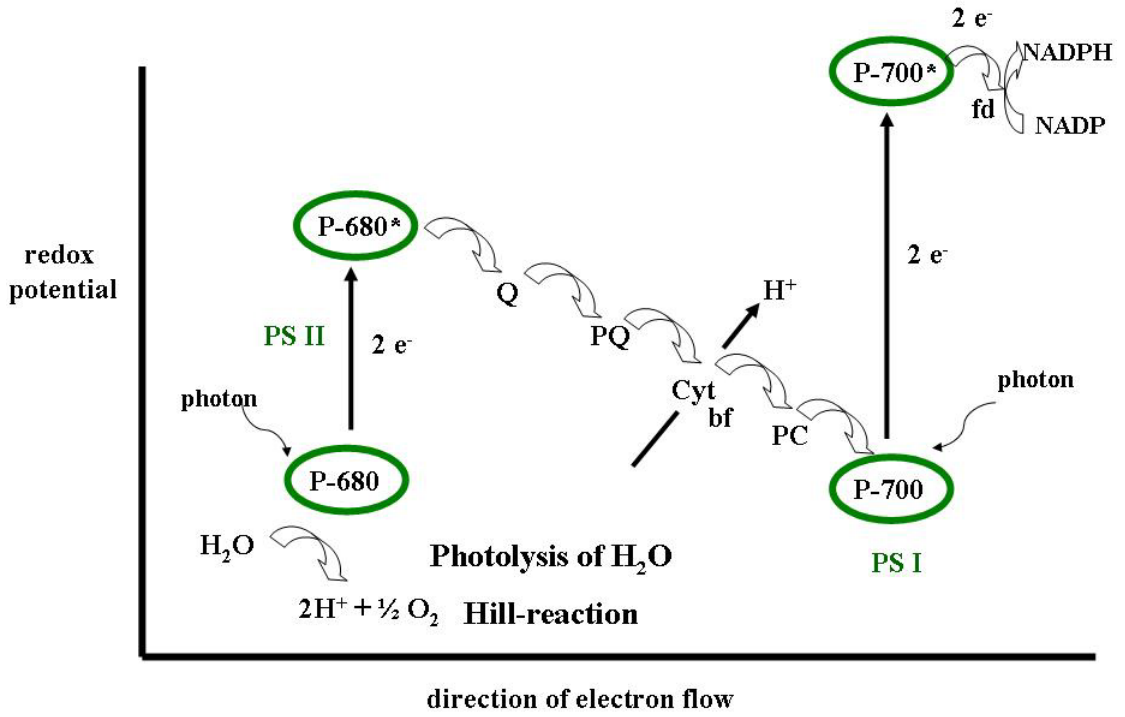


Figure 8.5
Outline of photophosphorylation

Plastoquinone is a mobile electron carrier in the membrane, which transfers protons (H^+) and electrons (Figure 8.6). In the process plastoquinol is transformed into plastoquinone by losing 2 electrons and 2 protons.

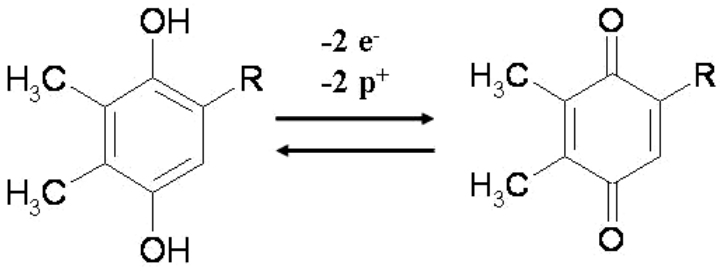


Figure 8.6
Electron-transport with plastoquinones

The cytochrome b/f is a transmembrane proton pump. Electrons from PSII are carried by plastoquinol to the cytochrome b/f complex (Figure 8.7), where they are removed one by one, reforming plastoquinone.

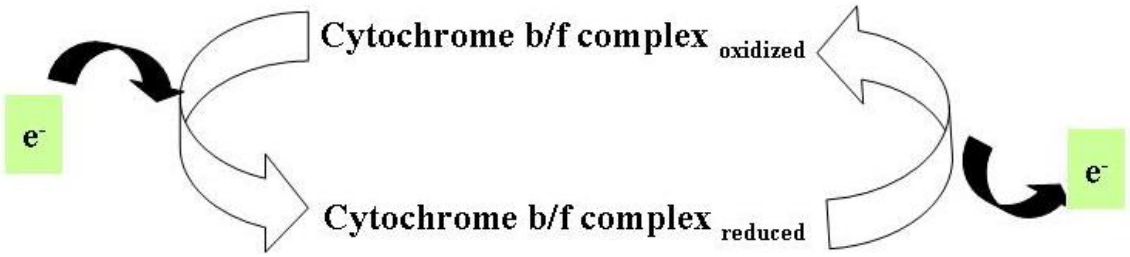


Figure 8.7
Electron-transport through the cytochrome b/f complex

Plastocyanine is a copper-protein complex with blue colour. It is a stable chromoproteid, which is synthetised in the cytoplasm. If functions as an electron transfer agent between the cytochrome b/f compex and the reaction centre of PSI.

Ferredoxins are iron-sulphur proteins with reddish-brown colour, mediating electron transfer in a range of metabolic processes, including photosynthesis. Ferredoxin reduces the final electron acceptor $NADP^+$.

8.5 Calvin cycle or "dark reactions" of photosynthesis

The so-called **dark reactions** of photosynthesis depend strongly on the light reactions, since they are driven by ATP and NADPH, which are the products of the light reactions (Figure 8.8). ATP and NADPH are essential for the reduction of carbon-dioxide to organic compounds such as glucose in the steps of the so-called Calvin cycle.

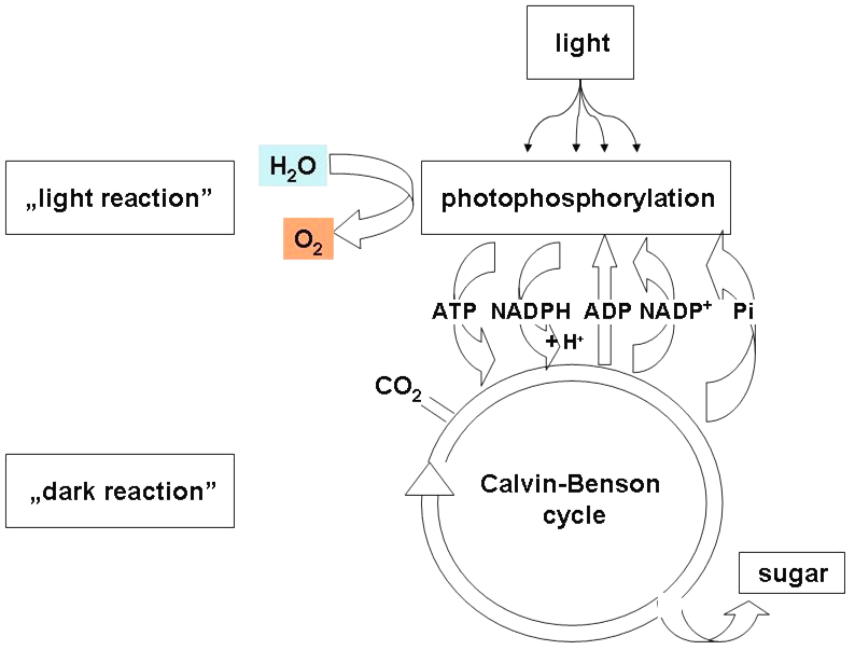


Figure 8.8
Summary and interrelations of light and dark reactions

The Calvin cycle, often referred to as "dark reactions" or the carbon reactions pathway (Figure 8.9), uses the energy of cleavage of phosphate bonds of ATP and the reducing

power of NADPH, to fix and reduce carbon-dioxide to form carbohydates. The enzymes and the intermediates of the Calvin cycle are located in the chloroplast stroma.

The enzyme Ribulose-Bisphosphate-Carboxylase (RuBp-Carboxylase) catalyses CO_2 fixation. The same enzyme can catalyse an oxygenase reaction, therefore it is frequently called RuBp-Carboxylase/Oxygenase or RuBisCo.

Carboxylation

Carbon dioxide is combined with the 5-carbon sugar ribulose 1,5-bisphosphate, creating a 6-carbon sugar. This in turn is broken down into 2 phosphoglycerate molecules.

Oxidation, dephosphorylation

The following steps include oxidation and dephosphorylation, using NADPH and ATP from the light reactions, resulting in triose phosphates.

Regeneration

Ribulose 5-monophosphate can be regenerated from these triose phosphate molecules, and with phosphorylation, using one more ATP the starting molecule of the Calvin cycle, ribulose 1,5-bisphosphate is regenerated.

The triose phosphates will be the precursors of important carbohydrates like fructose and glucose.

For details of the steps of the Calvin cycle see Chapter 10.

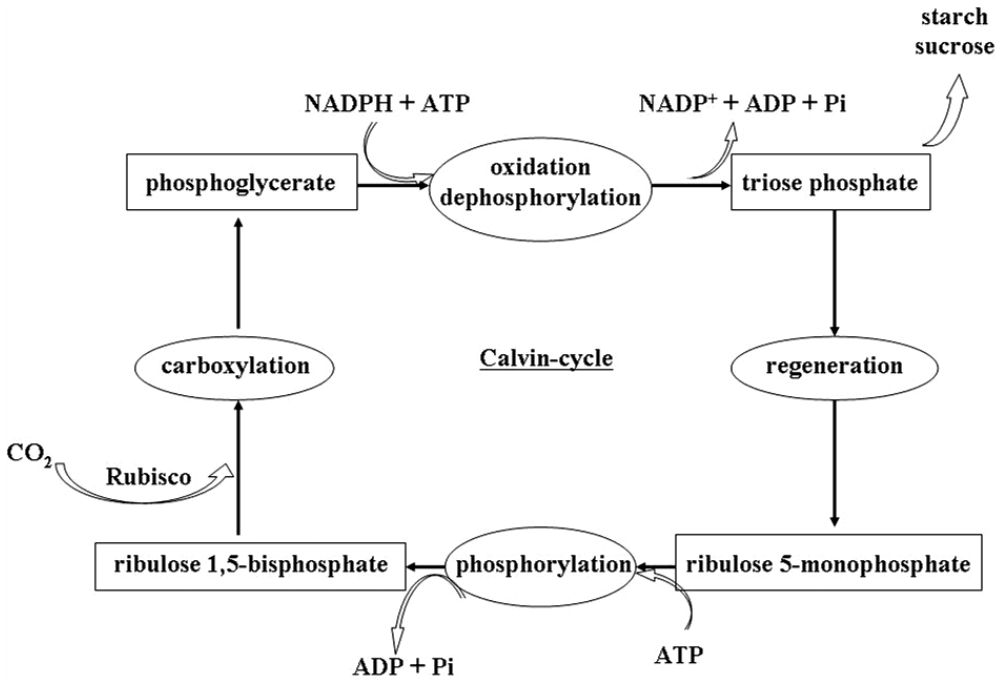


Figure 8.9
Summary of dark cycle reactions

8.6 Connection points of photosynthesis

fructose-6-phosphate → glucose-6-phosphate→ glucose → starch

erythrose-4-phosphate: one of the primary precursors of aromatic amino acids

(other precursor: PEP = **p**hospho**e**nol-**p**yruvate)

ribose-5-phosphate: precursor of ribonucleic acids and of the ribose in nucleotids (Figure 8.10)

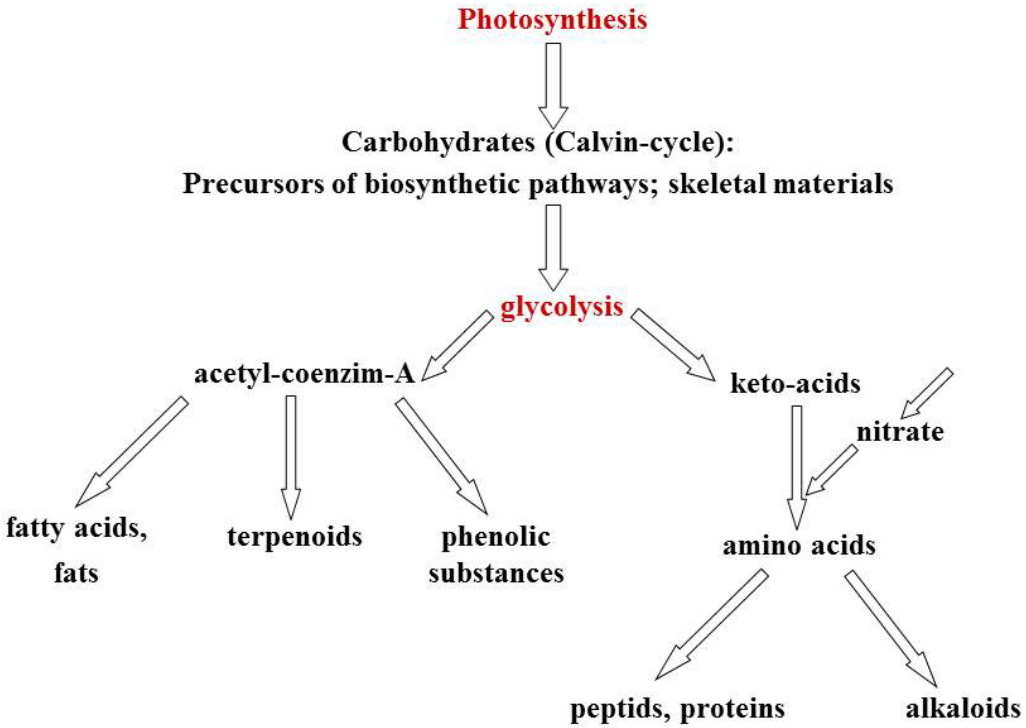


Figure 8.10
Outline of photosynthesis-related metabolic pathways

8.7 Differences between the C3 and C4 pathways of photosynthesis

In C4 plants the vascular bundle (vein) of the leaf is surrounded by two rings of cells. The cells of the inner ring, called bundle sheath, contain chloroplasts (Figure 8.12). These chloroplasts are rich in starch, but they are lacking grana, thus being different from chloroplasts present in the mesophyll cells of the outer ring. In contrast, in C3 plants the bundle sheath is lacking chloroplasts (Figure 8.11).

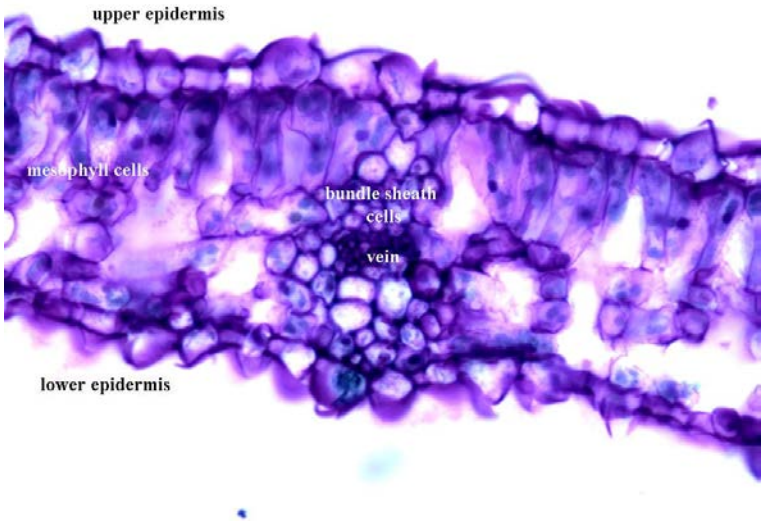


Figure 8.11
Leaf transversal section of a C3 plant

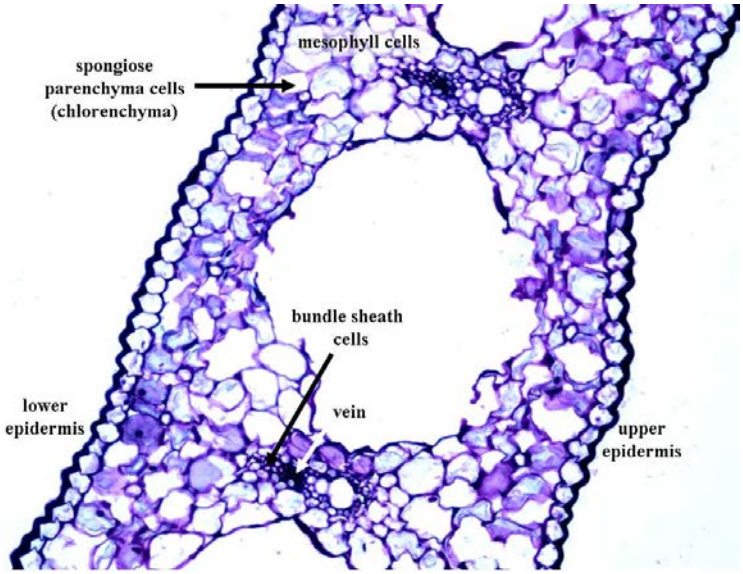


Figure 8.12
Leaf transversal section of a C4 plant

C4 pathway

The C4 photosynthetic pathway is typical in tropical, drought resistant plants, such as representatives of the Poaceae, Amaranthaceae, Chenopodiaceae, Euphorbiaceae and Portulacaceae family. The C4 pathway requires more energy in the form of ATP compared to the C3 pathway, however, it ensures a higher rate of photosynthesis, which is advantageous under tropical conditions with higher temperature and great light intensity.

The C4 pathway uses a more efficient enzyme to fix CO_2 in mesophyll cells. The fixed carbon is stored in the form of malic acid within leaf mesophyll cells. CO_2 is transferred to the bundle sheath cells, where it enters the Calvin cycle (Figure 8.13).

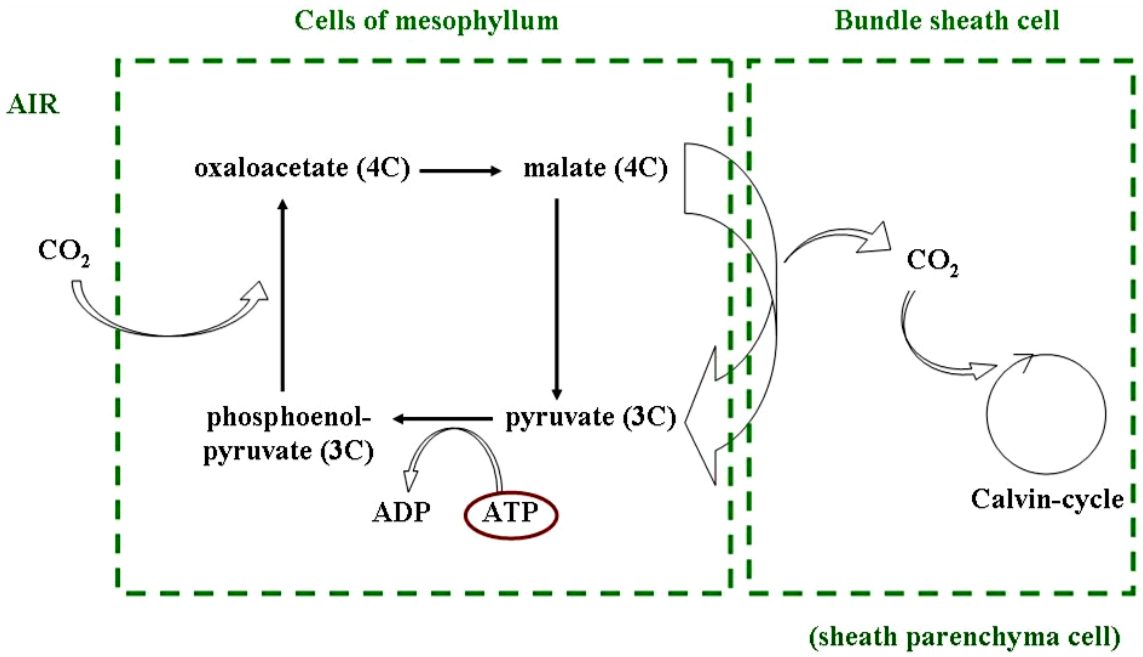


Figure 8.13
Summary of C4 photosynthetic pathway

Chapter 9
Role of nitrate- and sulphate-reduction in synthesis of effective substances

Photosynthesis, respiration: The most important chemical reaction of this process is the following:

keto acids → amino acids

The enzymes involved are: transaminases (reductive transaminases).

Formation of reduced (amino) form of nitrogen:

Nitrate (NO_3^-) is taken up by the plant.

N_2 is taken up from the air and fixed

Reduction of sulphate:

sulphate taken up by the plant → -SH (S-containing amino acids)

The base of nitrogen- and sulphur-assimilation is biological reduction (similarly to the reduction of CO_2 in photosynthesis).

9.1 Nitrogen metabolism in plants

Natural circulation of nitrogen by living organisms

The most important elements of nitrogen circulation are summarized in Figure 9.1.

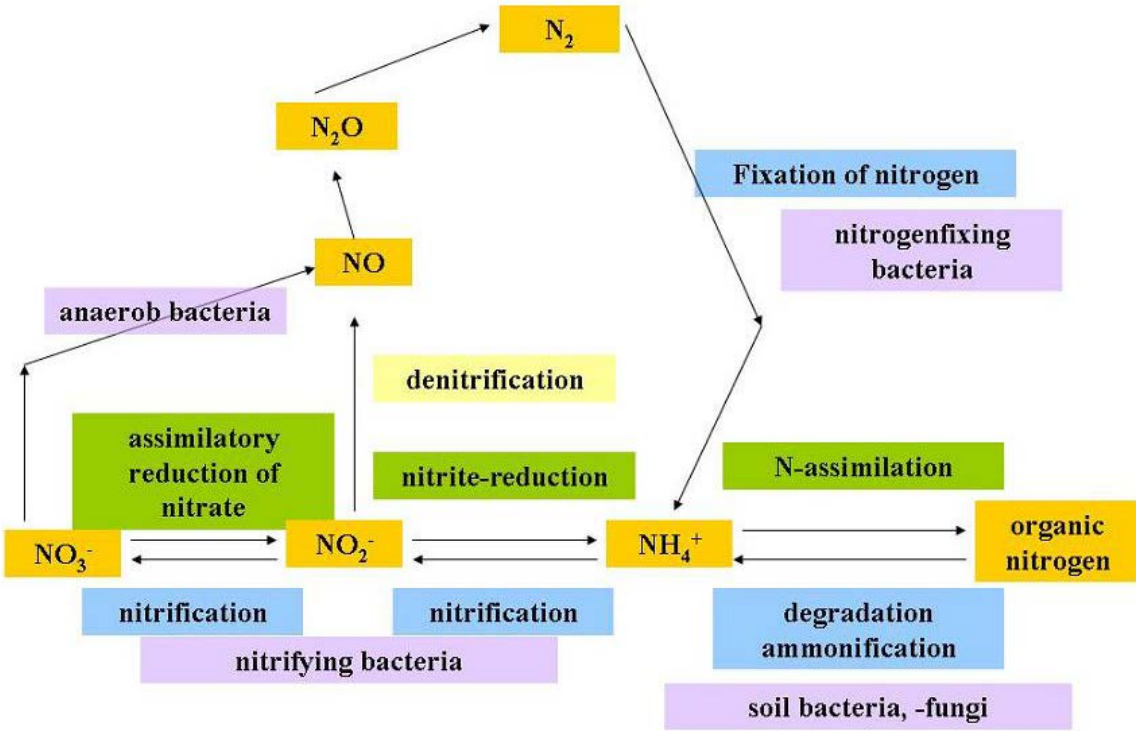


Figure 9.1
Biological circulation of nitrogen

Nitrogen fixation

Fixing of nitrogen is the following process: $N_2 \rightarrow NH_4^+$, which occurs with the participation of nitrogen-fixing bacteria (Figure 9.1).

Important: If plants are able to fix nitrogen, there is no need for N-containing artificial fertilizers!

Bacteria capable of nitrogen-fixing

Diazotroph bacteria

Non-symbiotic

- CYANOBACTERIA (e.g. *Nostoc*, *Anabaena*, *Oscillatoria*)
- PHOTOSYNTHETIC BACTERIA (e.g. *Rhodospirillium rubrum*)
- CHEMOTROPHIC BACTERIA (e.g. *Thiobacillus ferrooxidans*)
- EUBACTERIA (e.g. *Azotobacter*, *Klebsiella pneumoniae*, *Azospirillium* – the latter is in loose association with roots of grasses)

Symbiotic

- CYANOBACTERIA (in some angiosperms, ferns, mosses)
- ACTINOMYCETALES (ray fungi), e.g. *Frankia* – symbiosis with *Alnus*, *Elaeagnus*, *Hippophae*
- EUBACTERIUM (Rhizobiaceae: *Rhizobium*, *Bradyrhizobium*, *Azorhizobium* – endosymbiosis with legumes)

Legumes – endosymbiosis

- On the root of host plant: root nodules (tubers, nodules)
- Bacteria can penetrate through the root-hairs: → infection thread will be formed
- Bacteria penetrate through the cortical cells of the root into the internal part of the bark: → they induce mitosis
- In the polyploid cells: bacteria proliferate intensively
- The proliferated cells are demarcated by the membrane of the host cells (peribacteroid membrane)
- This enveloped formation is: **nodule** (Figure 9.2-3)

Root nodules

Formation of nodules – bacteria swell: **bacteroid phase**

In vitro: *Rhizobium* – they are not able to fix N_2 themselves (without the legume partner), but the bacteroids isolated from root nodules are!

The genetic ability of N_2-fixing is coded in *Rhizobium*. The host plant supplies bacteria with the suitable carbon skeleton.

Leghaemoglobin: located in the peribacteroid interstice, in plant cytosol (synthesis – hem: bacteroid; globin: host plant). It binds O_2 very strongly → under micro-aerophyl conditions (low, but necessary concentration of O_2).

The pink colour of a nodule shows that it contains leghaemoglobin, i.e. the nodule is viable.

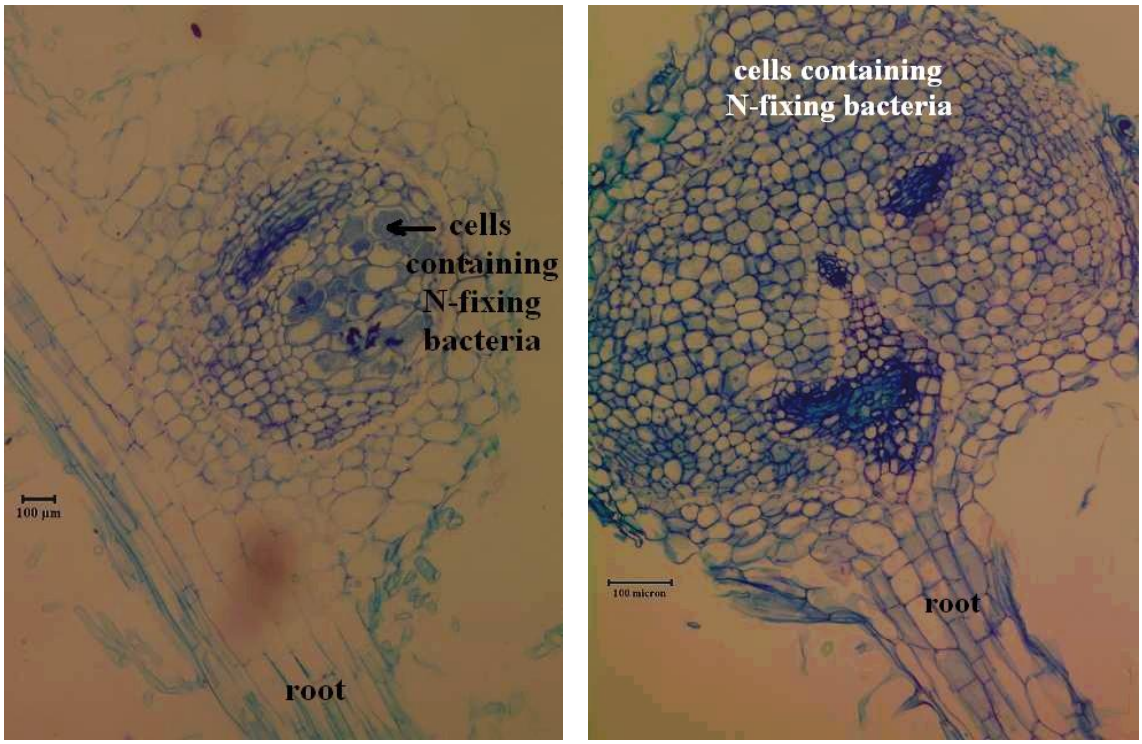


Figure 9.2-3
Root nodules with nitrogen-fixing bacteria in longitudinal section

Fixing of nitrogen with chemical equations

The energy of the diazo bond (:N ≡ N:): is very high.

Root nodule bacteria use about 20% of the ATP produced by the plant to break the diazo bonds.

Reduction of 1 mol N_2 requires 16 mol ATP, 8 mol electron:

$$N_2 + 10\ H^+ + 8\ e^- \xrightarrow[\textit{nitrogenase}]{} 2\ NH_4^+ + H_2$$

Nitrogenases: proteins containing Fe and Mo (Figure 9.4)

They are formed by two subunits, which dissociate readily, but neither of them is active in itself.

Nitrogenase-reductase: smaller unit, Fe-protein complex

Nitrogenase: bigger unit, Mo-Fe-protein complex, activity: $N_2 \rightarrow NH_4^+$

Outside the peribacteroid membrane (in cytosol) the following processes take place:

$$NH_4^+ \xrightarrow[\textit{transaminase}]{} \text{glutaminic acid}$$

$$NH_3 + \text{glutaminic acid} \xrightarrow[\text{ATP}]{} \text{glutamin} + ADP + Pi$$

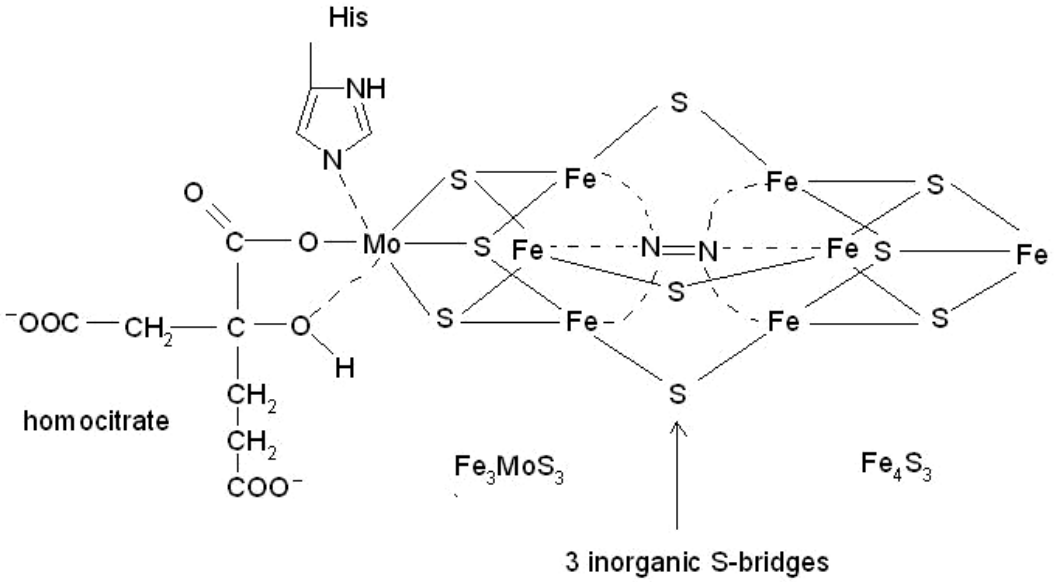


Figure 9.4
Structure of the nitrogenase subunit

Equation of N_2-fixing (reduction) (Figure 9.5)**:**

$$N_2 + 8\ e^- + 16\ MgATP + 16\ H_2O \rightarrow 2\ NH_4^+ + H_2 + 16\ MgADP + 16\ Pi + 6H^+$$

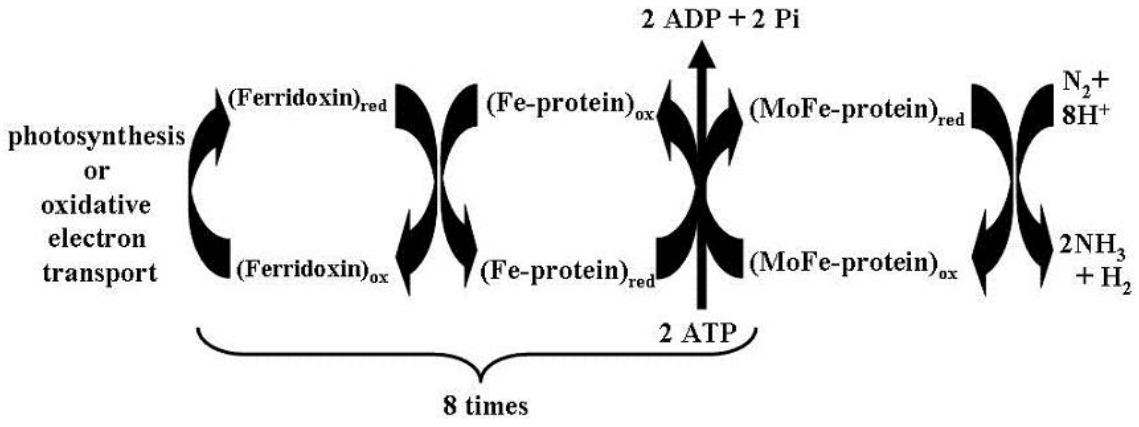


Figure 9.5
Nitrogenase electrontransport

Origin of ATP:

Photosynthetizing bacteria: → photosynthetic origin

Under *aerob conditions* (e.g. *Rhizobium* sp.): mitochondrial phosphorylation will take place.

Nitrification

Nitrification is an important step in the nitrogen cycle in the soil (Figure 9.1). It is an aerobic process performed by autotrophic bacteria, which are quite sensitive (e.g. to acidic pH, presence of tannins).

Nitrifying bacteria:

- *Nitrosomonas* sp.
- *Nitrobacter* sp.

Nitrification is the biological oxidation of ammonia with oxygen, into nitrite, followed by the oxidation into nitrate:

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$$

The function of nitrifying bacteria is hindered by this process:

$$NH_3 \rightarrow \text{atmosphere} \rightarrow NH_4^+ - \text{absorbed in the soil}$$

Denitrification

Denitrification is a process of nitrate reduction performed by heterotrophic facultative anaerobic bacteria that will eventually produce molecular nitrogen (N_2) through a series of intermediate nitrogen oxide products (Figure 9.1, 9.6).

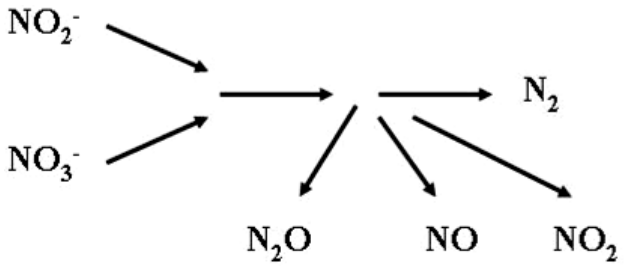


Figure 9.6
The process of denitrification

Anaerobic denitrifying bacteria:

- *Pseudomonas denitrificans*
- *Micrococcus dentirificans*
- *Thiobacillus denitrificans*

Nitrogen assimilation

Nitrogen assimilation is the **formation of organic nitrogen compounds** such as amino acids **from inorganic nitrogen compounds** present in the environment (Figure 9.1). Plants that cannot fix nitrogen gas (N_2) depend on the ability to assimilate nitrate or ammonia for their needs.

Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonia (NH_3). In aerobic soils where nitrification can occur, nitrate is the predominant form of available nitrogen (Figure 9.1). Nitrate is taken up by **nitrate transporters** that use a proton gradient to power the transport. The co-transport of NO_3^- and H^+ is directed through the plasmalemma into the root cells. Nitrogen is transported from the root to the shoot via the xylem in the form of nitrate, or dissolved ammonia and amino acids

(Figure 9.7). In some species NO_3^- can be reduced in the root cortex parenchyma cells; while in the majority of plants most of the nitrate reduction is carried out in the shoots, and the roots typically reduce only a small fraction of the absorbed nitrate to ammonia. Nitrate ions can be stored in the vacuoles until they are needed for the synthesis of amino acids (Figure 9.8). Ammonia is incorporated into amino acids via the glutamin synthetase-glutamate synthase pathway.

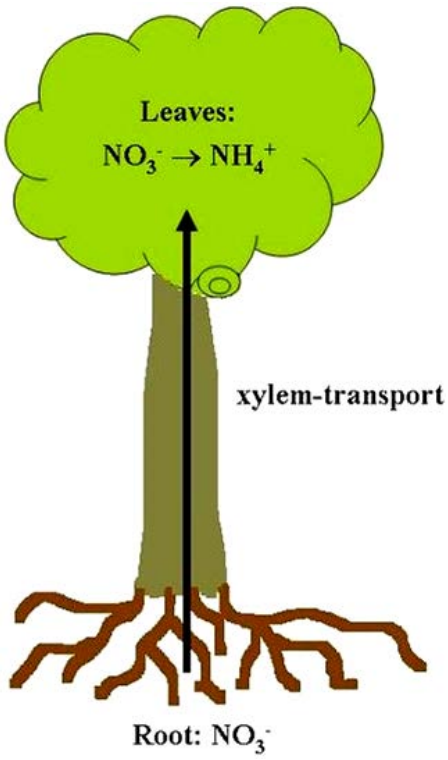


Figure 9.7
Transport ways of nitrate in plants

Reduction of nitrate

Nitrate reduction is carried out in two steps.

1) Nitrate is first **reduced to nitrite (**NO_2^-) in the cytosol by nitrate reductase using NADH or NADPH.

Nitrate-reductase:

$$NO_3^- + 2\ H^+ + 2\ e^- \xrightarrow[NADH]{} NO_2^- + H_2O$$

In the cytoplasm, during nitrate reduction, the nitrate-reductase enzyme is in close contact with the external membrane of the chloroplast, because in the next step further reduction will take place in the chloroplast by nitrite reductase. The presence of NO_3^- increases the activity of nitrate reductase.

The way of the electron:

$$\underset{}{NADH} \xrightarrow[e^-]{} \underset{}{NAD} \xrightarrow[e^-]{} \underset{}{\text{cytochrome } b_{557}} \xrightarrow[e^-]{} \underset{}{\text{Mo-cofactor}} \xrightarrow[e^-]{} \underset{}{NO_3^-} \rightarrow \underset{}{NO_2^-}$$

oxidation

$$NADH \rightarrow NAD^+$$

reduction

$$NO_3^- \rightarrow NO_2^-$$

$$NO_3^- + NADH + H^+ \rightarrow NO_2^- + NAD^+ + H_2O$$

2) Nitrite is then **reduced to ammonia** in the chloroplasts by a ferredoxin dependent nitrite reductase.

Nitrite-reductase:

$$NO_2^- + 8\ H^+ + 6\ e^- \xrightarrow[ferredoxine]{} NH_4^+ + 2\ H_2O$$

The electron donor is the e^- transport system in the chloroplast. Nitrate and light are the two most important factors in the regulation of nitrite reductase.

Transamination

Glutamin synthetase – glutamate synthase:

In the chloroplasts, **glutamin synthetase** incorporates ammonia as the amide group of glutamine, using glutamate as a substrate. Further **transaminations** are carried out to make other **amino acids** from glutamine.

The most important transport routes and pathways of nitrogen metabolism within the plant are summarized in Figure 9.8.

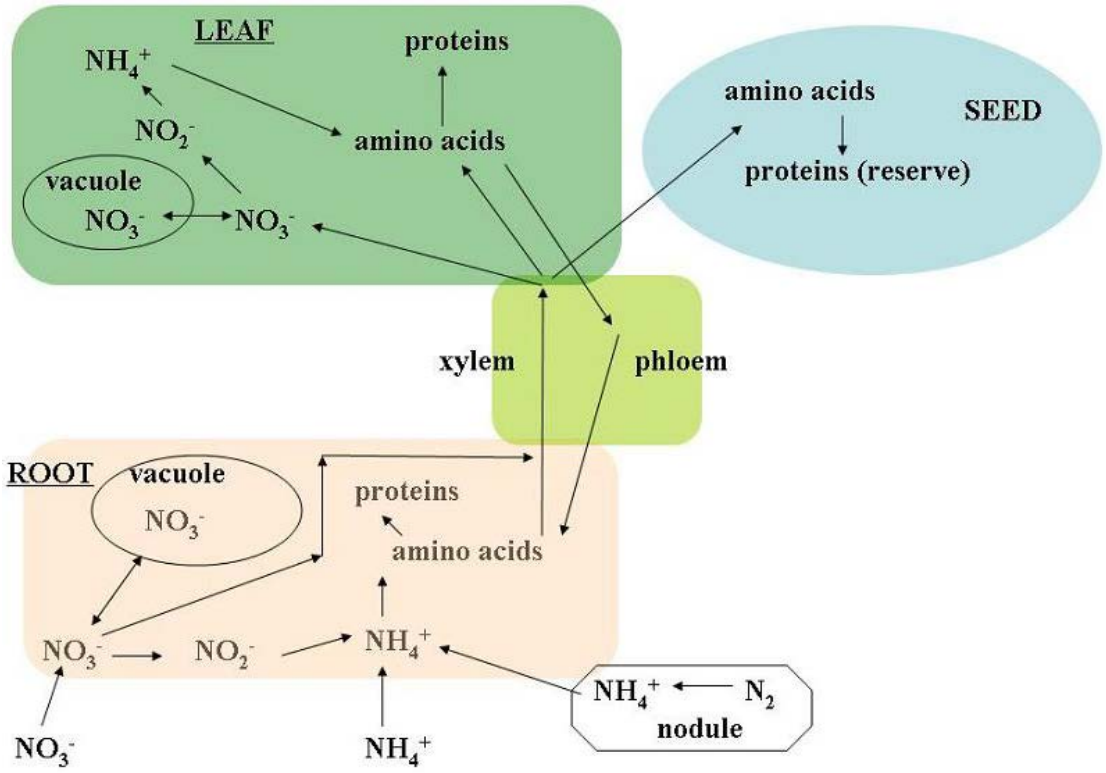


Figure 9.8
Transport routes and pathways of nitrogen metabolism in plants

9.2 Sulphate-reduction

Sulphate (SO_4^{2-}) is **reduced to thiols** by microorganisms and plants, and these are incorporated via amino acids into proteins. Sulphates taken up by the root are the major sulphur source, although they have to be reduced to sulphide before they are further metabolized. Root plastids contain all sulphate reduction enzymes, but typically SO_4^{2-} is transported by xylem-transport into the shoot (mainly the leaves), where the reduction of sulphate to sulphide and its subsequent incorporation into cysteine will take place, in the chloroplasts.

The main steps of sulphate reduction are summarized in the following equation. Further details of this process can be found below.

$$SO_4^{2-} + ATP + 8\ e^- + 8\ H^+ \rightarrow S^{2-} + 4\ H_2O + AMP + PP_i$$

(1) Activation of sulphate

Sulphate is first activated by adenosine triphosphate (ATP) before it is reduced (Figure 9.9).

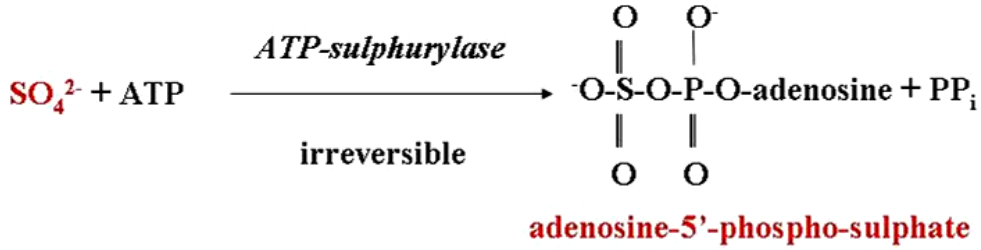


Figure 9.9
Activation of sulphate by ATP

A sulphur-containing nucleotide, adenosine-5'-phospho-sulphate (APS) is a carrier of sulphur during sulphate reduction in green plants (Figure 9.10-11).

(2) Reduction of sulphate

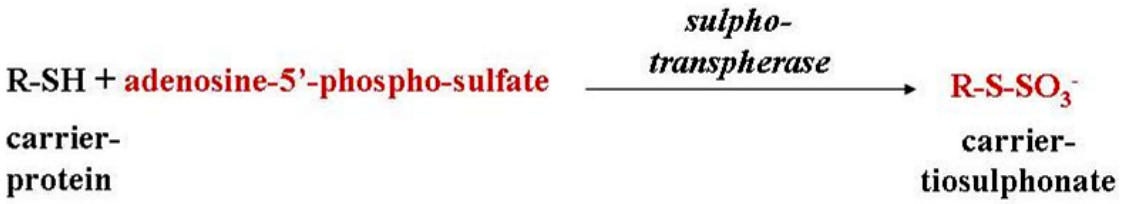


Figure 9.10
Formation of carrier-tiosulphonate complex

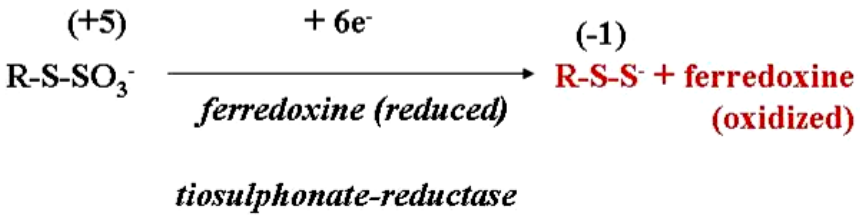


Figure 9.11
Reduction of tiosulphonate

(3) Formation of cysteine

Cysteine is the **precursor** or reduced sulphur donor of most **other organic sulphur compounds** in plants (Figure 9.12-13). The amino acids cysteine and methionine are highly significant in the structure, conformation and function of proteins.

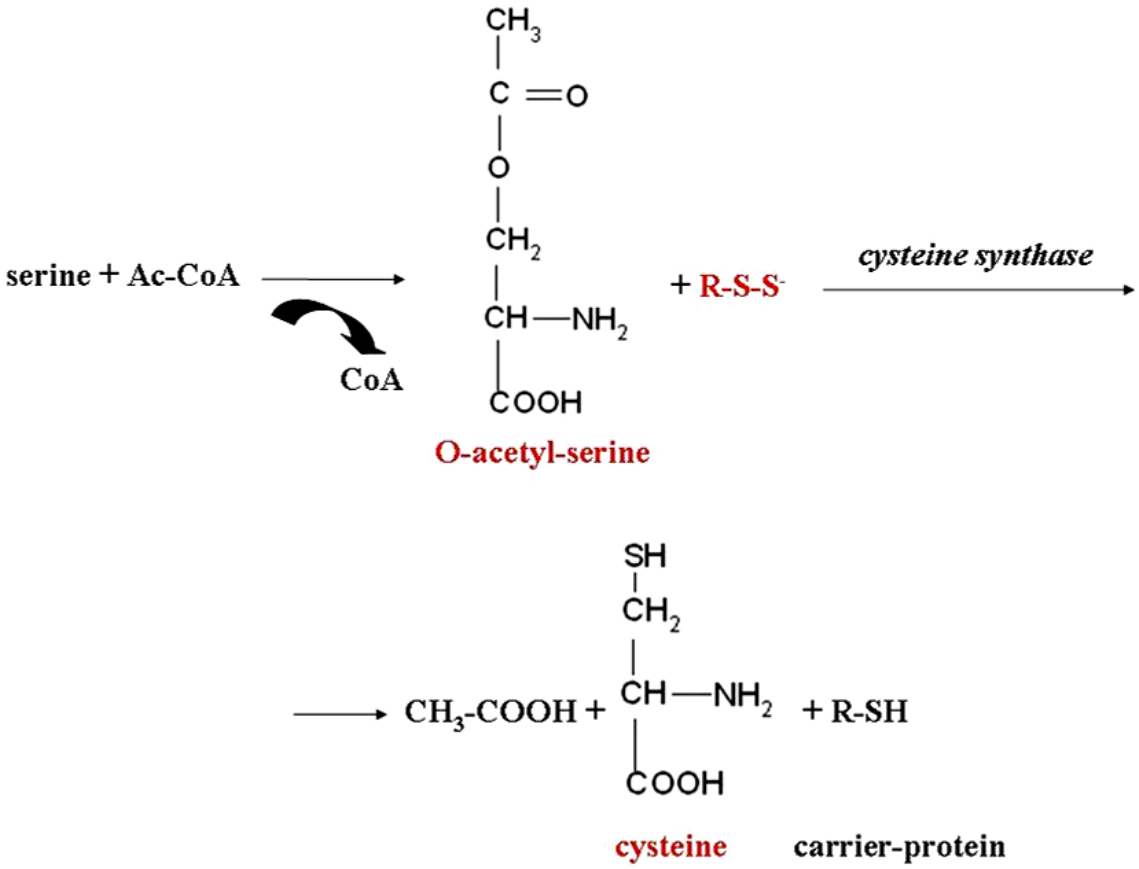


Figure 9.12
Incorporation of sulphide into cysteine

Importance of sulphate-reduction

Plants contain a variety of organic sulphur compounds, as thiols, sulpholipids and secondary sulphur-containing metabolites like alliin, glucosinolates etc. (Figure 9.13), which play an important role in plant physiology and are of great importance for pharmaceutical applications.

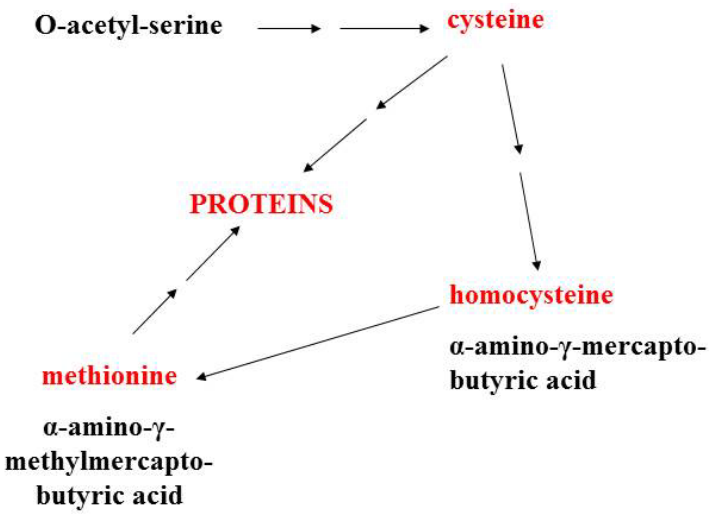


Figure 9.13
Interrelations of various sulphur-containing compounds

Chapter 10
Synthesis, role and usage of carbohydrates

10.1 Formation of carbohydrates

Carbohydrates are formed in the process of photosynthesis.

- Carbohydrates ← CO_2 and H_2O + solar energy
- **Primary processes** of photosynthesis: photolysis of water – light energy (for details see Chapter 8)
- **Secondary process:** CO_2 reduced to carbohydrates; hydrogen ← photolysis of water
- **Equation of the fundamental process:** $6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$
- The steps of CO_2 fixing and reduction were clarified by Calvin and co-workers (Nobel-price; 1961) → **Calvin-cycle** (Figure 10.1-3)

The Calvin-cycle – Fixation and reduction of carbon dioxide

CO_2 fixation: ***ribulose-1,5-diphosphate*** (or -bisphosphate) – phosphorylated by the primary ATP (Figure 10.1)

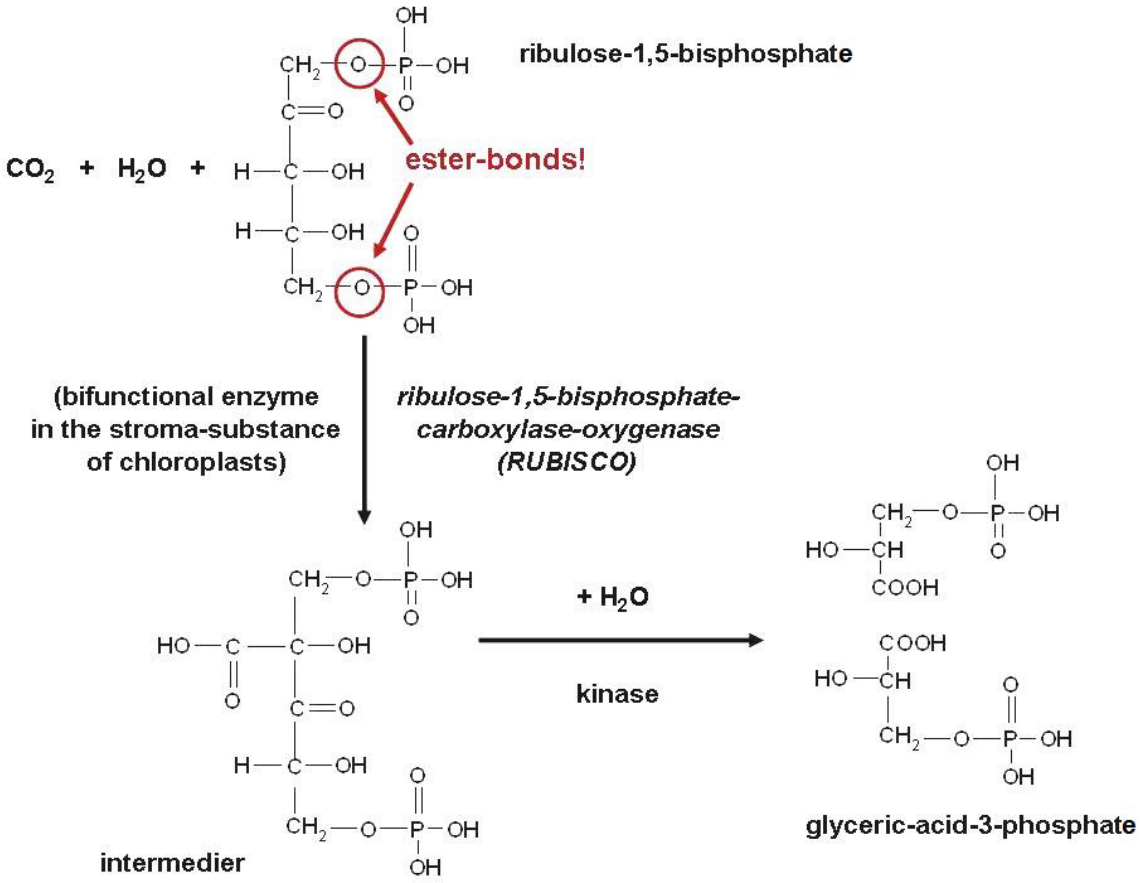


Figure 10.1
Fixation of carbon-dioxide by ribulose-1,5-bisphosphate-carboxylase-oxygenase

The product of the RuBP carboxylase reaction, glyceric-acid-3-phosphate (3-phosphoglcyerate) is converted to glyceraldehyde-3-phosphate. Triose-phosphate-isomerase catalyses the formation of dihydroxy-aceton-3-phosphate (Figure 10.2).

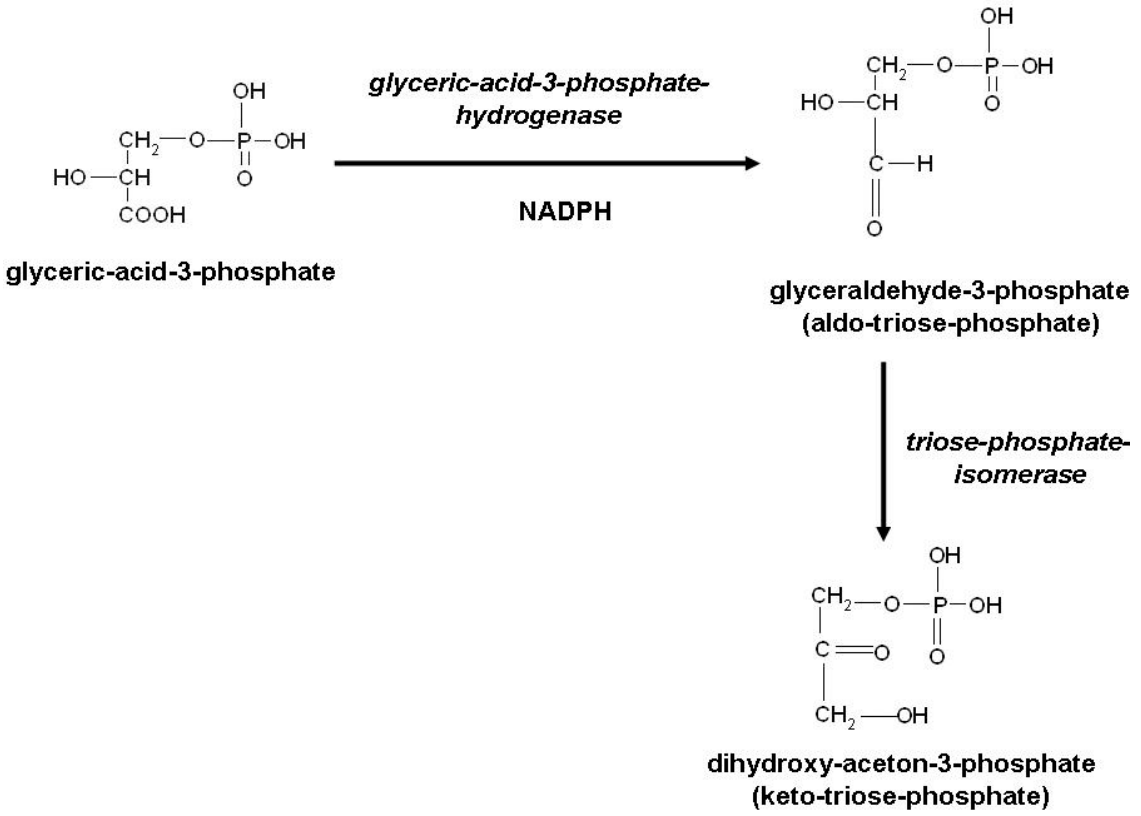


Figure 10.2
The C-3 reactions of the Calvin-cycle

Further reactions of the Calvin-cycle are catalysed by various aldolase and trans-ketolase enzymes, and yield a variety of carbohydrates important in several plant physiological processes (Figure 10.3-5). From glucose-phosphate molecules **primary** or **photosynthetic starch** is formed, which serves as a reserve substance.

aldo- and keto-triose-phosphates

↓ ***aldolase*** **and** ***trans-ketolase*** **enzymes**

important carbohydrates
(sugars with 4-7 C-atoms):

fructose (C-6)
glucose (C-6)
erythrose (C-4)
ribulose (C-5)
xilulose (C-5)
ribose (C-5)
sedoheptulose (C-7)

subsequent phosphorilation of ribulose - cycle repeated

Figure 10.3
Carbohydrates formed in the Calvin-cycle

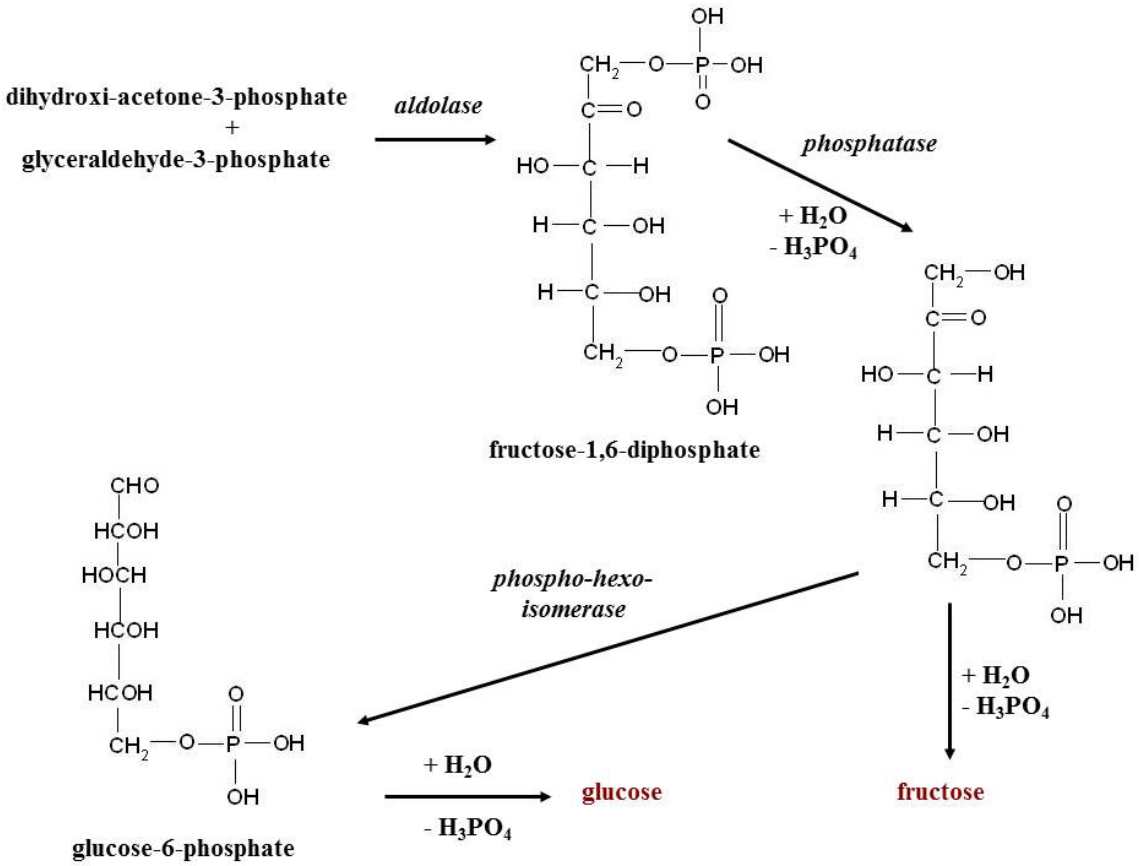


Figure 10.4
Synthesis of various carbohydrates in the Calvin-cycle

Fixation of carbon dioxide in C4 plants

C4 plants bind CO_2 in a combined way, where **p**hospho-**e**nol-**p**yruvate (PEP) (Figure 10.5) plays an important role. The bound CO_2 is temporarily stored as malic acid (Figure 10.6), which in turn is transported to neighbouring tissues. Here it can be used in the Calvin-cycle, yielding starch as the end product. C4 type plants include e.g. maize (*Zea mays*) and sugar beet (*Beta vulgaris*).

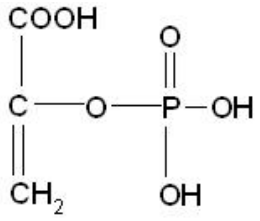


Figure 10.5
Phospho-enol-pyruvate (PEP)

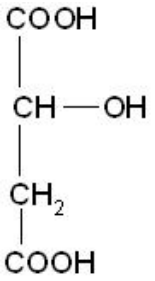


Figure 10.6
Malic acid (monohydroxy-succinic acid) → dicarboxylic acid

Fixation of carbon dioxide in CAM plants

Crassulaceae Acid Metabolism (CAM) is typical in members of the family Crassulaceae (Figure 10.8), but also in cacti (family Cactaceae) and other succulents, including representatives of the family Euphorbiaceae (Figure 10.7) and the genus *Sempervivum*. Here the process is separated in time (e. g. during the night the stomata are open); the chloroplast-containing cells store CO_2 as malic acid in the vacuole, which results in an acidic pH of the cell sap.

Figure 10.7
Euphorbia monteiroi (Angola)

Figure 10.8
Crassula falcata (South-Africa)

10.2 Di- and polysaccharides, role, usage

Saccharose / sucrose (beet sugar, cane sugar)

- the most important disaccharide
- saccharose = α-D-glucose-1,2-β-D-fructose (α-D-glucopyranosyl-β-D-fructofuranose)
- α-D-glucopyranose and β-D-fructofuranose units: 1,2-glycosidic bond

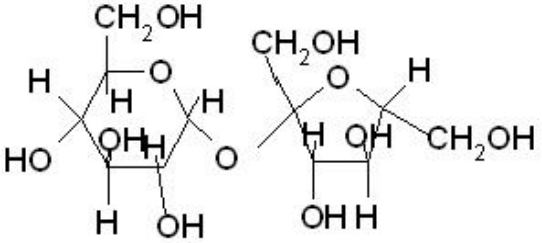


Figure 10.9
Chemical structure of sucrose (saccharose)

- basic component of syrups, important vehicle and flavouring compound
- sources: sugar beet (*Beta vulgaris*), sugar-cane (*Saccharum officinarum*)

- **inverted sugar**: 1 : 1 mixture of glucose (grape sugar, dextrose) and fructose (fruit sugar, laevulose);
- it is formed from saccharose → by dilute acidic hydrolysis, or by the enzyme invertase
- industrial sugar → used after purification in food industry, and in the falsification of honey!

$$\text{saccharose} + H_2O \xrightarrow[\text{invertase}]{H^+} \text{glucose} + \text{fructose}$$

$$(C_{12}H_{22}O_{11}) \qquad\qquad 2\ C_6H_{12}O_6$$

Figure 10.10
Simplified equation of sugar invertation

Sugar alcohols

Sugar alcohols are formed by reduction of monosaccharides, e.g. D-sorbitol is formed from D-glucose (Figure 10.11). D-sorbitol frequently occurs in members of the Rosaceae family, e.g. *Malus* sp., *Pyrus* sp., *Prunus* sp., and enhances peristaltic action of the bowels.

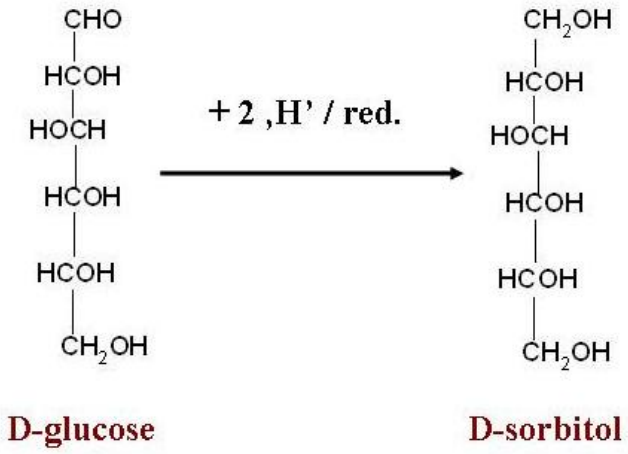


Figure 10.11
D-glucose and D-sorbitol

In plants **ascorbic acid (vitamin C)** is formed from D-sorbitol, e.g. in *Rosae pseudofructus* (rose hips, Figure 10.12). The jam prepared from rose hips contains pectin, too, which contributes to the mild laxative activity of the jam.

Figure 10.12
False fruit of *Rosa canina*

Reduction of D-mannose yields D-mannitol (Figure 10.13), which is the effective substance of *Manna* (source plant: *Fraxinus ornus*, Oleaceae).

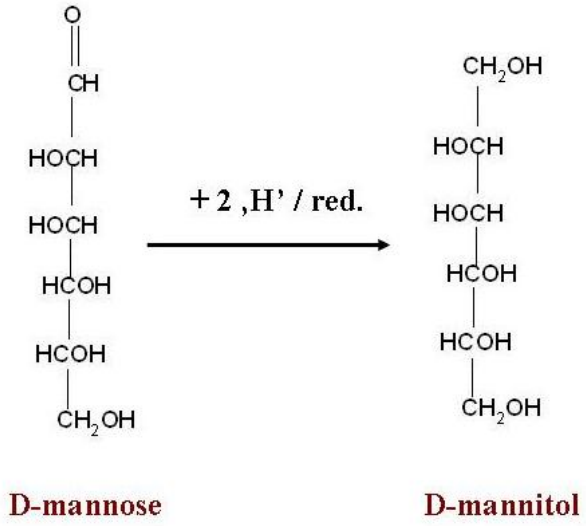


Figure 10.13
D-mannose and D-mannitol

Polysaccharides

- **in water:**
  - form colloid solutions
  - cannot be dissolved in water
- **Classification on the basis of their functions:**
  - reserve nutrients
  - structural components in plant cell wall
  - osmotic regulators
- **According to their chemical structure:**
  - homo-polysaccharides (homoglycanes)
  - hetero-polysaccharides (heteroglycanes)

Reserve nutrients

***Starch* (amylum):** amylose + amylopectine;

- total hydrolysis (boiling in solution with mineral acids) → D-glucose (homoglycane)
- **amylose**: glucose units linked by α (1→4) bonds; linear, unbranched chain with a helical structure,1 spiral unit: 6 glucose molecules (Figure 10.14)
- with I_2 (iodine) it forms an inclusion-complex with intensive blue colour – stability decreases at higher temperature

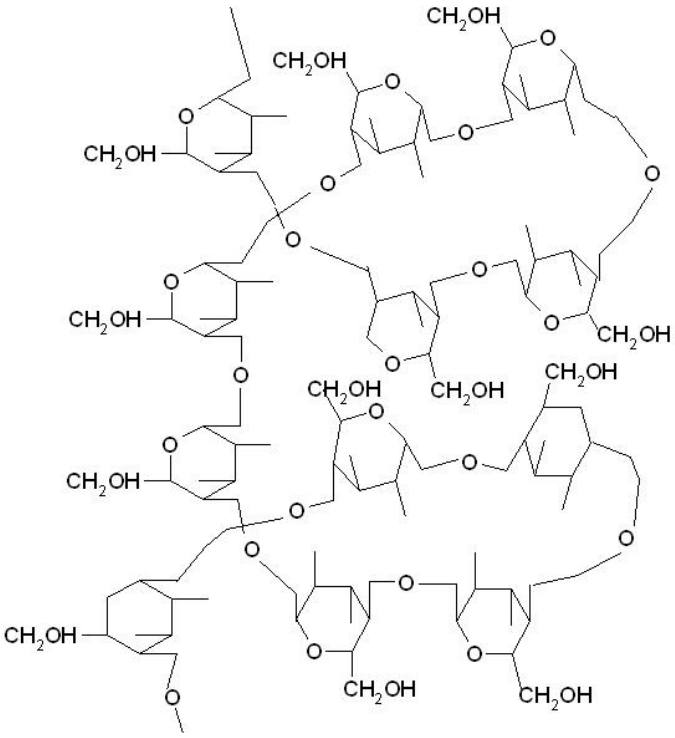


Figure 10.14
Structure of amylose

- **amylopectin:** glucose units are connected by α(1→4)-bonds, but per 10-20 glucose units there are α(1→6)-branches, too (Figure 10.15)

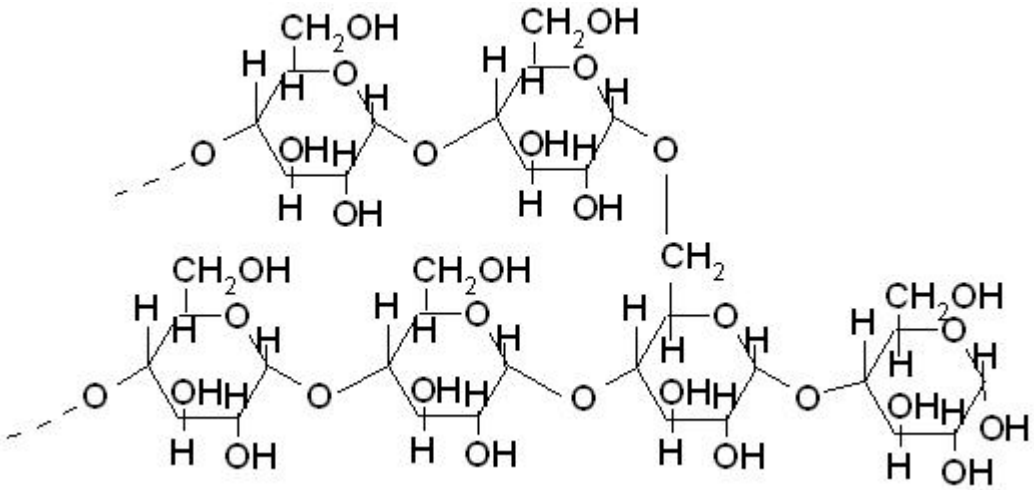


Figure 10.15
Structure of amylopectin

- partial hydrolysis results in **maltose**: 2 D-glucose-units connected by α-glycosidic bond (Figure 10.16)

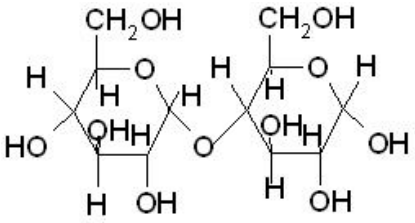


Figure 10.16
Chemical formula of maltose

Hydrolysis of starch: enzyme *α-amylase* (saliva, pancreas) cleaves the α(1→4) bonds → maltose + glucose + dextrin, containing α(1→6) bonds (the hydrolysis of dextrin is catalyzed by the enzyme α(1→6)-glycosidase)

- **refined starch:** tablets and powders (talcums), e.g.:
  - *Solani amylum* (potato starch),
  - *Tritici amylum* (wheat starch),
  - *Oryzae amylum* (rice starch)
- **maltodextrines:** formed during an enzymatic hydrolysis, and after refining used in the food industry (e.g.: instant cacao, coffee)
- **inulin** ← D-fructose-units – β (2→1) bonds; e.g. Jerusalem artichoke (*Helianthus tuberosus*) → fructose syrup; purified inulin used as excipient (binding material) in tablets

Structural / Cell-wall carbohydrates

Cellulose

- nearly half of the organic materials of the biosphere
- the main constituent of plant fibres
- the **purest cellulose** is **scoured cotton** (*Lana gossypii*) from the seed hairs of cotton (*Gossypium hirsutum*, Malvaceae, Figure 10.17)

Figure 10.17
Gossypium hirsutum (cotton)

- structure of cellulose: D-glucose-units connected by β (1 → 4) bonds (Figure 10.18)

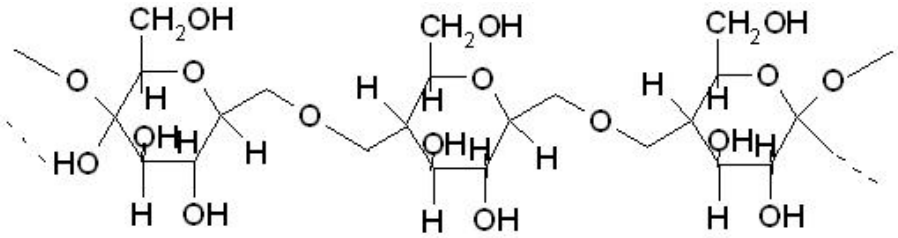


Figure 10.18
Chemical structure of cellulose

- 3000-10 000 glucose-units, M ≈ 500 000 – 1 500 000
- insoluble in water
- hydrolysis: **cellulose** $\xrightarrow[\textbf{\textit{cellulase}}]{H_2O/H^+}$ **cellubiose** (Figure 10.19)

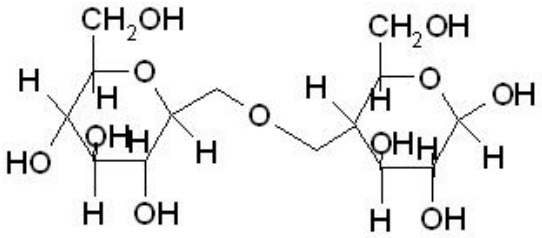


Figure 10.19
Chemical structure of cellobiose

Characteristics of cellulose

- cellulose chains stabilized by **H-bonds (H-bridges)** between C-3 OH groups and ring O-atoms
- chains arranged as **parallel bundles,** between them H-bonds
- **X-ray structure-analysis:** a high degree of regularity → mechanical stability
- ***enzym cellulase*:** produced by bacteria, fungi – able to utilize cellulose
- in the stomach of ruminants: protozoa help the digestion of cellulose (originating from plants, fodder)
- **cellulose can be accompanied by:**
  - **lignin:**
    - in the lignified (woody) secondary cell wall; consisting of polymerized aromatic alcohols, too
  - **xylane:**
    - D-xylose with β (1→4) bonds
    - important skeleton material, giving the hardness of woods (e.g.: corn-stalk, corn-cob)

Pectins

- they are present in the soft tissues of plants (e.g.: fruits, Figure 10.20)
- their building stones are: **D-galactose** and **L-arabinose**
- they dissolve in boiling water, after cooling down form gel
- employment: food industry

Figure 10.20
Cerasus avium (sweet cherry)

Agar-agar

- polysaccharide (Figure 10.21) of marine algae containing sulphate and phosphate groups
- it dissolves in boiling water
- at room temperature forms a gel, which solidifies
- employment: culture medium of bacteria , electrochemistry (salt-bridge), electrophoresis (excipient)

Figure 10.21
Agar

Mannanes

- polymers of **D-mannose**, formed by β (1→4) bonds
- occurrence: higher plants (e.g.: kernel of stone-fruits, e.g. *Prunus domestica*, Figure 10.22), lignified part of conifers
- in yeasts: 1→2, resp. 1→3 branched mannanes

Figure 10.22
Prunus domestica (plum)

Gums

- *Acaciae gummi:* acacia (gum arabic) ← *Acacia senegal* (Mimosaceae, Figure 10.23)
  constituents: D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid
- *Tragacantha* ← *Astragalus gummifer* (*Fabaceae*)
  employment: glues, emulgeating agents and stabilizing materials in the preparation of drugs

Figure 10.23
Acacia senegal (gum acacia)

Heteropolysaccharides

Gluco- and galacto-mannanes, xylanes, rhamnogalacturonanes

- their solutions are viscous, but not sticky
- action: form a protective layer on mucous membrane
- immunostimulants in the case of respiratory and gastro-intestinal diseases
- occurrence: plant mucilages

A few examples for mucilage-containing drugs are shown in Figure 10.24-30.

Figure 10.24
Lichen islandicus (Iceland moss)

Figure 10.25
Althaeae radix (marshmallow root)

Figure 10.26
Malvae folium (mallow leaf)

Figure 10.27
Malvae flos (mallow flower)

Figure 10.28
Plantaginis lanceolatae folium (ribwort plantain leaf)

Figure 10.29
Farfarae folium (colt's-foot leaf)

Figure 10.30
Lini semen (flax seed or linseed)

E. Minker (1996): a number of water-soluble polysaccharides show antiphlogistic and gastroprotective action by intraperitoneal administration.

A few examples for **drugs containing water-soluble polysaccharides** are shown in Figure 10.31-33.

Figure 10.31
Matricariae flos (matricaria / chamomile flower)

Figure 10.32
Tiliae flos (lime flower)

Figure 10.33
Cucurbitae semen (pumpkin seed)

Chapter 11
Synthesis, role and usage of lipids. Oils and waxes

The majority of lipids **dissolve in nonpolar solvents** (chloroform, ether, CCl_4), because they are **hydrophobic**.

11.1 Biological functions

(1) reserve sources of energy
(2) building stones of cell membranes
(3) isolation and mechanical protection
(4) hormones, vitamins → regulation of metabolic processes

11.2 Classification on the basis of their reaction with bases (alkali)

(1) not saponifiable lipids
- terpene- and carotenoid hydrocarbons
- steroids (stigmasterine and sytosterine)
- liposoluble vitamins (D, E, K, A)
- prostaglandins

(2) saponifiable lipids
- simple lipids: neutral fats, plant oils, waxes
- combined lipids: phosphoglycerides, sphyngolipids, glycolipids

the special transformation of fatty acids results polyalkines (polyacetylenes)

Saponifiable combined lipids

(1) Phosphoglycerides

- fundamental compound: **L-α-phosphatidic acid** (Figure 11.1)

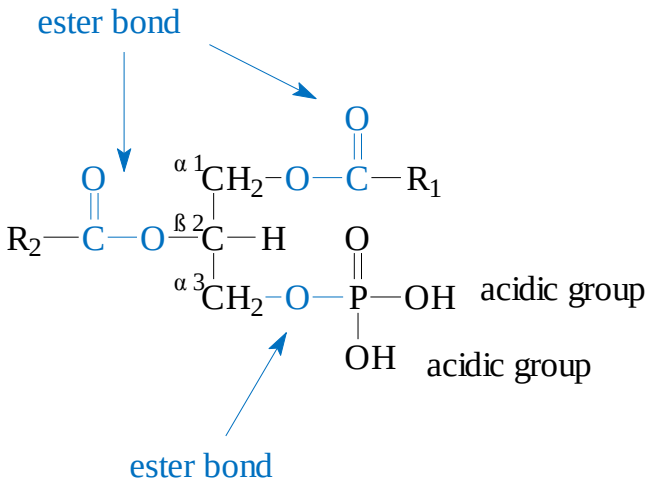


Figure 11.1
L-α-phosphatidic acid

- further alcohol components can bind to the phosphoric acid molecule part by ester bonds
- if the alcohol is **cholamine** (ethanolamine = 1-amino-2-hydroxy-ethan, Figure 11.2), the forming phosphoglyceride is **cephaline** (phosphatidyl-ethanol-amine, Figure 11.3)

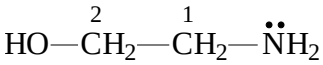


Figure 11.2
Cholamine

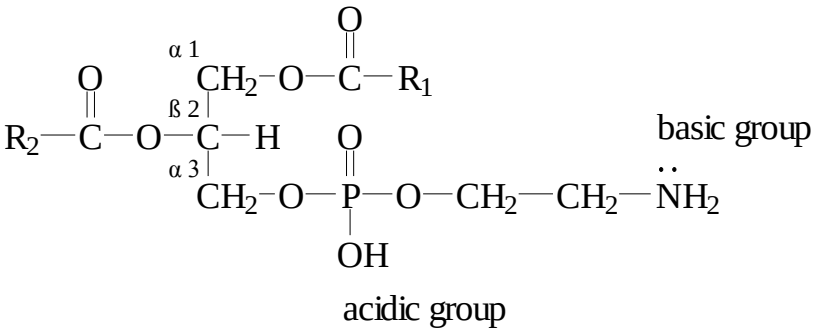


Figure 11.3
Cephaline

- if the alcohol component is **choline** (trimethyl-2(or β)-hydroxyethyl-ammonium-hydroxide, Figure 11.4), the forming phosphoglyceride is **lecithin** (Figure 11.5)

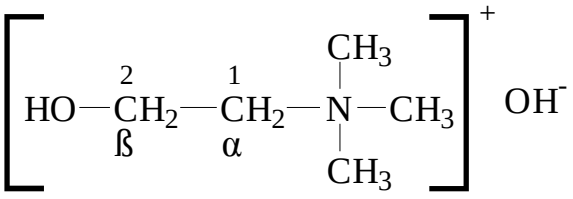


Figure 11.4
Choline

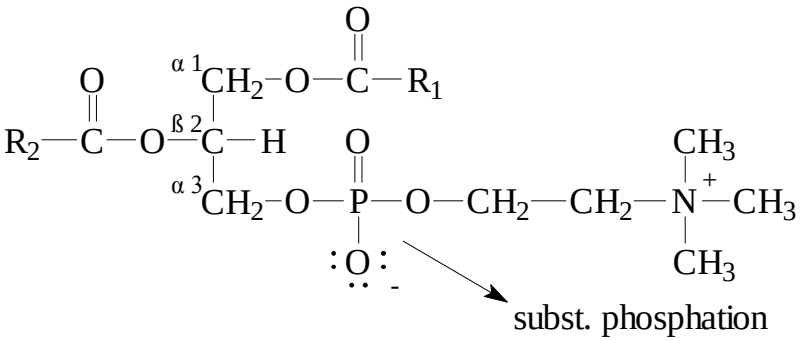


Figure 11.5
Lecithin

- **cephaline and lecithin**: the main building stones of membranes in animal and plant cells
- cephaline occurs in the lipoid materials of the brain
- the egg-yolk contains lecithin in high quantities
- from soybean oil: **soya-lecithin** → it is used by food- and pharmaceutical industry as emulgent and emulsion stabilizing material
- if the alcohol component is **serine** (α-amino-β-hydroxy-propionic acid, Figure 11.6), **phosphatidyl-serine** (Figure 11.7) will be formed

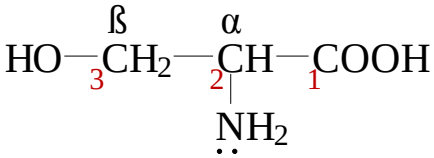


Figure 11.6
Serine

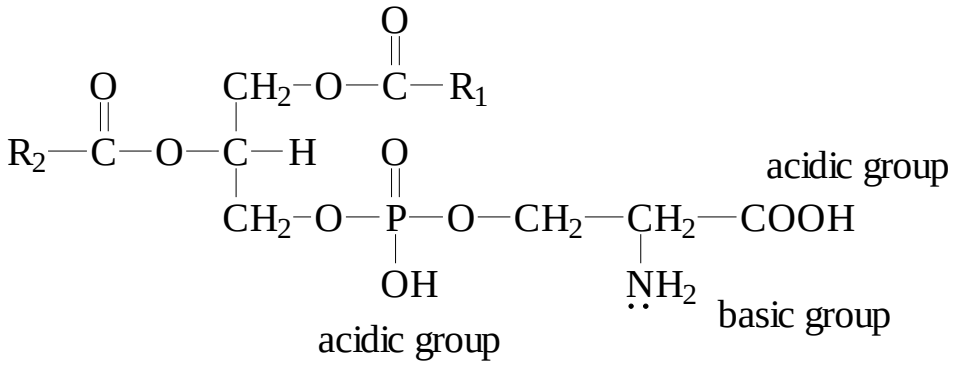


Figure 11.7
Phosphatidyl-serine

- if the alcohol component is **inositol** (Figure 11.8), **phosphatidyl-inositol** (Figure 11.9) will be formed

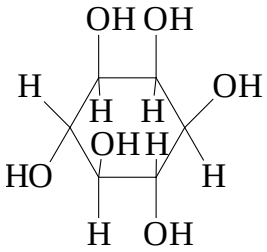


Figure 11.8
Inositol (hexa-hydroxy-cyclohexane)

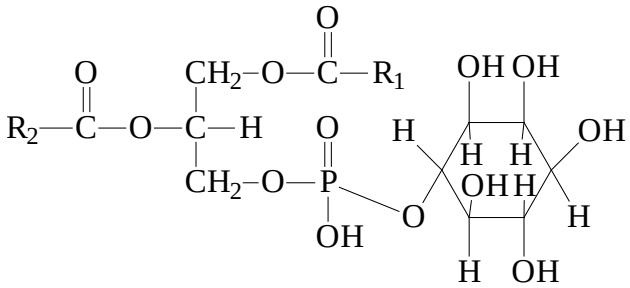


Figure 11.9
Phosphatidyl-inositol

- **phosphatidyl-serine and phosphatidyl-inositol:** building blocks of cell membranes
- **phosphatidyl-inositol:** important role in signal transformation, activated by hormone receptors → secondary messenger molecules will be formed

(2) Sphyngolipids

- occurrence: in the membrane of plant and animal cells; a fundamental compound is ***sphyngosine*** = unsaturated amino-diol with long (C18) carbon-chain (Figure 11.10)

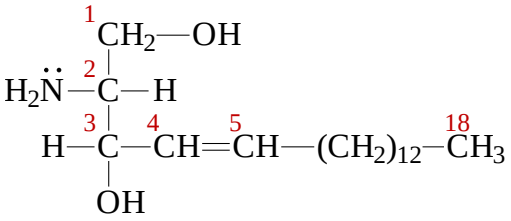


Figure 11.10
Sphyngosine

(3) Glycolipids

- sugar- or sugaralcohol-molecules connect to one of the OH groups of glycerol by **ether-bond**; the other two OH groups are **esterified** by long-chain fatty acids
- **mono- and digalactosyl-diglycerols**: components of photosynthetic membranes

Saponifiable simple lipids

(1) Fats and fatty oils

- esters of fatty acids formed with glycerol
- ***solid*** state of matter (fat): the esterifying acid components are saturated
- ***liquid*** state of matter (fatty oil): the esterifying acid components are unsaturated
- place of biosynthesis: **chloroplast**, but the enzymes *elongase* and *desaturase* work in the **cytoplasm**
- fatty acids are formed from acetyl-CoA and malonyl-CoA (Figure 11.11), and they are always even- numbered:

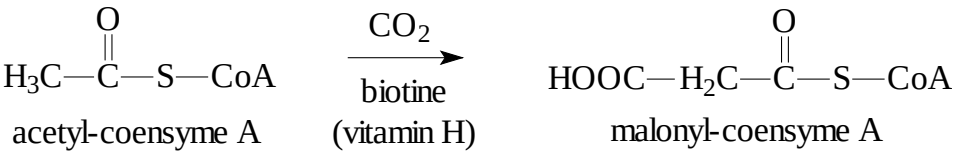


Figure 11.11
Acetyl-CoA and malonyl-CoA, precursors of fatty acids

- malonyl-CoA can be formed also from **oxalacetic acid**; the reaction is catalysed by the enzyme *peroxydase in the presence of* Mn^{2+} ions; **malonic acid** is formed as intermedier (Figure 11.12)

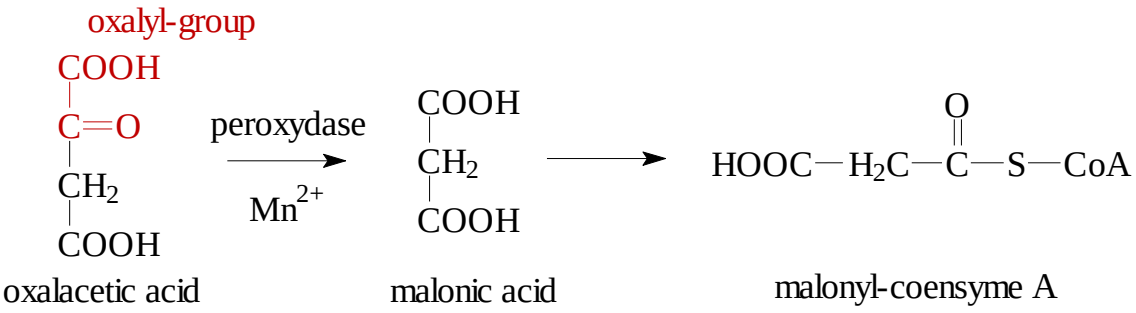


Figure 11.12
Synthesis of malonyl-CoA from oxalacetic acid

- the most commonly occurring **saturated fatty acids** in plants are listed in Figure 11.13.

$CH_3 – (CH_2)_{10} – COOH$ – **laurinic acid** = dodecane acid

$CH_3 – (CH_2)_{12} – COOH$ – **myrystinic acid** = tetradecane acid

$CH_3 – (CH_2)_{14} – COOH$ – **palmitinic acid** = hexadecane acid

$CH_3 – (CH_2)_{16} – COOH$ – **stearinic acid** = octadecane acid

$CH_3 – (CH_2)_{18} – COOH$ – **arachinic acid** = eicosane acid

Figure 11.13
Saturated fatty acids in plants

- the most commonly occurring **unsaturated fatty acids** in plants are shown in Figure 11.14-17.

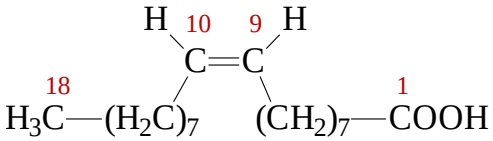


Figure 11.14
Oleic acid (9-*cis*-octadecen acid)

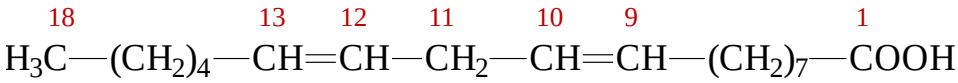


Figure 11.15
Linolic acid (9,12-di-*cis*-octadeca-dien acid)

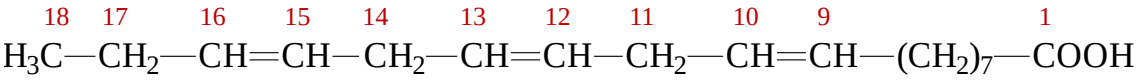


Figure 11.16
α-linoleic acid (9,12,15-tri-*cis*-octadeca-trien acid)

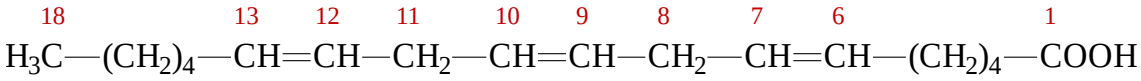


Figure 11.17
γ-linoleic acid (6,9,12-tri-*cis*-octadeca-trien acid)

- **Omega-3 fatty acids** (ω-3 fatty acids or *n*-3 fatty acids) are polyunsaturated fatty acids with a double (C=C) bond at the third carbon atom (in *n*-3 position) from the end of the carbon chain. In **ω-6 fatty acids** this double bond can be found at the sixth carbon atom from the methyl group at the end of the carbon chain. Each double bond is in *cis* position both in ω-3 and ω-6 fatty acids.
- **Omega-3 fatty acids** include α-linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); while **omega-6 fatty acids** contain linolic acid and arachidonic acid.
- The human body is not able to synthesize the above compounds, therefore they are considered **essential fatty acids** and **vitamin-like materials** (vitamin F), and they should be taken with food.
- **Sources of ω-3 and ω-6 fatty acids** include milk, fish, meat, eggs; various plant oils such as linseed oil, soy oil, rapeseed oil, olive oil, sunflower oil, corn (maize) oil, poppyseed oil; as well as oily seeds like nuts and peanuts. The optimal intake of **ω-3 : ω-6 fatty acids** would be 1:3 – 1:5.
- The **omega-3 and omega-6 fatty acids** enhance quality of life and lower the risk of premature death. They function via cell membranes, in which they are anchored by phospholipid molecules. They have a beneficial effect on the cardiovascular system, lowering the level of triglycerides, as well as pulse rate, blood pressure and the risk of atherosclerosis. They are needed for the proper functioning of the immune system, and are valued also for their anti-inflammatory character.
- DHA was proven essential to pre- and postnatal brain development, whereas EPA seems to be more influential on behaviour and mood. Both DHA and EPA generate neuroprotective metabolites (Kidd 2007).
- **Arachidonic acid** is a polyunsaturated omega-6 fatty acid (Figure 11.18-19), which is the starting material of the biosynthesis of prostaglandins

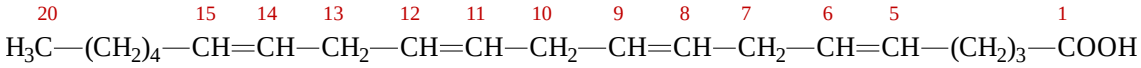


Figure 11.18
Arachidonic acid (5,8,11,14-tetra*cis*-eikosa-tetraen acid)

- its correct structure:

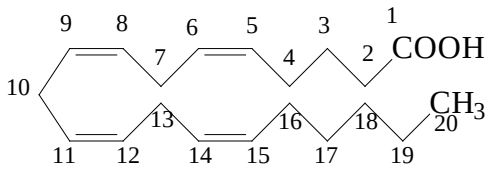


Figure 11.19
Arachidonic acid

- one of the special and rarely occurring representatives of unsaturated fatty acids is **ricinolic acid** (Figure 11.20), which can be found in castor-oil bean (*Ricini semen*, source plant: *Ricinus communis*)

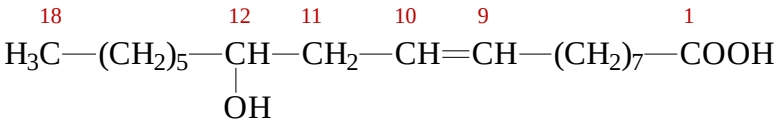


Figure 11.20
Ricinolic acid

- 80-90% of **castor oil** (*Ricini oleum*) is the glycerol-ester of ricinolic acid; the oil is known for its purgative action; **it cannot contain ricinine** (toxic pyridine-derivate, acid nitrile, Figure 11.21) **and ricine** (toxic protein)

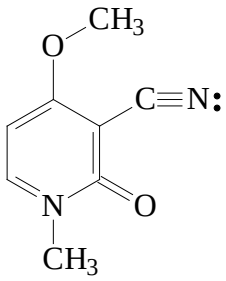


Figure 11.21
Ricinine

- **fats** can be stored as reserve nutrients mainly in seeds, from which the fats and fatty oils are recoverable by pressing for food and pharmaceutical purposes
- fats and fatty oils are insoluble in water and in alcohols, but they are well-soluble in nonpolar solvents (hexane, benzene, ether, chloroform, ethylacetate)
- under the influence of air and light they can be quickly oxydized, and become rancid → during this process **organic peroxy-derivatives** and **free radicals** are formed
- storage: in a cool place, in tightly closed dark (brown) flask → protection from light

- **the alkaline hydrolysis of fats and fatty oils** results the salts of corresponding fatty acids (soaps) and glycerol → **saponification**

Plants containing fats and fatty oils

A few examples are shown in Figure 11.22-31.

Figure 11.22
Amygdalus communis − sweet or bitter almond (Rosaceae)

Figure 11.23
Brassica napus − oilseed rape (Brassicaceae / Cruciferae)

Figure 11.24
Glycine soja – soy(a) bean (Fabaceae)

Figure 11.25
Gossypium hirsutum – mountain cotton (Malvaceae)

Figure 11.26
Seeds with cover hairs from *Gossypium hirsutum* − mountain cotton (Malvaceae)

Figure 11.27
Helianthus annuus − sunflower (Asteraceae / Compositae)

Figure 11.28
Linum usitatissimum − cultivated flax (Linaceae)

Figure 11.29
Olea europaea − common olive (Oleaceae)

Figure 11.30
Fruits of *Olea europaea* – common olive (Oleaceae)

Figure 11.31
Fruits and seeds of *Theobroma cacao* − cacao(tree) (Sterculiaceae)

Plant oils – Olea herbaria (Ph.Eur.)

Virgin oil (Oleum virginale): oil obtained with mechanic processes (cold pressing, centrifugation).

Refined oil (Oleum raffinatum): oil obtained with pressing and/or with solvent (hexane) extraction; followed by chemical refinement (with a base) or physical refinement

Hydrogenised oil (Oleum hydrogenatum): pressing or extraction with solvent, refined chemically or physically; hydrogenised

List of plant oils in Ph.Eur. 6.:

Amygdalae oleum raffinatum – almond oil, refined

Amygdalae oleum virginale – almond oil, virgin

Arachidis oleum hydrogenatum – peanut oil, hydrogenated

Arachidis oleum raffinatum – peanut oil, refined

Carthami oleum raffinatum – safflower oil, refined

Cocois oleum raffinatum – coconut oil, refined

Gossypii oleum hydrogenatum – cotton oil, hydrogenated

Helianthi annui oleum raffinatum – sunflower oil, refined

Lini oleum virginale – flaxseed oil, virgin

Maydis oleum raffinatum – maize/corn oil, refined

Olivae oleum raffinatum – olive oil, refined

Rapae oleum raffinatum – rape oil, refined

Ricini oleum virginale – castor oil, virgin

Sesami oleum raffinatum – sesame oil, refined

Soiae oleum raffinatum – soy oil, refined

Tritici aestivi oleum raffinatum –wheat germ oil, refined

Tritici aestivi oleum virginale –wheat germ oil, virgin

Cod-liver oil

"cod-liver oil" ***(Iecoris aselli oleum)***: oil of animal origin ← melting, cooling and filtering the fatty oil from the **liver of *Gadus morhua***

contains:

- oleic acid (85 %)
- linolic acid
- palmitoleic acid
- **gadolinic acid** (9-*cis* eicosen acid, Figure 11.32)

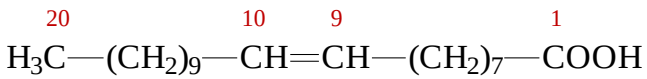


Figure 11.32
Gadolinic acid

- glycerolesters of palmitinic- and myristinic acid
- **vitamin A (retinol)** (Figure 11.33)

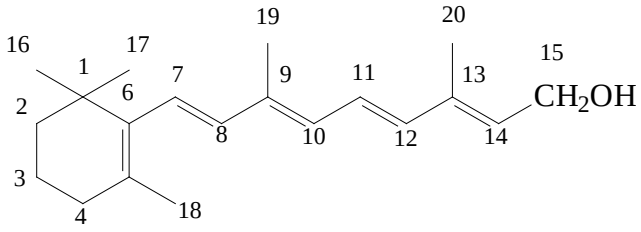


Figure 11.33
Vitamin A (retinol)

- **vitamin D_2 (ergocalciferol)** (Figure 11.34)

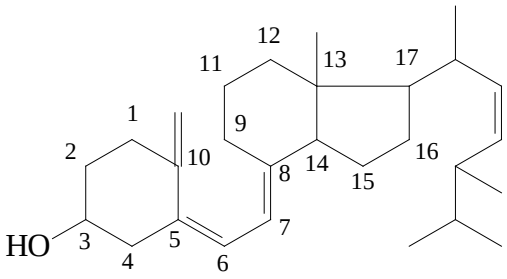


Figure 11.34
Vitamin D_2 (ergocalciferol)

- **vitamin D_3 (cholecalciferol)** (Figure 11.35)

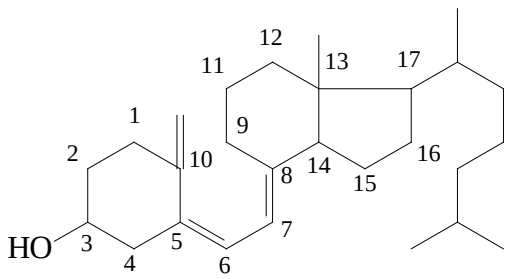


Figure 11.35
Vitamin D_3 (cholecalciferol)

- "cod-liver oil" is used for the prevention and treatment of deficiency diseases (A- and D-avitaminosis), and as component of ointments (creams)

(2) Waxes

- esters of long-chain fatty acids with long-chain monovalent alcohols (wax-alcohols)
- some important wax-alcohols are shown in Figure 11.36.

$CH_3 – (CH_2)_{14} – CH_2 – OH$ – **cetylalcohol**

$CH_3 – (CH_2)_{16} – CH_2 – OH$ – **stearylalcohol**

$CH_3 – (CH_2)_{24} – CH_2 – OH$ – **cerylalcohol**

$CH_3 – (CH_2)_{28} – CH_2 – OH$ – **myricyl- or melissylalcohol**

Figure 11.36
Wax-alcohols

- esterifying acid-components include: *laurinic acid, palmitinic acid, stearinic acid, melissynic acid, myristynic acid*
- waxes are solid; their solubility relations are similar to those of fats, but they dissolve to a lesser degree
- they are less sensitive to oxydation than fats, they are saponifiable
- **therapeutical usage:** basic materials of plasters and ointments, covering of dragées
- some widely used waxes are listed below:
  - *Cera flava* (yellow wax or beeswax)
  - *Cera alba* (white wax)
  - *Cera carnauba* (carnauba wax)
  - *Cetaceum* (its main components: cetyllaurate, cetylmirystate, cetylpalmitate,
  - cetylstearate)

Not saponifiable lipids

Prostaglandins

- discovery: **1935** Euler (Swedish) and Goldblatt (English): → in human sperm cells and in tissues of ram seminal vesicle
- the first isolation in pure crystalline state: Bergström and co-workers in the **1960s**
- They can be found in all living organisms; mainly in animal and human organism, but they occur in plants, too (e.g. *Allium* sp.)
- Prostaglandins are unsaturated fatty acids, containing 2-3 OH groups and a cyclopentene or cyclopentane ring
- the cyclopentene- or cyclopentane-ring is always formed from the C-atoms 8, 9, 10, 11 and 12
- between the C-atoms 13-14 there is a double bond in each case
- to the C-atom C-15 a OH group is bound in each case, having *S* configuration
- on the basis of structure and substitution of cyclopentene and cyclopentane rings
- **6 fundamental types (groups) of prostaglandins** can be distinguished (Figure 11.37-38):

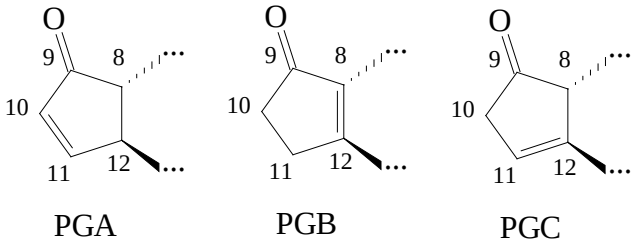


Figure 11.37
Cyclopentene rings

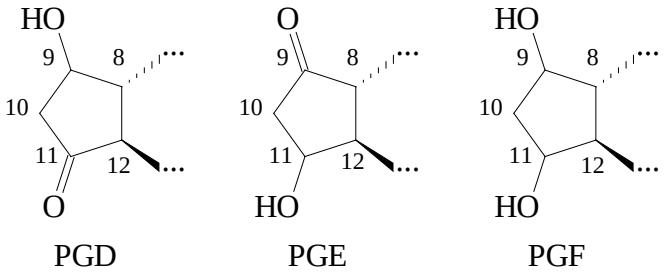


Figure 11.38
Cyclopentane rings

- the **lower index-numbers** (e.g. PGE_2) show the number of double bonds in the molecule:
  - **1**: double bond is between C-13, C-14
  - **2**: double bonds are between C-5, C-6 and C-13, C-14
  - **3**: double bonds are between C-5, C-6 and C-13, C-14 and C-17, C-18
- the carbon chains connected to the rings are arranged in *trans* (*E*) configuration
- **the precursor of prostaglandin-biosynthesis is arachidonic acid** (Figure 11.39)

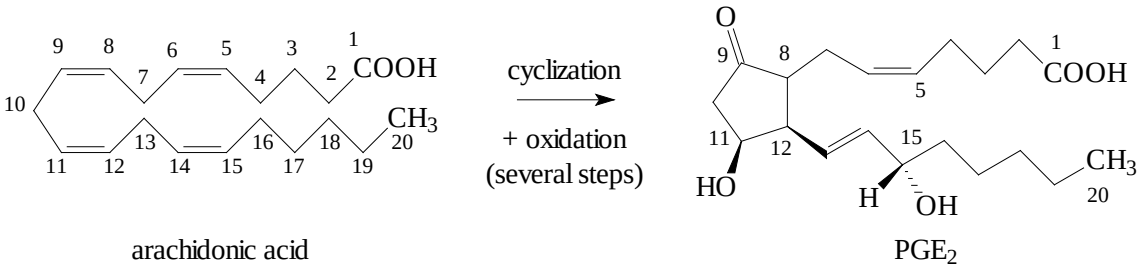


Figure 11.39
Biosynthesis of prostaglandins from arachidonic acid

- prostaglandins are used in therapy, too:
  - **PGE_2**: *Dinoproston* – abortive; derivatives of PGE_2 are used in the treatment of gastrointestinal ulcers, due to their antisecretoric and cytoprotective activity
  - **PGE_1** and **prostacyclin** are applied in disorders of blood circulation, for inhibition of platelet-aggregation and in the case of asthma bronchiale
  - **$PGF_{2\alpha}$**: used in treatment of intestinal diseases
- their biological activity depends on the structure!

Polyalkines (polyacetylenes)

- they are formed from ***fatty acids*** (mainly from linolic acid), through special transformations
- first **polyenes** are formed, from which **polyines** are formed – enhanced by different *desaturase* enzymes
- their structure is often linear, but they can form rings; in this case they form spiroether and thiophen-derivatives with oxygen and sulphur atoms, respectively
- they are ***phototoxic***: on the effect of **UV-A irradiation** their electron system gets into excited state → they become toxic
- they are important **resistance factors**, play a role in tolerance; transported to the root system and to the shoots they hinder the proliferation of microfungi (e.g. *Candida*), bacteria and viruses
- **effective compounds of several medicinal plants are polyalkine-derivatives,** e.g.: *Arctium lappa* (greater burdock), *Echinacea* species
- a number of toxic plants cause **contact dermatitis or allergy** due to the photoactivated compounds of polyalkines, e.g.: *Heracleum sphondylium* (pigweed), *Oenanthe* sp. (water-dropwort, hemlock), *Cicuta* sp. (water-hemlock, cowbane), *Hedera helix* (ivy, Figure 11.40)

Figure 11.40
Hedera helix (ivy)

Chapter 12
Biosynthesis and classification of terpenoids

12.1 Compounds having isoprene skeleton

- **isoprene-principle** is manifested in their structure → they look like polymers of **isoprene (2-methyl-1,3-butadiene)** (Figure 12.1) → Wallach (1883)
- Ruzička (1921): the connection of isoprene units is well-regulated

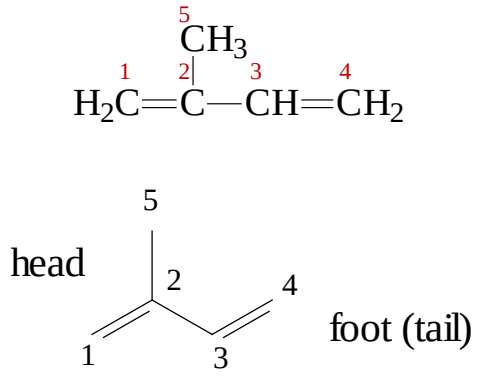


Figure 12.1
Isoprene

- isoprene is the building block of naturally occurring polyisoprenes (caoutchouc, guttapercha), terpenes, carotenoids and steroids

Connection types of isoprene units

Various **types of the connection of isoprene units** are demonstrated in Figure 12.2-6.

(a) ***linear***

1 - 4 (or 4 – 1) connection: head-foot

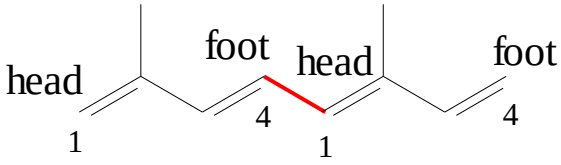


Figure 12.2
Head-foot connection of isoprene units

4 - 4 connection: foot-foot

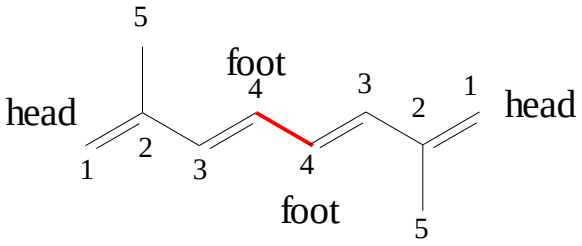


Figure 12.3
Foot-foot connection of isoprene units

(b) *cyclic*: mono- and bicyclic terpenes (Figure 12.4):

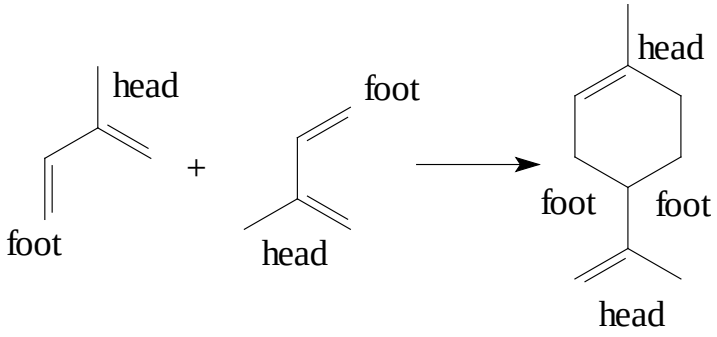


Figure 12.4
Limonene or dipentene (fundamental compound of monocyclic monoterpenes)

caoutchouc : natural **poly-isoprene** having all-*cis* connections (Figure 12.5-6):

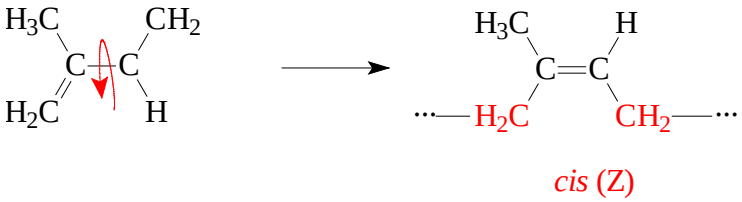


Figure 12.5
Poly-isoprene structure with all-*cis* connections

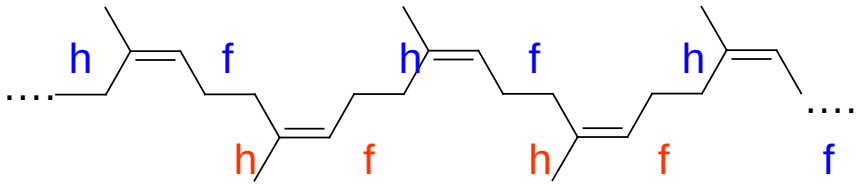


Figure 12.6
Structure of caoutchouc

- caoutchouc is a substance with thread-like structure containing about 40 000 isoprene units; it occurs in the milky sap (*latex*) of the caoutchouc tree (*Hevea brasiliensis*); can be precipitated with acids
- **rubber: vulcanized caoutchouc** → the molecule chains are kept together by S-bridges (Figure 12.7):

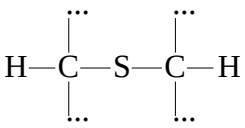


Figure 12.7
Structure of vulcanized caoutchouc

- artifical caoutchouc: ***polyisoprene*** (Figure 12.8)

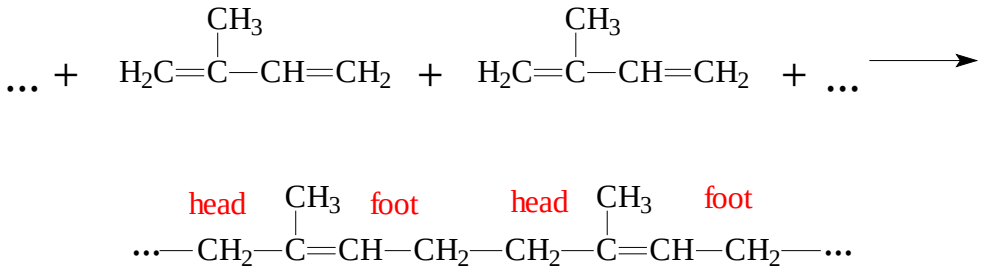


Figure 12.8
Polyisoprene structure of artificial caoutchouc

guttapercha: naturally occurring polyisoprene having all-*trans* configuration (Figure 12.9)

- its chain has an almost elongated structure
- not elastic
- the connection of isoprene units is **foot – foot**, or **head – head**:

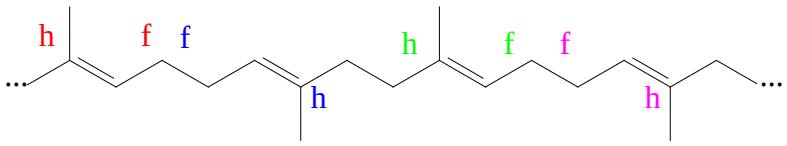


Figure 12.9
Guttapercha

12.2 Terpenes (terpenoids)

Terpenoids are hydrocarbons and derivatives containing oxygen, having $(C_5)_n$ carbon atoms, isoprene units; isolated generally from volatile oils of plant origin.

Biosynthesis

- from **isopentenyl-pyrophosphate** (= **IPP**; active isoprene), which is formed from mevalonic acid (Figure 12.10) by repeated phosphorylation, decarboxylation and by loss of water

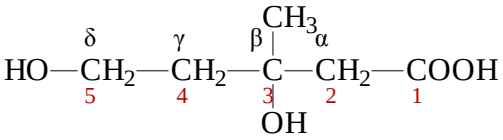


Figure 12.10
Mevalonic acid
(3,5-dihydroxy-3-methyl-pentanoic acid = β,δ-dihydroxy-β-methyl-valerianic acid)

- the biosynthesis of **mevalonic acid,** as precursor of isoprenoids, starts from **acetyl-coensyme A**, similarly to the biosynthesis of fatty acids
- fundamental difference: from acetyl-coensyme A not malonyl-coensyme A is formed, but **2 mols of acetyl-coensyme A** are condensed under the catalytic effect of the enzymes *acyl-transpherase* and *thiolase*, resulting in **aceto-acetyl-coensyme A** (Figure 12.11)

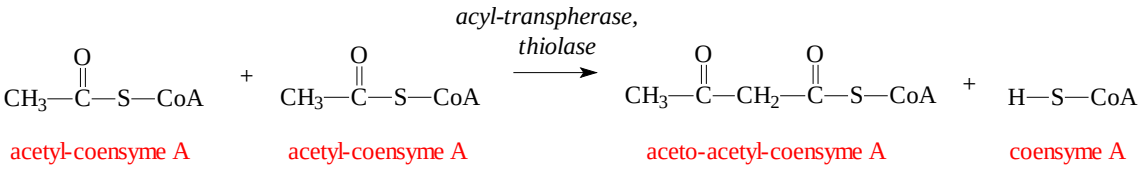


Figure 12.11
Synthesis of aceto-acetyl-coensyme A

- the process mentioned above is the first step of the biosynthesis of fatty acids;
- from this step branches the biosynthesis of isoprenoids (terpenes, carotenoids, steroids) into the direction of the formation of mevalonic acid
- the first step from the fatty acid cycle branching into the direction of mevalonic acid is the special, enzyme-catalyzed *addition reaction* of aceto-acetyl-coensyme A with a new molecule of acetyl-coensyme A (Figure 12.12)
- This reaction is followed by hydrolysis, which results in the formation of **H – S – CoA**

(coensyme A) and **β-hydroxy-β-methyl-glutaryl-CoA (HMG-CoA)**:

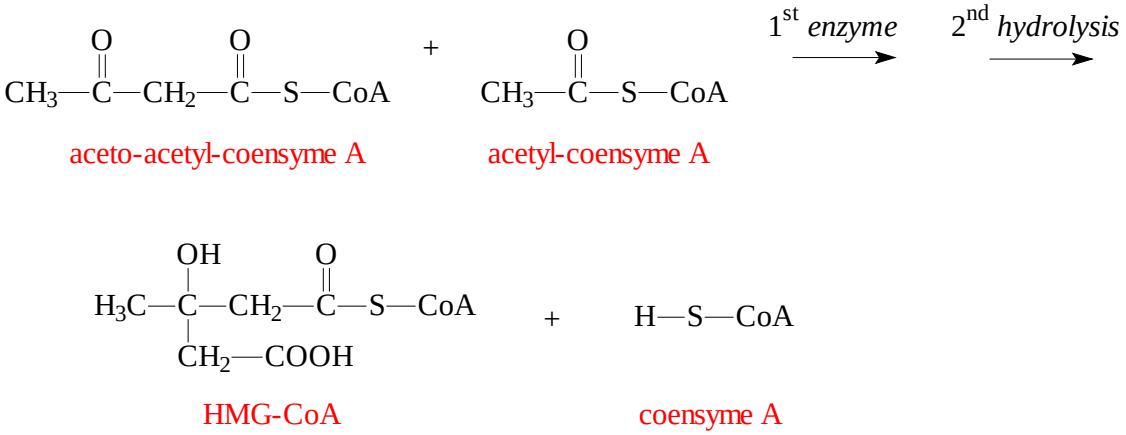


Figure 12.12
Addition reaction of aceto-acetyl-CoA with acetyl-CoA, followed by hydrolysis

HMG-CoA will be transformed into **(3*R*)-mevalonic acid (MVA)** with the catalysis of the HMG-CoA-reductase enzyme (Figure 12.13)

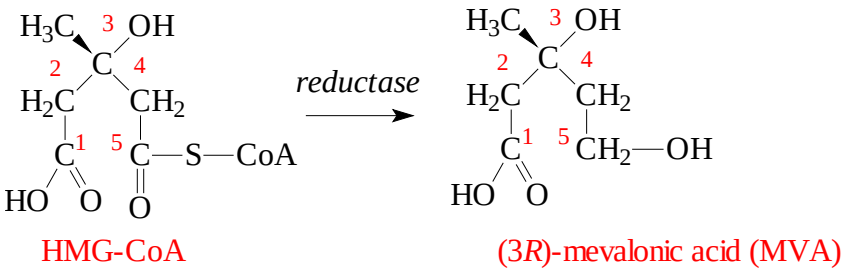


Figure 12.13
The reaction catalyzed by *HMG-CoA reductase*

- In the next step of the reaction, the primary alcoholic OH-group of **MVA** is phosphorylated by **ATP**, so **MVA-pyrophosphate** will be formed. This reaction is followed by loss of water and decarboxylation, resulting in the formation of isopenthenyl-pyrophosphate (**IPP**), which is considered as "active isoprene" (Figure 12.14-15).

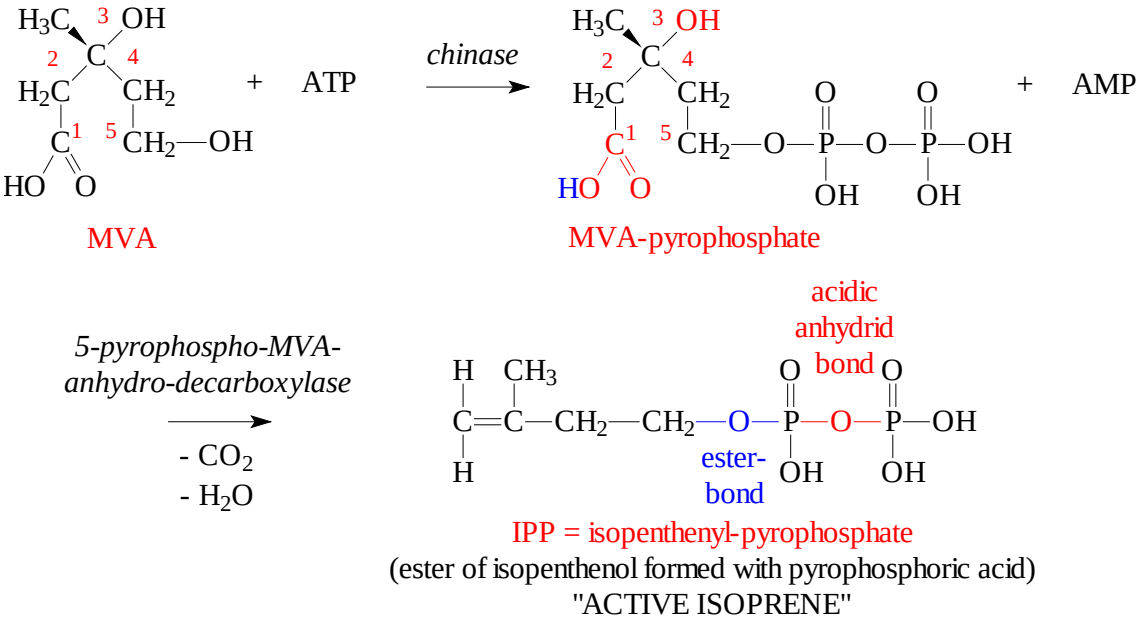


Figure 12.14
Phosphorylation of MVA, followed by decarboxylation

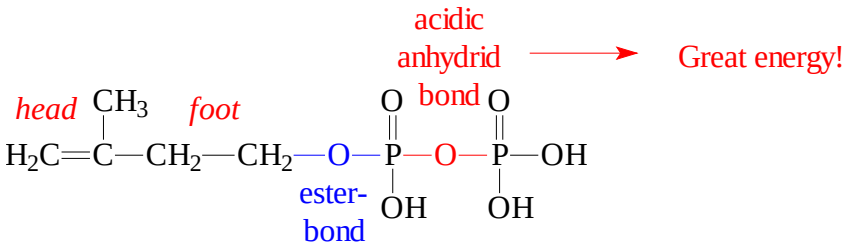


Figure 12.15
isopentenyl-pyrophosphate (IPP)

IPP can then be isomerized to dimethyl-allyl-pyrophosphate (DMAPP) by the enzyme isopentenyl pyrophosphate isomerase. IPP and DMAPP will form geranyl-pyrophosphyate (a C10 molecule). With an additional IPP molecule, pharnesyl-pyrophosphate (C15) can be formed (Figure 12.16).

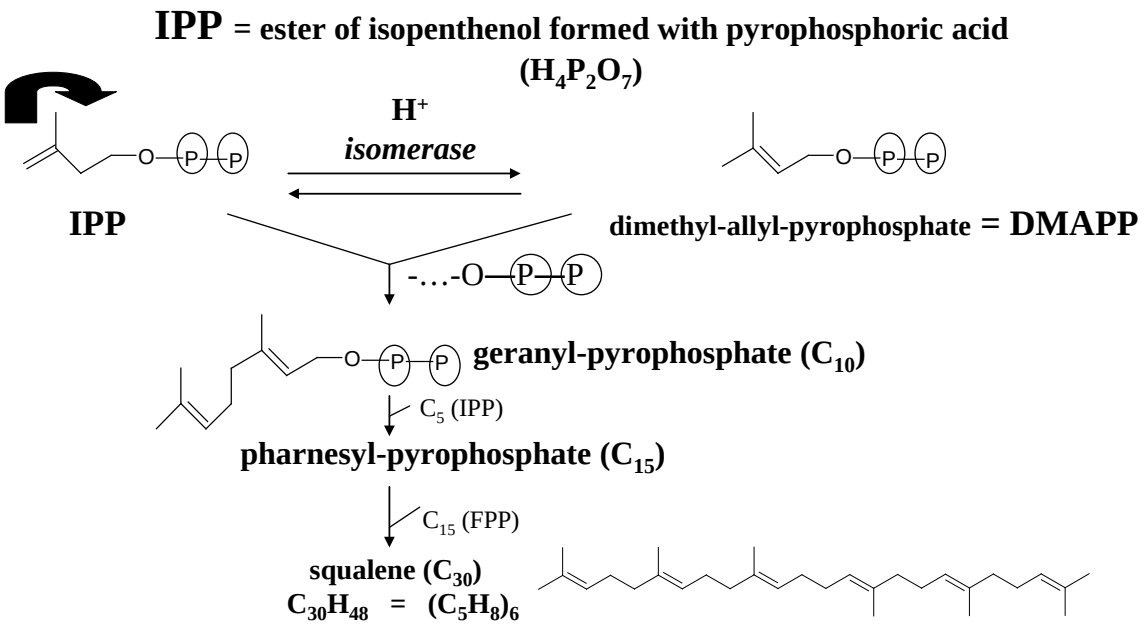


Figure 12.16
Formation of dimethyl-allyl-pyrophosphate and geranyl-pyrophosphate

Classification of terpenes

Terpenes can be classified based on the number of their isoprene units (Table 12.1).

Table. 12.1. Classification of terpenes

Name	Number of isoprene units	Formula of fundamental hydrocarbons
Monoterpenes	**2**	**2 x C_5H_8**
Sesquiterpenes	**3**	**3 x C_5H_8**
Diterpenes	**4**	**4 x C_5H_8**
Triterpenes	**6**	**6 x C_5H_8**
Tetraterpenes, Carotenoids*	**8** **8**	**8 x C_5H_8** ***$C_{40}H_{56}$**
Polyterpenes	**n**	**n x C_5H_8**

* Containing more double bonds than the corresponding tetraterpenes

(1) Monoterpenes

Classification of monoterpenes based on their structural properties

Monoterpenes can be open-chain or cyclic; they are mostly components of volatile oils.

(a) Open-chain monoterpenes

Myrcene (Figure 12.17) and ocymene (Figure 12.18) occur in the volatile oil of laurel/bay tree (*Laurus nobilis*).

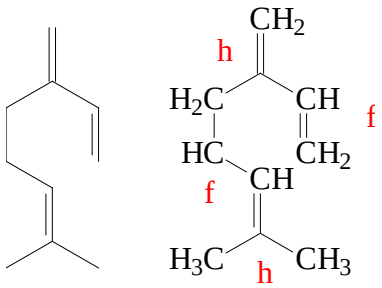


Figure 12.17
Myrcene

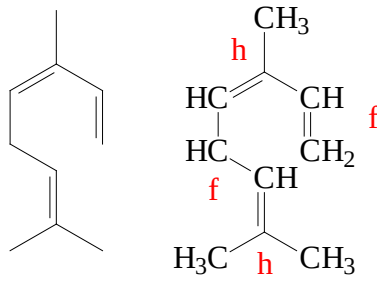


Figure 12.18
Ocymene

Geraniol (Figure 12.19) can be detected e.g. in rose (*Rosa* sp.) oil and geranium oil (*Geranium* and *Pelargonium* sp., e.g. *P. odoratissimum*).

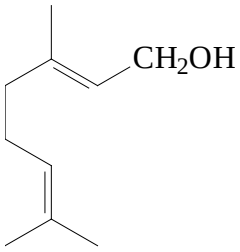


Figure 12.19
Geraniol

(b) Monocyclic monoterpenes

Cymene (Figure 12.20) can be detected in the essential oil of eucalyptus (*Eucalpytus*) and cypress (*Cupressus*) species. P-cymene was first isolated from cumin (*Cuminum cyminum*), hence the name cymene. P-menthane (Figure 12.20) can also occur in eucalyptus oil.

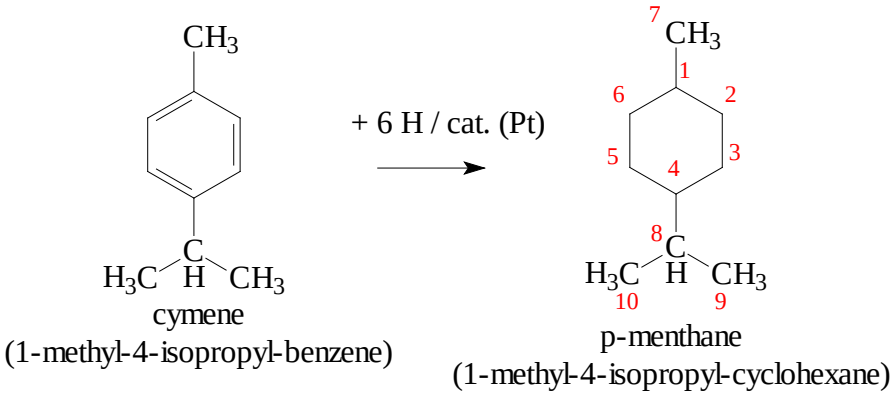


Figure 12.20
p-cymene and p-menthane

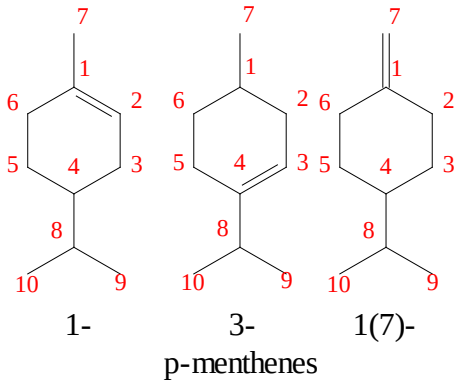


Figure 12.21
P-menthenes

Limonene (Figure 12.22) occurs in the volatile oil of lemon (*Citrus limon*) peel.

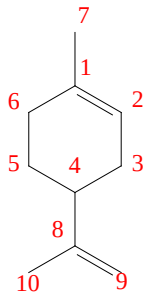


Figure 12.22
Δ - 1,8 – para-menthadiene = limonene = dipentene

α-terpinene (Figure 12.23) is one of the components of tea tree (*Melaleuca*) oil, responsible for its antioxidant activity.

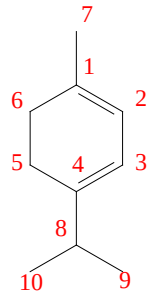


Figure 12.23
α-terpinene

Thymol (Figure 12.24) is a characteristic compound in thyme (*Thymus*) essential oil, with antiseptic and antifungal activity. Menthol (Figure 12.24) can be detected in mint (*Mentha*) oil, with antimicrobial and refreshing features.

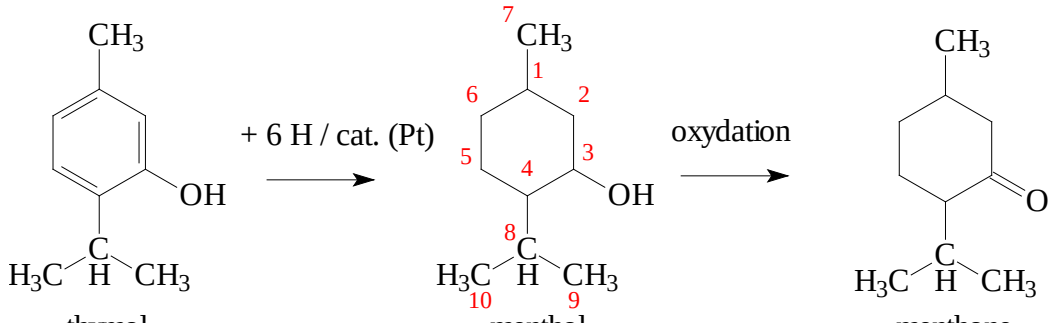
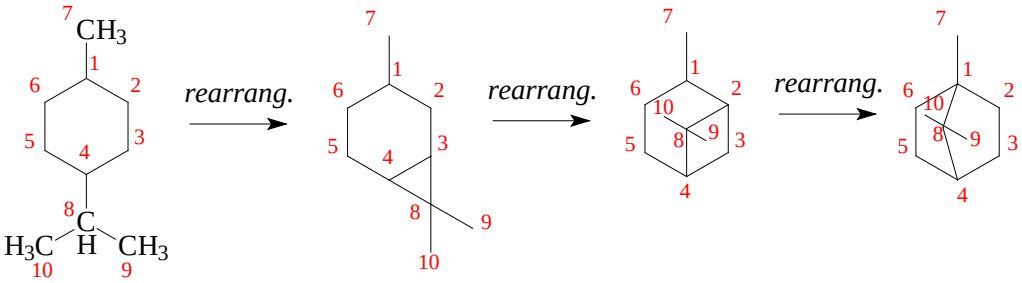


Figure 12.24
Thymol, menthol (3-hydroxy-p-menthane) and menthone

(c) Bicyclic monoterpenes

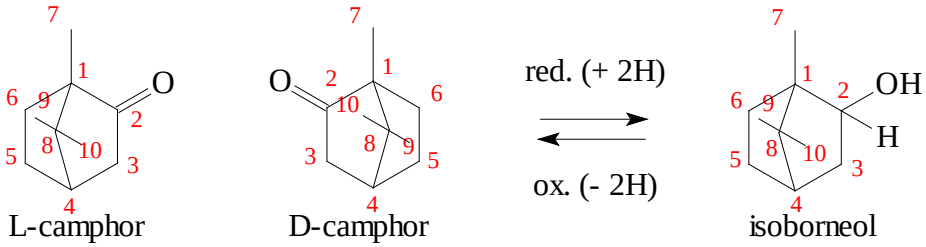
Examples include pinenes, carene, camphene, sabinene.



p-menthane (1-methyl-4-isopropyl-cyclohexane) — carane — pinane — camphane

Figure 12.25
Basic skeletons of bicyclic monoterpenes

D-camphor (Figure 12.26-27) is a characteristic compound of the camphor tree (*Cinnamomum camphora*), with refreshing, antiseptic and analgesic properties.



L-camphor — D-camphor — isoborneol

Figure 12.26
D-camphor and isoborneol

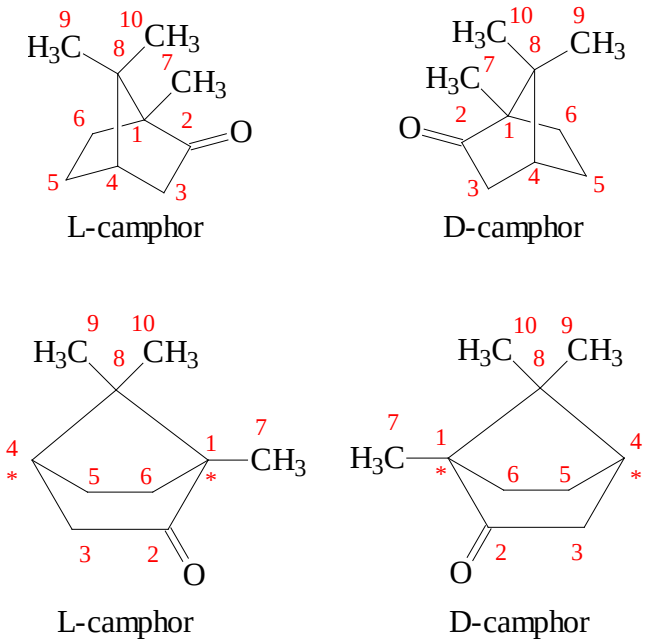


Figure 12.27
Possible conformations of camphor

- although camphor contains two differently saturated chiral centers, it has **only a single pair of diastereoisomers**
- reason: the dimethyl-methylene bridge can be attached only in *cis*-position to the cyclohexane skeleton, which will be forced into the **bathtub conformation**

Pinenes (Figure 12.28) are characteristic compounds in the essential oils of pines (*Pinus* sp.), e.g. the Scots pine (*P. sylvestris*).

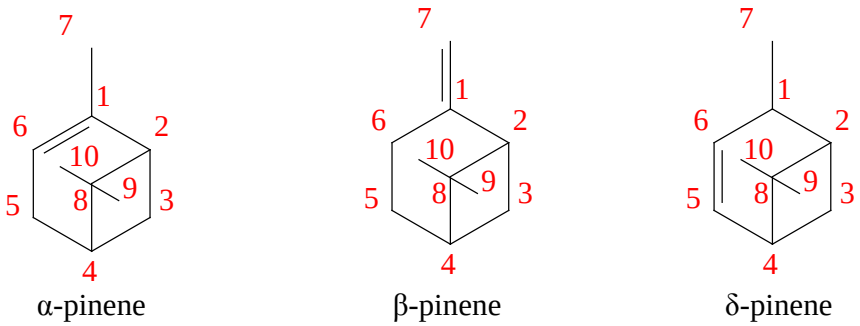


Figure 12.28
Pinenes

Carene (Figure 12.29) occurs in the volatile oil of *Pinus longifolia*, a pine species native to India.

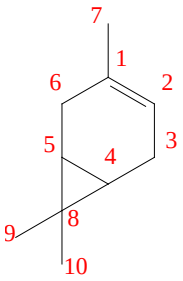


Figure 12.29
Carene

Camphene (Figure 12.30) can be found in the volatile oil of Siberian fir (*Abies sibirica*).

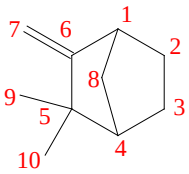


Figure 12.30
Camphene

Sabinene (Figure 12.31) is characteristic in the volatile oil of savin juniper (*Juniperus sabina*).

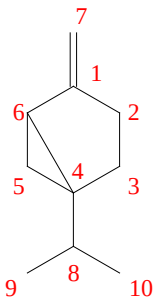


Figure 12.31
Sabinene

Classification of monoterpenes according to their functional groups

- **alcohols** (geraniol, linalool, citronellol)
- **aldehydes** (geranial, neral, citronellal)
- **ketones** (tagetone, menthone, carvone, pulegone)
- **esters** (linalyl-acetate, citronellyl-acetate)
- **ethers** (cineol)
- **peroxydes** (ascaridol)
- **phenols** (thymol, carvacrol)

Monoterpene-alcohols

Geraniol (Figure 12.19) belongs to the group of monoterpene-alcohols.

Linalool (Figure 12.32) can be detected in the essential oil of lavender (*Lavandula*).

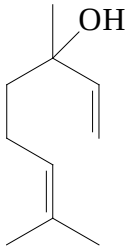


Figure 12.32
Linalool

Citronellol (Figure 12.33) is naturally occurring in the oil of rose, geranium and lemongrass (*Cymbopogon* / *Andropogon*) species.

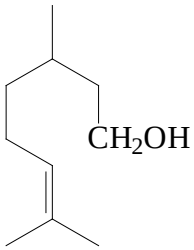


Figure 12.33
citronellol

Monoterpene-aldehydes

The monoterpene-aldehydes shown in Figure 12.34-36 occur in the volatile oil of lemon grass (*Andropogon nardus*), native to Indonesia.

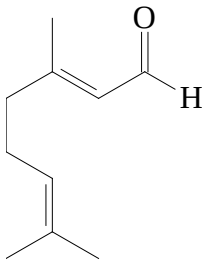


Figure 12.34
Geranial (citral A)
cis (*Z*) isomer

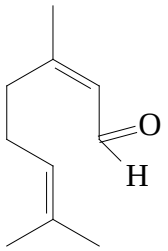


Figure 12.35
Neral (citral B)
trans (*E*) isomer

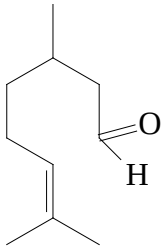


Figure 12.36
Citronellal

Monoterpene-ketones

- menthone (Figure 12.24) occurs together with menthol in peppermint (*Mentha piperita*)
- carvone (Figure 12.37) can be found in the essential oil of caraway (*Carum carvi*) and dill (*Anethum graveolens*)
- pulegone (Figure 12.38) can be detected in the volatile oil of pennyroyal (*Mentha pulegium*)

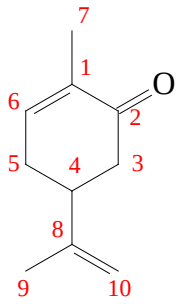


Figure 12.37
Carvone

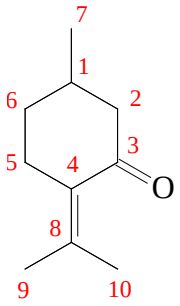


Figure 12.38
Pulegone

Monoterpene-ethers

Cineol (Figure 12.39), also called eucalyptol, was isolated for the first time from the oil of the seeds of santonica (*Artemisia cina*), but it occurs in substantial amounts in the volatile oil of eucalyptus (*Eucalyptus globulus*). It can be detected in rosemary (*Rosmarinus officinalis*) oil, as well.

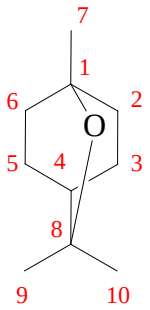


Figure 12.39
Cineol

Monoterpene-peroxydes

Ascaridol (Figure 12.40) is a characteristic compound in the goose-foot (Chenopodiaceae) family, e.g. in white goose-foot (*Chenopodium album*).

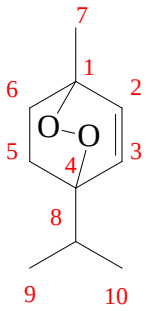


Figure 12.40
Ascaridol

Phenolic monoterpenes

Thymol (Figure 12.24) occurs in thyme (*Thymus*) oil, while carvacrol (Figure 12.41) is typical in caraway (*Carum carvi*) oil.

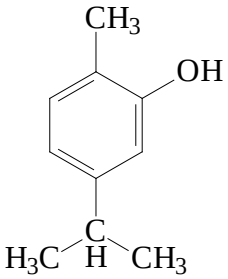


Figure 12.41
Carvacrol

Iridoids

- biosynthetically they are **monoterpenes**
- characteristic skeleton: **cyclopenta-pyranoid skeleton** (Figure 12.42)

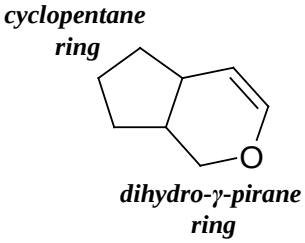


Figure 12.42
The cyclopenta-pyranoid skeleton of iridoids

- they occur in plants as ***glycosides***
- **biosynthesis:** in the 1st step **10-hydroxy-geraniol** is formed from 2 mols of dimethyl-allyl-pyrophosphate (DMAPP), and from this compound 7-de(s)oxy-loganinic acid and other iridoids will be formed :

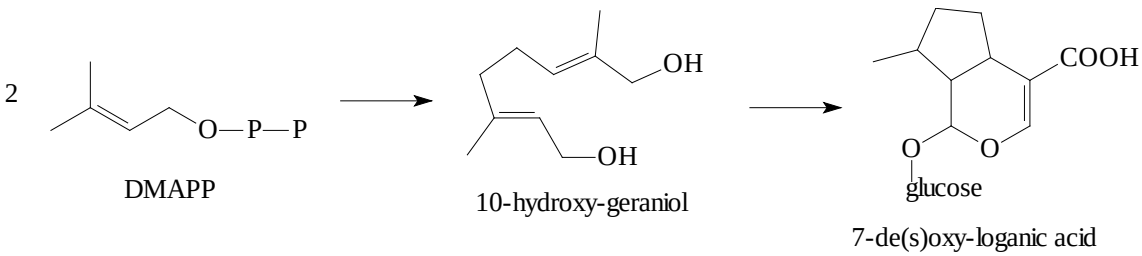


Figure 12.43
Biosynthesis of iridoids

- the **alkaloid-derivatives of iridoids** are well-known
- ecological role: they are **toxic** for certain animals, e.g. **aucubin** for insects and birds
- **Medicinal plants containing iridoids** include valerian (*Valeriana officinalis*, Figure 12.44), figworts (*Scrophularia* sp.), gentians (*Gentiana* sp., Figure 12.45), centauries (*Centaurium* sp., Figure 12.46), bogbean (*Menyanthes trifoliata*, Figure 12.47), common vervain (*Verbena officinalis*), as well as *Galium* and *Asperula* species (Figure 12.48).

Figure 12.44
Valeriana officinalis (valerian)

Figure 12.45
Gentiana lutea (yellow gentian)

Figure 12.46
Centaurium minus (annual centaury)

Figure 12.47
Menyanthes trifoliata (bogbean)

Figure 12.48
Galium odoratum (woodruff)

Pyretroids

- **not regular monoterpenes; esters** of **cyclopropane carboxylic acids**
- they are toxic for insects, but not for mammals
- occurrence: e.g. insect-powder-flower (*Chyrsanthemum cinerariifolium*)

(2) Sesquiterpenes

formula of the fundamental hydrocarbon: $C_{15}H_{24}$

- they may be **hydrocarbons** (e.g. β-bisabolene, β-caryophyllene) and derivatives containing **oxygen** (e.g. pharnesol)
- they occur in nature mainly as lactones (intramolecular cyclic internal esters) → **sesquiterpene-lactones**
- about 3000 compounds with known structure; they are bitter
- occur in mushrooms and mosses, but mainly in the species of Asteraceae, Apiaceae, Lauraceae and Menispermaceae
- **sesquiterpene-lactones are enzyme-inhibitors**: they hinder the activity of the enzymes DNA-polymerase or thimidyl-synthase; they are irreversible alkylation agents, antibacterial; some of them can cause allergy and dermatitis
- active ingredients of antiparasitic, insecticidal and digestive medicinal plants
- Sesquiterpene-lactones can be detected e.g. in St. Benedict's thistle (*Cnicus benedictus*, Figure 12.49), ragweeds (*Ambrosia* sp.), horse-heal (*Inula helenium*, Figure 12.50), mountain arnica (*Arnica montana*), tansy (*Tanacetum vulgare*, Figure 12.51) and wormwoods (*Artemisia* sp., Figure 12.52).

Figure 12.49
Cnicus benedictus (St. Benedict's thistle)

Figure 12.50
Inula helenium (horse-heal)

Figure 12.51
Inflorescence of *Tanacetum vulgare* (tansy)

Figure 12.52
Artemisia vulgaris (mugwort, common wormwood)

Figure 12.53
Artemisia absinthium (absinthe wormwood)

Bisabolene (Figure 12.54) was first isolated from the **resin** of Asteraceae sp., myrrh (*Commiphora abyssinica*) and frankincense (Burseraceae).

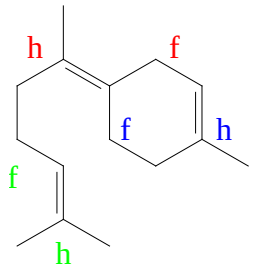


Figure 12.54
bisabolene

Caryophyllene (Figure 12.55) was first isolated from **clove** (*Syzygium aromaticum*, syn. *Eugenia caryophyllata*) by Wallach (1892).

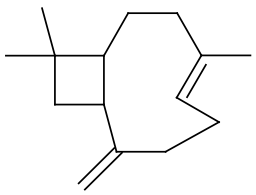


Figure 12.55
Caryophyllene

Pharnesol (Figure 12.56) is a sesquiterpene-alcohol with open chain, which occurs in the volatile oil of lime flower (*Tiliae flos*) and cyclamen (*Cyclamen*).

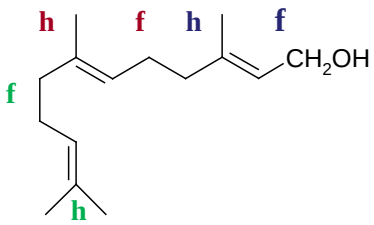


Figure 12.56
Pharnesol

Guaiazulene (Figure 12.57) is a bicyclic sesquiterpene, which occurs in chamomile oil, and has antiphlogistic effect.

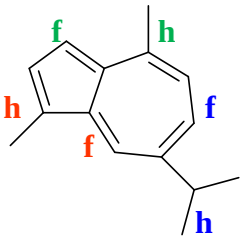


Figure 12.57
S - guaiazulene

Volatile oils

- main components of **volatile oils** are monoterpenes and sesquiterpenes;
- they can be **extracted** by:
  (1) water/steam distillation
  (2) solvent extraction
  (3) pressing
  (4) fluid CO_2 → **supercritical extraction**
- volatile oils containing pinenes and cineol (= monoterpenes): attract pollinating insects → **pheromone-like attractants**
- there are volatile oils with **repellent** action, too (containing e.g. limonene, menthone, myrcene and camphor)

(3) Diterpenes

Formula of the fundamental hydrocarbon: $C_{20}H_{32}$

- these compounds are mainly **cyclic**
- they are **inhibitors or toxic**
- about 1500 compounds with known structure
- diterpenes and diterpene-alkaloids toxic to humans occur in Thymelaeaceae (*Daphne* sp.), Euphorbiaceae (*Euphorbia* sp.) and Ericaceae (*Rhododendron* sp.).
- **taxol** and its semi-synthetic derivatives are active ingredients of anticancer drugs – taxol can be isolated from Taxaceae species, particularly from the bark of *Taxus brevifolia* (North-America)
- **Vitamin A**: see carotenoids, vitamins
- **Phytol**: a component of **chlorophyll**, formed during the hydrolysis of chlorophyll; built up from 4 isoprene units connected by head-foot coupling (Figure 12.58)

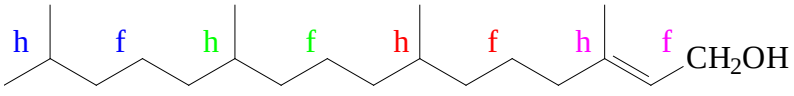


Figure 12.58
Structure of phytol

(4) Triterpenes

formula of the fundamental hydrocarbon: $C_{30}H_{48}$

- plants: about 4000 triterpenes with known structure
- **cucurbitacines** (Cucurbitaceae): tetracyclic triterpenes, **laxatives**
- **saponins**: triterpene saponins (five-membered ring skeleton) or steroid saponins (six-membered ring skeleton);

  → they occur in plants as glycosides

Squalene: intermedier of the biosynthesis of steroids (Figure 12.59)

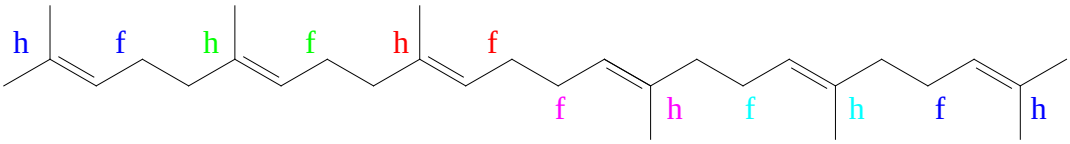


Figure 12.59
Structure of squalene

- **Medicinal plants containing triterpene saponins** include liquorice (*Glycyrrhiza glabra*, Figure 12.60), horse chestnut (*Aesculus hippocastanum*, Figure 12.61), senega root (*Polygala senega*, Figure 12.62), ivy (*Hedera helix*), cowslip (*Primula* sp., Figure 12.63), Siberian ginseng (*Eleutherococcus senticosus*), soap bark tree (*Quillaja saponaria*, Figure 12.64), common soapwort (*Saponaria officinalis*, Figure 12.65) and Hungarian soap root (*Gypsophila paniculata*, Figure 12.66).

Figure 12.60
Glycyrrhiza glabra (liquorice)

Figure 12.61
Aesculus hippocastanum (horse chestnut)

Figure 12.62
Polygalae radix (senega root)

Figure 12.63
Primula veris (primula = cowslip)

Figure 12.64
Bark of *Quillaja saponaria* = *Quillajae cortex* (soapbark)

Figure 12.65
Saponaria officinalis (common soapwort)

Figure 12.66
Gypsophila paniculata (Hungarian soap root, baby's breath)

Chapter 13
Biosynthesis and role of amino acids and proteins

13.1 Occurrence and significance of amino acids

- **Amino acids** are present in plants in free and bound (fixed) form (e.g. peptides, proteins)

13.2 Proteinogenic amino acids

Proteinogenic amino acids (shown in alphabetical order in Figure 13.1-21) are **α-amino acids having L-configuration**

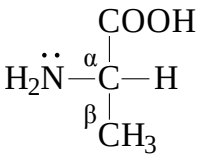


Figure 13.1
Alanine (α-amino-propionic acid, 2-amino-propanoic acid)

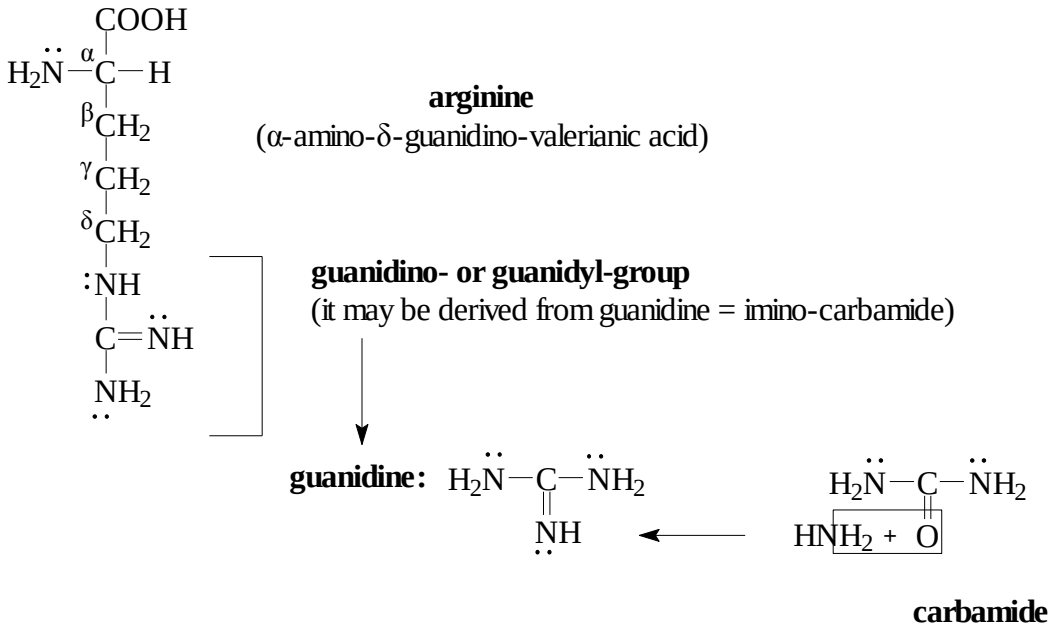


Figure 13.2
Arginine (α-amino-δ-guanidino-valerianic acid)

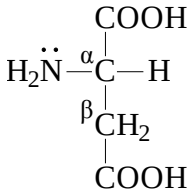


Figure 13.3
Asparagic acid (aspartic acid, amino succinic acid)

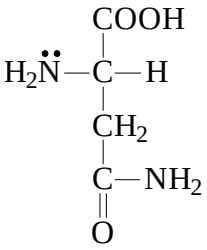


Figure 13.4
Asparagine (monoamid of asparagic acid)

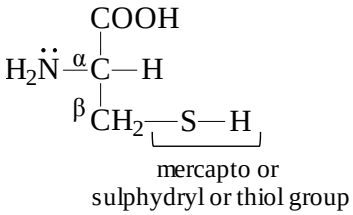


Figure 13.5
Cysteine (α-amino-β-mercapto-propionic acid)

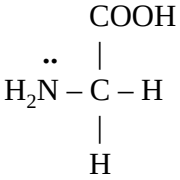


Figure 13.6
Glycine (amino acetic acid)

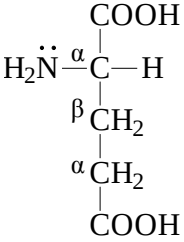


Figure 13.7
Glutamic acid (α-amino-glutaric acid)

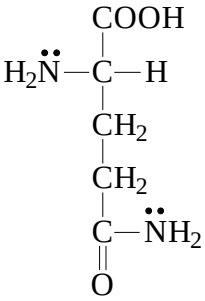


Figure 13.8
Glutamine (monoamid of glutamic acid)

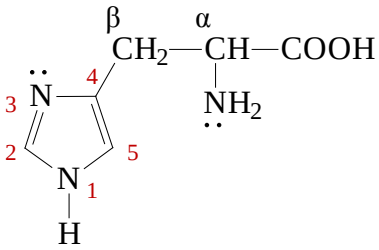


Figure 13.9
Hystidine (α-amino-β-(4)-imidasolyl-propionic acid)

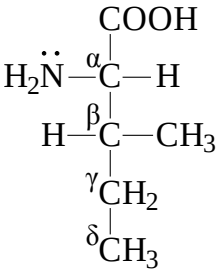


Figure 13.10
Isoleucine (α-amino-β-methyl-valerianic acid)

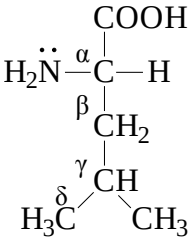


Figure 13.11
Leucine (α-amino-γ-methyl-valerianic acid)

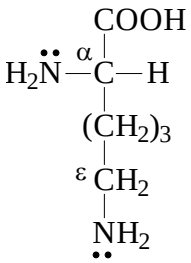


Figure 13.12
Lysine (α,ε-diamino-capronic acid)

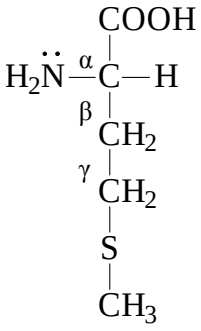


Figure 13.13
Methionine (α-amino-γ-methyl-mercapto-butyric acid)

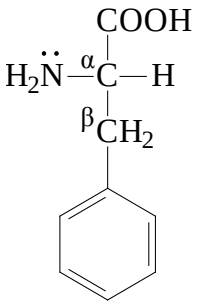


Figure 13.14
Phenylalanine (α-amino-β-phenyl-propionic acid)

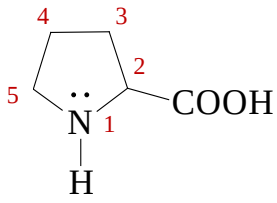


Figure 13.15
Proline (pyrrolidine-2-carboxilic acid or 2-carboxy-pyrrolidine)

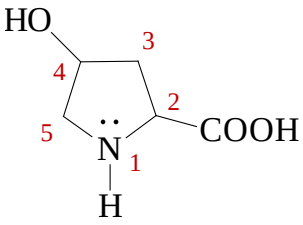


Figure 13.16
Hydroxyproline (4-hydroxy-pyrrolidine-2-carboxylic acid)

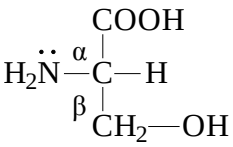


Figure 13.17
Serine (α-amino-β-hydroxy-propionic acid)

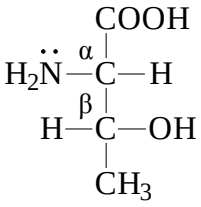


Figure 13.18
Threonine (α-amino-β-hydroxy-butyric acid)

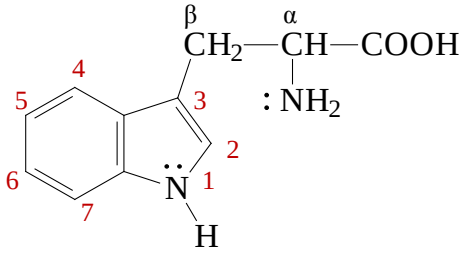


Figure 13.19
Tryptophan (α-amino-β-(3)-indolyl-propionic acid)

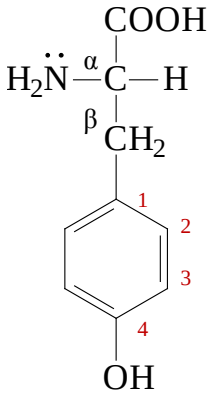


Figure 13.20
Tyrosine (α-amino-β-4-hydroxyphenyl-propionic acid = para-hydroxy-phenylalanine)

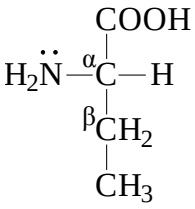


Figure 13.21
Valine (α-amino-butyric acid)

- Hydroxyproline (Figure 13.16) is exceptional in that it is synthesized by post-translational modification, and this amino acid is present only in the proteins of connective tissues in animals
- **glutamic acid** (an important precursor) is formed from 2-oxo(keto)-glutaric acid (Figure 13.22) and glutamine (Figure 13.8) during reductive transamination

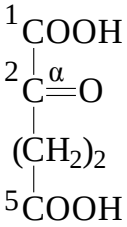


Figure 13.22
2-oxo(keto)- or α-oxo(keto)-glutaric acid

13.3 Reactions of amino-acids

Transamination

- the fundamental reaction of **transamination** is shown in Figure 13.23.

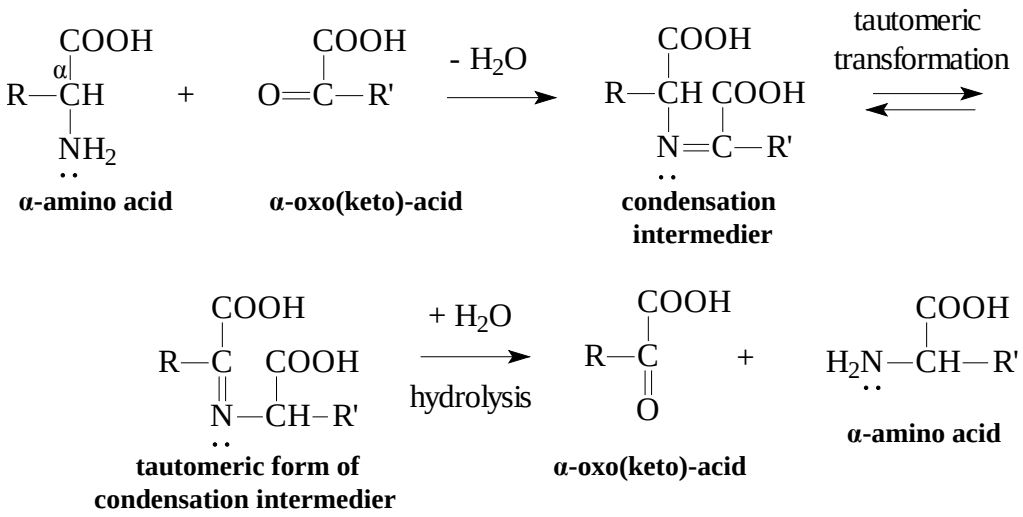


Figure 13.23
Transamination

- the original (starting) α-amino acid is transformed into the corresponding α-oxo(keto)-acid, the original (starting) α-oxo(keto)-acid into the corresponding α-amino acid
- the reaction is catalyzed by the enzyme ***transaminase*** - both in plants and animals
- **the amino acid composition of proteins is characteristic to the species, and depends on the genotype**
- the number of non-proteinogenic, **free amino acids** is more than **100**
- the skeleton of amino acids is provided by the intermediers of photosynthesis and of respiration

Biosynthesis of amino-acids from α-oxo-acids

- in plants: amino acids are synthetized in the chloroplasts from α-oxo(keto)-acids (Figure 13.24)

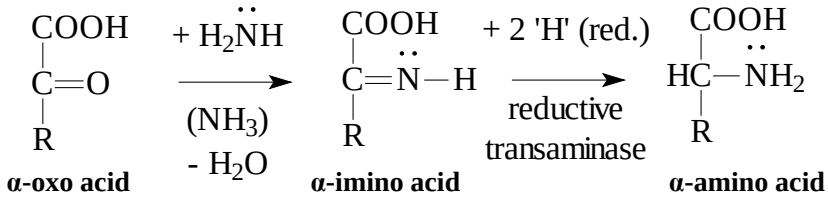
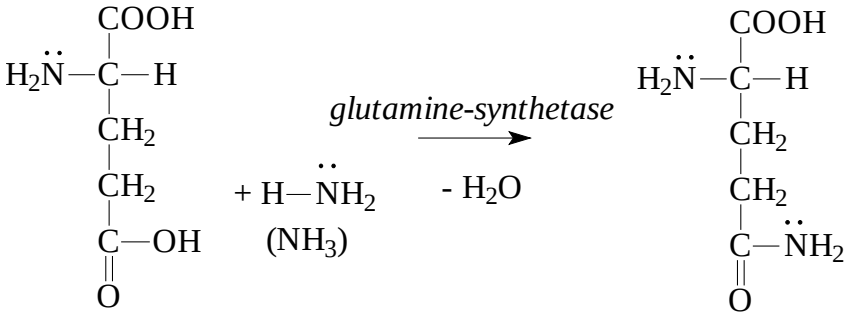


Figure 13.24
Synthesis of α-amino acids

Formation of glutamine from glutamic acid

- the reaction of glutamic acid with **ammonia** results in glutamine (Figure 13.25) – in the presence of the enzyme glutamine synthetase
- Ammonia is formed by reduction of nitrate (NO_3^-) and of nitrite (NO_2^-) ions or by means of the reduction of atmospherical N_2.



glutamic acid
(α-amino-glutaric acid)

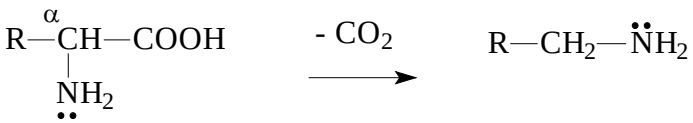
glutamine
(monoamide of glutamic acid)

Figure 13.25
Synthesis of glutamine

- Fabaceae sp. (e.g. bean, lentil, alfalfa, melilot): symbiosis with *Rhisobium* bacteria – form root nodules – atmospheric N_2 is fixed and reduced by **nitrogen reductase** → ammonia
- similarly, **reduction of sulphate** (taken up from the soil) → **cystein**

Decomposition and oxidation of amino acids in plants

- by ***decarboxylation*** (Figure 13.26) and ***desamination***; the latter process is preceded by oxidation (Figure 13.27)



α-amino acid **primer amine**

Figure 13.26
Decarboxylation of α-amino acids

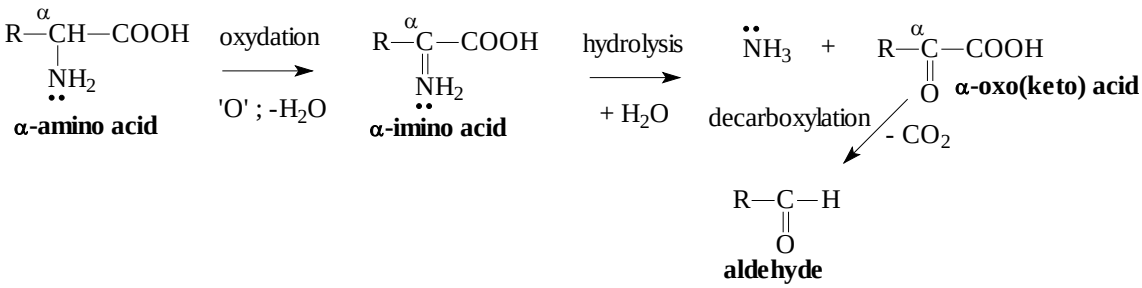


Figure 13.27
Oxydative desamination of α-amino acids

- during the decomposition of xanthine (Figure 13.28), caffeine (Figure 13.29) and other effective substances with purine skeleton, **ureides** (N-acyl derivatives of carbamide, Figure 13.31), **glycolic acid** (Figure 13.30)**, glyoxylic acid** (Figure 13.32) **and carbamide** are formed
- the most well-known ureide of plant origin is **allantoin** (Figure 13.33), which is the effective substance of *Symphyti radix* (comfrey root)

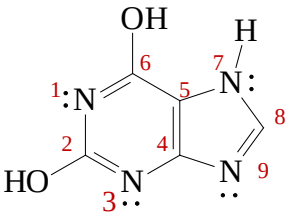


Figure 13.28
Xanthine (2,6-dihydroxy-purine)

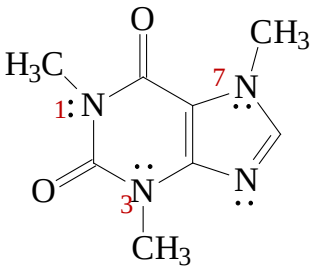


Figure 13.29
Caffeine (1,3,7-trimethyl-xanthine)

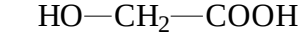


Figure 13.30
Glycolic acid (hydroxy acetic acid)

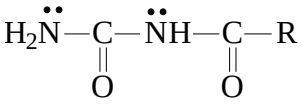


Figure 13.31
General formula of ureide

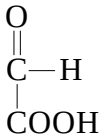
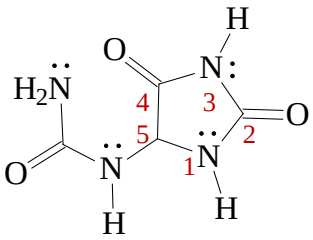


Figure 13.32
Glyoxylic acid



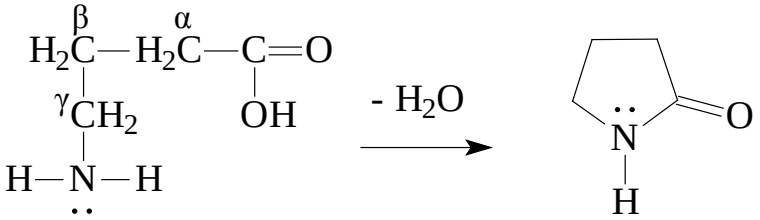
5-membered ring: 2,4-dioxo (diketo) derivative of
imidasolidine (saturated derivative of imidasole) → **hydantoine ring**

Figure 13.33
Allantoin

13.4 Classification of amino acids with respect to the formation of alkaloids

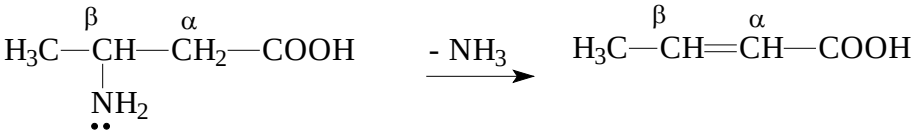
(1) **Pyruvate origin**: alanine, valine, leucine
(2) **Formed during photorespiration and sulphate-reduction**: glycine, serine, cysteine
(3) **Citrate-cycle origin**: asparagic acid, threonine, isoleucine, methionine, lysine, glutaminic acid, proline, ornithine, arginine
(4) **Aromatic amino acids: formed during the carbohydrate metabolism:** phenylalanine, tyrosine, tryptophan, hystidine
(5) **Special amino acids:** do not take part in protein synthesis; several special amino acids accumulate in the taxon to which they are characteristic;

- ***γ*-amino-butyric acid (GABA):** formed in plants as a response to stress; protective role together with β-amino-butyric acid (BABA) against certain virulent pathogens (Figure 13.34-35)



γ-amino-butyric acid (GABA) **γ-butyro-lactam**
(intramolecular, cyclic amide)

Figure 13.34
γ-amino-butyric acid (GABA)



β-amino-butyric acid (BABA) **β-methyl-acrylic acid = crotonic acid**

Figure 13.35
β-amino-butyric acid (BABA)

- **β-alanine:** (**β-amino-propionic acid,** component of coensyme-A) (Figure 13.36)

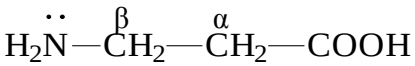


Figure 13.36
β-alanine

- **Occurrence***: Canavalia ensiformis* (sword-bean), Fabaceae; its enzymatic degradation yields important proteinogenic amino acids; insecticide.
- **L-dihydroxy-phenylalanine = L-DOPA**: toxic for most mammals; occurs in great quantity in the seed coat and fruit wall of horse-bean (*Vicia faba*).

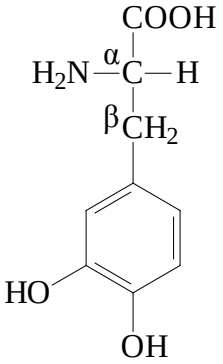


Figure 13.37
L-DOPA

- **alkylated derivatives of cysteine:** in *Allium* species (Alliaceae)
- from the allyl-derivative of cysteine, **allicine** is formed by enzymatic way; occurrence: garlic (*Allium sativum*) and wild garlic (*A. ursinum*, Figure 13.38), responsible for characteristic smell

Figure 13.38
Allium ursinum (wild garlic)

- in the essential oil of garlic: **diallyl-sulphide** and **diallyl-disulphide**

- **propenyl-cysteine-derivatives**: precursors of the compounds responsible for the smell of onion (*Allium cepa*, Figure 13.39)

Figure 13.39
Allium cepa (onion)

13.5 Peptides, proteins

- peptides built up from amino acids by means of enzymes,
- proteins synthesis is regulated by DNA
- molecular mass of peptides is not > 10 000; that of proteins may be > 100 000, even > 1 000 000
- certain peptides are toxic, e.g. **phalloidine**, a cyclic octapeptide, the effective substance of death cap or deadly amanita (*Amanita phalloides*), which causes lethal intoxication

Classification of proteins on the basis of their solubility

(1) **albumines**: soluble in distilled water; stored reserve nutrients

(2) **globulines**: insoluble in water, soluble only in salt solutions

(3) **glutelines**: soluble only in week acids and week bases

(4) **histones**: soluble in diluted acids; basic proteins, mainly in the nucleus

(5) **prolamines**: soluble in ~ 60-80 % ethanol, proteins containing mainly proline and asparagic acid

Enzymes

According to function, the most important proteins are the **enzymes**, which catalyse the chemical processes taking place in living organisms → **biocatalysts**

Classification of enzymes on the basis of their functions:

(1) ***hydrolases:*** breaking of C – O and C – N bonds by means of hydrolysis
- esterases: substrate-specific
- lipases: cleave fats
- glycosydases: cleave carbohydrates
- proteases: cleave proteins

(2) ***lyases*****:** reversible addition of water (H – OH) to double bonds
(3) ***ligases*****:** can join and break C – C bonds
(4) ***transferases*****:** transfer atomic groups and radicals from one compound to the other; e.g. transmethylation, transcarboxylation
(5) ***isomerases*****:** transform phosphorylated aldoses or ketoses into each other
(6) ***oxydoreductases*****:** catalyse redox processes, e.g. lactate-pyruvate reaction

Enzymes used in therapy:

(1) papaine: occurs in papaya (*Carica papaya*, Caricaceae), proteolytic enzyme, cleaves proteins; built up from 185 amino acids; aids digestion of proteins; used for the cleaning of wounds

(2) bromelaine: occurs in the ripe and unripe fruits of pineapple (*Ananas comosus*, Bromeliaceae) and in the pressed juice of its floral axis; enzyme with proteolytic activity; treatment of digestion disorders and against postoperative oedemas and inflammations

(3) ficine: *Ficus* species (Moraceae); e.g. fig (*Ficus carica*); proteolytic enzyme

Biosynthesis of proteins

Biosynthesis of plant proteins in the cytoplasm; Characteristic steps of biosynthesis:

(1) Activation of amino acids:

formation of enzyme – AMP – amino acid complex

(2) Activated amino acid:

binding of the above complex to t-RNA

(3) Initiation and elongation
(4) Termination: the biosynthesized polypeptide reaches the corresponding codon and breaks away from the m-RNA

Storage proteins

- reserve nutrients
- Monocotyledonous plants: store mainly gluteline (~45 % glutaminic acid content) and prolamine
- **glutenine**: the gluteline of wheat and rye
- **gliadine**: the prolamine of wheat and rye
- **glutenine + gliadine = glutene**
- ***glutene intolerance (allergy)*** – sensitivity may develop also to proteins that are characteristic for dicotyledonous plants
- bean-seed: albumine, globuline, gluteline are present in high quantities
- most legumes contain the globuline **viciline**
- a number of enzymes, glycoproteins and lectins are globulines
- **legumeline**: albumine present in the seeds of leguminous plants → not toxic

Lectins (phytohemagglutinines)

- form a precipitate with hemoglobine
- specific sugar-binding capacity: proteins which "recognize" the sugar components characteristic to the cell membrane and bind to them
- they play a role e.g. in the **symbiosis** between legumes and *Rhizobium* bacteria (formation of root nodules)
- **they are stored in seeds and metabolised during germination**
- several lectins have mitosis-stimulating action, **mitogen**
- because of their **selective recognizing-ability** several lectins are used in immune biology and immune therapy ; e.g. lectin of wheat-germ, soy, castor plant, mistletoe, bean
- lectins are particularly characteristic to the plant family **Fabaceae**
- lectin of ***Phytolacca americana*** **(American pokeweed)** has antiviral action

Toxic proteins

Ricine: toxic protein (toxalbumine) occurring in the seeds (***Ricini semen***) of the **castor oil plant** (*Ricinus communis*)*;* insoluble in fats, therefore it cannot occur in purified castor-oil; ricine present in only 3-4 seeds cancause death

- ricine and other toxic lectins bound to monoclonal antibodies are used in "directed cancer therapy"

Proteins having antidigestive, antinutritive and trypsin-inhibitory properties

- their molar mass < the molar mass of lectins
- occur mainly in legumes
- the trypsin-inhibitor of soy causes pancreas-hypertrophy
- lectins and trypsin-inhibitors are thermosensitive; they decompose during thermal action and cooking

Chapter 14
General features of alkaloids

14.1 Definition of alkaloids

According to the ***old definition***, alkaloids are end products of plant metabolism, organic heterocyclic bases containing a N-atom, and characterised by marked physiological action.

According to the ***new definition***, alkaloids are cyclic organic compounds, which contain the N-atom in the state of negative oxydation grade; occurring in various living organisms in limited quantities.

14.2 Distribution of alkaloids in plants

Alkaloids can be found predominantly in plants, but they occur also in animals (salamanders, frogs, insects, sea animals etc.), as well as in fungi and bacteria. Alkaloids are more typical in dicotyledonous plants, where a huge variety of alkaloids can be detected in substantial amounts; whereas only a few monocotyledonous families are characterised by the presence of alkaloids. Alkaloids frequently occur in the dicot plant families Annonaceae, Apocynaceae, Fumariaceae, Lauraceae, Loganiaceae, Magnoliaceae, Menispermaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae and Solanaceae; as well as in the monocot families Amaryllidaceae and Liliaceae. Several alkaloid-containing plants keep off certain herbivorous animals, which in turn accumulate these alkaloids in their bodies, frightening away predators. Alkaloids exert a strong physiological effect, being mostly toxic to animals and humans, already in low concentrations. Alkaloid-containing drugs typically have narrow therapeutic range, which means that there is little difference between therapeutic and toxic doses.

The highest quantities of alkaloids are present in the organs of fully developed plants (root, leaf, flower, seed) or in their tissues (e.g. bark). In plants alkaloids occur in the form of their water soluble salts, or bind to phenolic acids in the vacuole of the cells. Alkaloids form salts mainly with organic acids, such as citric acid, malic acid, tartaric acid and benzoic acid.

14.3 Alkaloid biosynthesis

Alkaloids are typically synthetized from amino acids. The most important starting materials of biosynthesis include ornithine, lysine, phenylalanine, tyrosine, tryptophan and hystidine. The biosynthesis of alkaloids is a genotypic property; precursors and end products can already be detected in cell- and tissue cultures.

The most characteristic N-containing heterocycles of alkaloids include pyrrol, pyrrolidine, pyridine, piperidine, indole, quinolone and isoquinoline.

Alkaloids can be classified according to their origin (derived from amino acids or other substances); whether they possess a heterocyclic ring with nitrogen, or contain the N-atom outside the ring; and according to the type of the N-containing heterocycle.

14.4 Classification of alkaloids

(1) Protoalkaloids (Nonheterocyclic alkaloids)

Protoalkaloids (e.g. mescaline and ephedrine) do not have a heterocyclic ring with nitrogen, they contain the N-atom in the form of amino group. This group also includes pigments containing quaternary N-atom; e.g.: chromoalkaloids, betalains and their glycosides, betanines are present in Chenopodiaceae (goose-foot sp.). These compounds are pigments with antioxidant activity, not being toxic.

Phenyl-ethylamin alkaloids

Ephedrine (Figure 14.1) can be found in *Ephedra vulgaris* (ephedra). Its biological activities include increasing blood-pressure, stimulating the nervous system and dilating the bronchi.

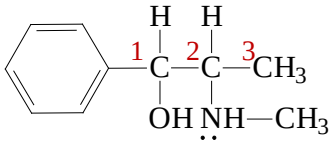


Figure 14.1
Ephedrine – 1-Phenyl-2-methylamino-propanol-(1)

The antibiotic Chloromycetin or chloramphenicol (Figure 14.2) produced by *Streptomyces venezuelae* has a similar structure to ephedrine, but it is not an alkaloid.

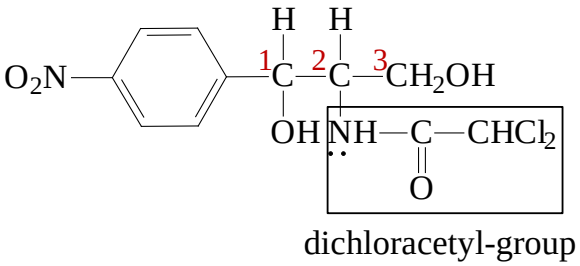


Figure 14.2
Chloramphenicol

Mescaline (Figure 14.3) is a derivative of pyrogallol-trimethyl-ether or of ethylamine. It is not heterocyclic, therefore it can be classified into the group of protoalkaloids. Mescaline can be found in peyote (peyotl) or mescaline-cactus (*Lophophora williamsii*) and is responsible for causing hallucinations.

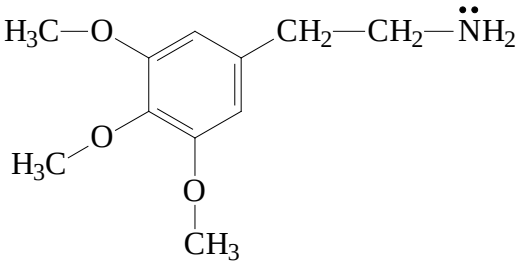


Figure 14.3
Mescaline

Colchicine (Figure 14.4), naturally found in autumn crocus or meadow saffron (*Colchicum autumnale*), has a tropolone nucleus with nitrogen in side-chain, containing an amid-group.

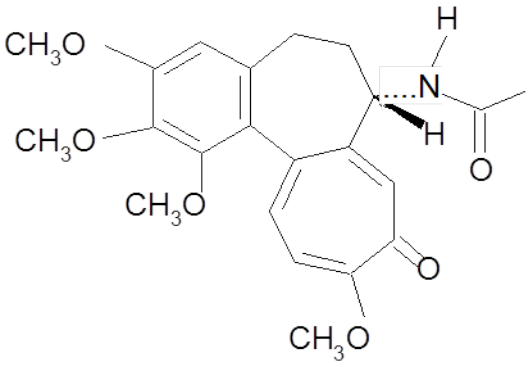


Figure 14.4
Colchicine

(2) True alkaloids

Alkaloids containing pyridine or piperidine ring

Nicotine (Figure 14.5) is one of the best-known examples of alkaloids with a pyridine ring. Nicotine is found in high amounts in various tobacco (*Nicotiana*) plants, e.g. *Nicotiana tabacum*.

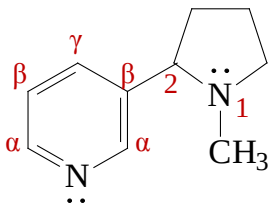


Figure 14.5
Nicotine (1-methyl-2(β)-pyridil-pyrrolidine)

Coniine (Figure 14.6), one of the few liquid alkaloids, is the main active compound of poison hemlock (*Conium maculatum*). Coniine is highly toxic, and extracts of poison

hemlock were once used as a means of execution (e.g. Socrates was sentenced to death by drinking a hemlock-based liquid).

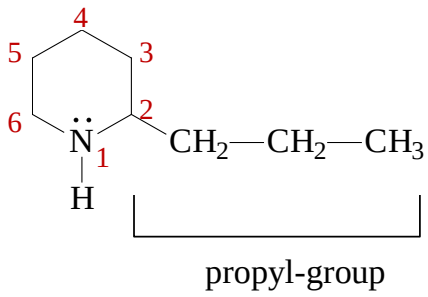


Figure 14.6
Coniine (2-Propyl-pyperidine)

Similar types of alkaloids include **lobeline**, which can be detected in various *Lobelia* sp. (lobelias, e.g. *Lobelia inflata*); and **piperine**, a pseudoalkaloid in *Piper* sp. (pepper, e.g. *Piper nigrum* - black pepper).

Tropane alkaloids

Tropane alkaloids are alkaloids with pyrrolidine- and piperidine condensated ring systems.

Tropane alkaloids are a class of compounds that are derived from the amino acid ornithine, and contain a tropane ring in their chemical structure. Their basic structures include 8-azabicyclo-[3,2,1]-octane (Figure 14.7), nortropane (Figure 14.8) and tropane (Figure 14.9). Tropane alkaloids occur in several members of the nightshade (Solanaceae) family.

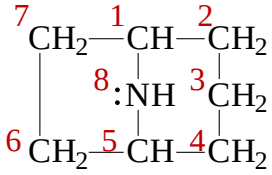


Figure 14.7
8-azabicyclo-[3,2,1]-octane

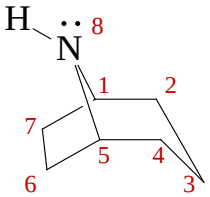


Figure 14.8
Nortropane

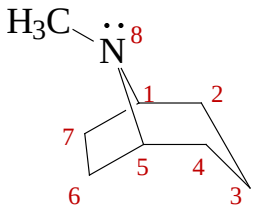


Figure 14.9
Tropane (N-methyl-8-azabicyclo-[3,2,1]-octane)

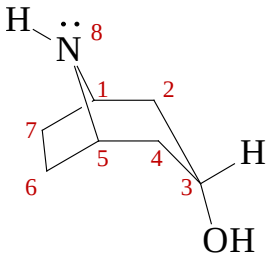


Figure 14.10
Tropine (3-α-hydroxy-tropane)

Atropine (Figure 14.11) is the ester of tropine (Figure 14.10) formed with racemic tropic-acid (α-phenyl-β-hydroxy-propionic acid). Atropine is the characteristic alkaloid of deadly nightshade or belladonna (*Atropa belladonna*).

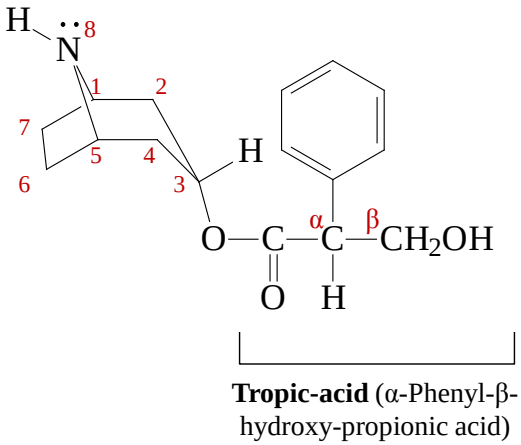


Figure 14.11
Atropine

Hyosciamine is the ester of tropine with levorotatory (—)-tropic-acid (α-phenyl-β-hydroxy-propionic acid).

Scopolamine (Figure 14.12) is the 6,7-epoxyde of atropine, exerting a particularly strong effect on the central nervous system; due to its narcotic action it causes a feeling of exhaustion. However, if overdosed, it will induce strong excitement. Scopolamine can be found in various representatives of the Solanaceae family, including henbane (*Hyosciamus niger*) (Figure 14.13) and *Datura* species (Figure 14.14-16).

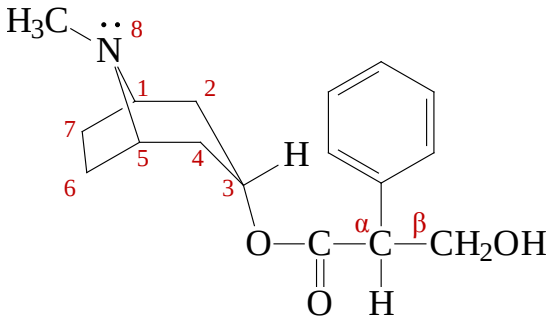


Figure 14.12
Scopolamine

Figure 14.13
Hyosciamus niger (henbane)

Figure 14.14
Datura metel (angel's [devil's] trumpet) flower

Figure 14.15
Datura metel (angel's [devil's] trumpet) fruit

Figure 14.16
Datura stramonium (thorn apple) flower

Ecgonine (Figure 14.17) is a tropane alkaloid found naturally in coca leaves. It is the 2-carboxy-derivative of pseudotropine (3-β-hydroxy-tropane). The ester of ecgonine with benzoic acid and methanol is called **cocaine**, which is the main active compound of the coca shrub (*Erythroxylon coca*). Cocaine has local anaesthetic effect, which can be attributed to the benzoylester group (Figure 14.18).

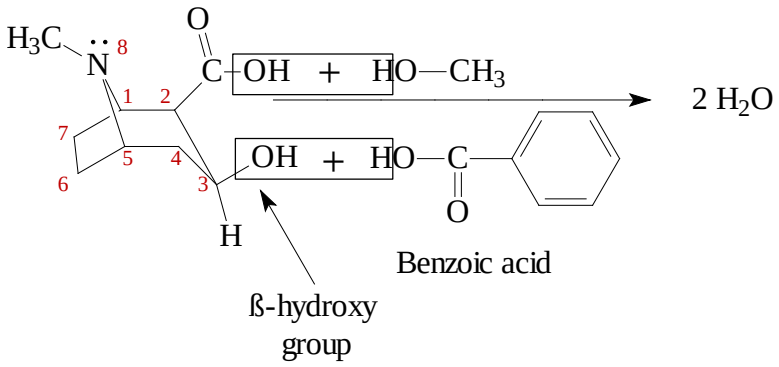


Figure 14.17
Ecgonine

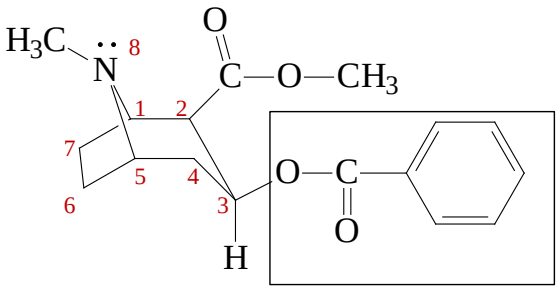


Figure 14.18
Cocaine

The well-known local anaesthetic drug, Novocain, is the ester of PABA (p-amino-bensoic-acid) with diethylamino-ethanol (Figure 14.19).

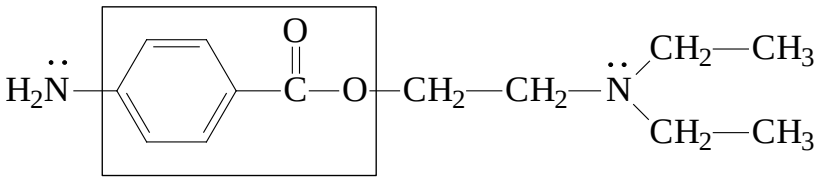


Figure 14.19
Novocain

Quinoline alkaloids

Quinine (Figure 14.20) is isolated from Cinchona bark, collected from various *Cinchona* species (*C. succirubra*, *C. pubescens*). Quinine is valued for its antimalarial and antipyretic property.

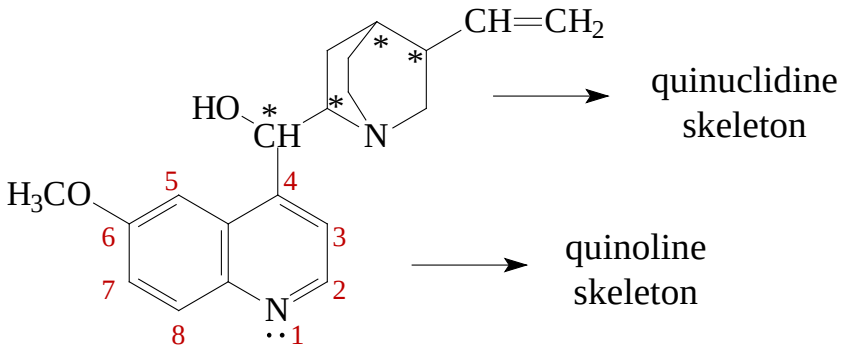


Figure 14.20
Quinine

Opium alkaloids: alkaloids of poppy (*Papaver somniferum*)

The six opium alkaloids which occur naturally in the largest amounts are morphine, narcotine, codeine, thebaine, papaverine and narceine. Of these, three are phenanthrene alkaloids (morphine, codeine and thebaine), all three used in the drug industry; thebaine usually for conversion into some derivative which is more useful medically.

The first major alkaloid formed in the plant is thebaine, this is irreversibly converted to codeine and then to morphine. Morphine (Figure 14.21), the main alkaloid of opium, is the derivative of N-methyl-morphinane. Structurally, the molecule of morphine contains three units: 1. partially hydrogenated phenanthrene skeleton, 2. piperidine ring, 3. dihydrofuran ring.

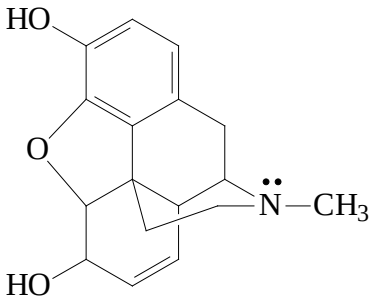


Figure 14.21
Morphine

Opium and morphine are widely used to relive pain and are particularly valuable as hypnotics.

Codeine, the monomethyl-ether of morphine, is a milder sedative than morphine and is useful as a cough suppressant. Thebaine, the 3,6-dimethoxy-derivative of 6,7,8,14-tetradehydro-N-methyl-morphinan, is the most poisonous opium alkaloid and is scarcely used medically.

The illegal drug heroine is chemically diacetyl-morphine.

Papaverine (Figure 14.22) is approved to treat spasms of the gastrointestinal tract, bile ducts and ureter and for use as a cerebral and coronary vasodilator.

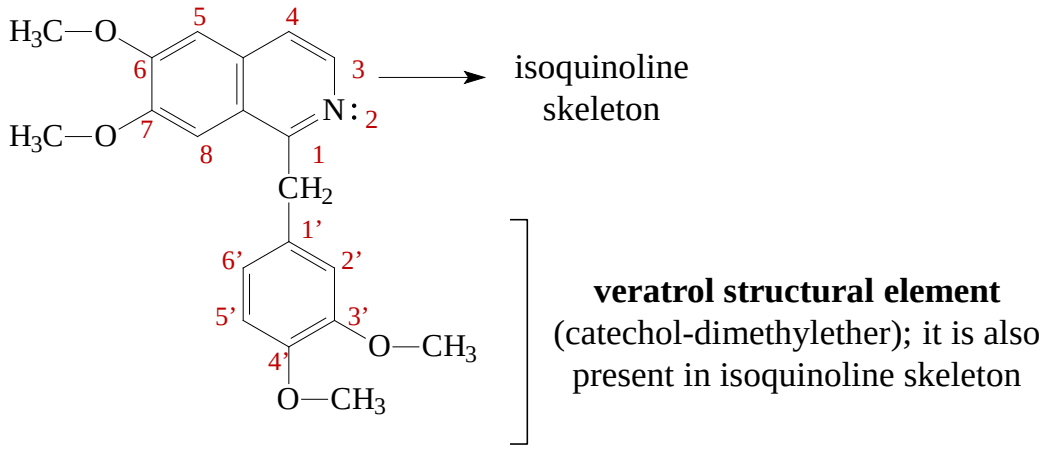


Figure 14.22
Papaverine (6,7,3',4'-Tetramethoxy-1-benzyl-isoquinoline)

Ergot-alkaloids

Ergot alkaloids are produced by ergot, the sclerotium of a fungus, *Claviceps purpurea*. The pharmacologically active alkaloids of ergot are the derivatives of lysergic acid. Whole ergot preparations were traditionally used in labour to assist delivery and to reduce post-partum haemorrhage, today ergot has been replaced by the isolated alkaloids. Ergometrine produces an oxytocic effect, while ergotamine is employed as specific analgesic for the treatment of migraine. Lysergic acid diethylamide (LSD, Figure 14.23), prepared by partial synthesis from lysergic acid, is a potent psychotomimetic, used illegally as a recreational drug.

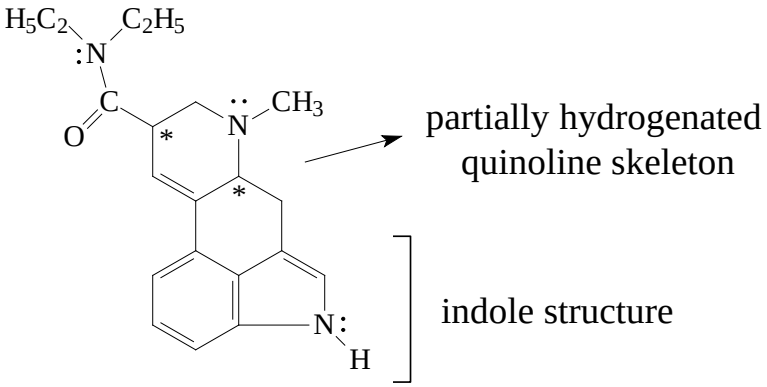


Figure 14.23
LSD = Liserg-Säure-Diethyl-amide (German)

Purine-alkaloids

Purine alkaloids are N-methylated derivatives of xanthine (2,6-dihydroxy-purine) (Figure 14.24).

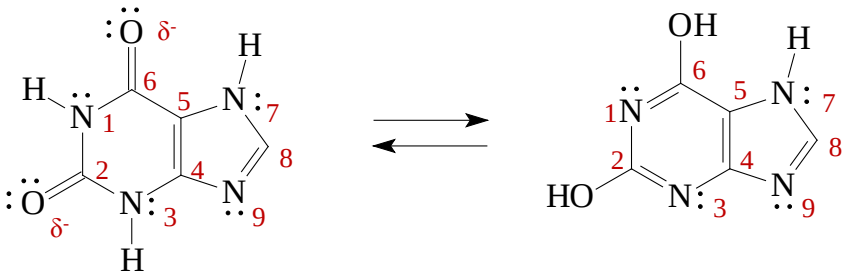


Figure 14.24
Xanthine (2,6 – dihydroxy – purine)

Xanthine itself does not occur in nature, but the N-methyl-derivatives of the lactame form are naturally occurring compounds. Three well-known examples are caffeine (1,3,7-trimethyl-xanthine), theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine). Beverages such as tea and coffee owe their stimulant properties to these substances.

Caffeine (Figure 14.25) stimulates the central nervous system and has a weak diuretic action, whereas theobromine acts in the reverse way. Theophylline exerts a shorter, but more powerful diuretic action than caffeine; it relaxes involuntary muscles more effectively than either caffeine or theobromine.

Caffeine occurs naturally in the seeds of various coffee shrubs (*Coffea arabica, C. liberica, C. canephora*), in cola tree (Cola nut) (*Cola acuminata, C. nitida, C. verticillata*), and in the leaves of tea shrubs (*Camellia* or *Thea sinensis*).

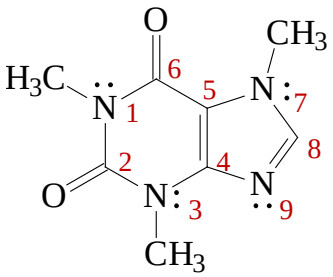


Figure 14.25
Caffeine (1,3,7-trimethyl-xanthine)

Theophylline (Figure 14.26) is the main alkaloid in the leaves of tea (*Camellia sinensis*).

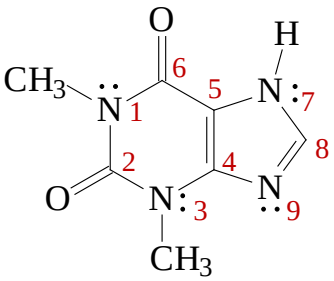


Figure 14.26
Theophylline (1,3-dimethyl-xanthine)

Theobromine (Figure 14.27) is synthesized abundantly in the seeds (beans) of cacao tree (*Theobroma cacao*).

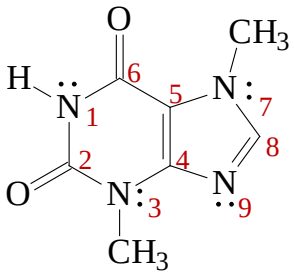


Figure 14.27
Theobromine (3,7-dimethyl-xanthine)

(3) Pseudodalkaloids

Pseudoalkaloids are compounds, whose biosynthesis starts not from amino acids, but other substances. This group includes terpenoid alkaloids such as aconitine, a diterpenoid alkaloid in *Aconitum* species; steroid-alkaloids of *Solanum species*; and coniine, having a piperidine skeleton in poison hemlock (*Conium maculatum*).

Steroid-alkaloids

Steroid alkaloids arise by the inclusion of basic nitrogen at some point in the steroid molecule. These compounds can be employed in the partial synthesis of steroidal drugs (including several hormones). Species so exploited are *Solanum laciniatum* and *S. aviculare*. Solanidine (Figure 14.28), the glycoside of which is solanine, occurs also in the leaves and seeds of potato (*Solanum tuberosum*).

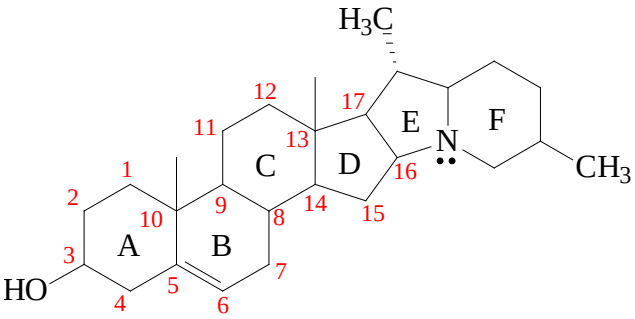


Figure 14.28
Solanidine

Tomatidine (Figure 14.29), the glycoside of which is tomatine, occurs naturally in the leaves of tomato (*Lycopersicon esculentum*).

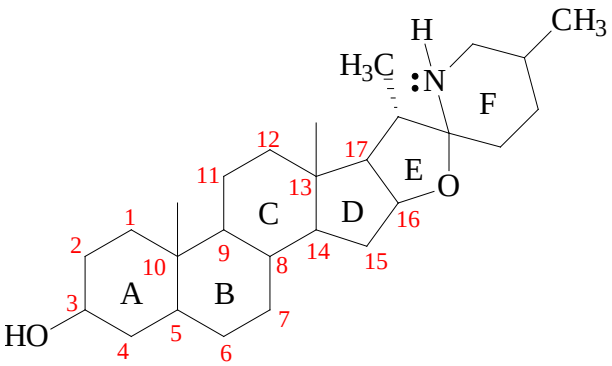


Figure 14.29
Tomatidine

Chapter 15
General features of phenoloids

Phenoloids constitute one of the largest groups of plant secondary metabolites. They are widespread in nature, ranging from simple structures with one aromatic ring to highly complex polymeric substances such as tannins and lignins.

15.1 Biosynthesis of phenolic compounds

Their biogenesis starts mainly from aromatic amino acids (phenylalanine, tyrosine). During the biosynthesis phenylpropionic acid (Figure 15.1) derivatives, then cinnamic acid (Figure 15.2-3) derivatives (cinnamoids) are formed; e.g. cinnamic acid, p-coumaric acid (Figure 15.4), caffeic acid (Figure 15.5), ferulic acid and chlorogenic acid (Figure 15.6). Cinnamates are the salts of cinnamic acid, occurring in essential oils and resins.

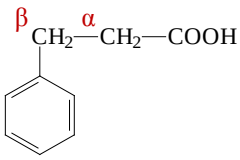


Figure 15.1
β-phenylpropionic acid

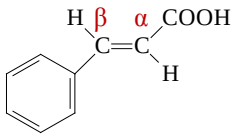


Figure 15.2
Cinnamic acid (*trans*, *E*) (β-phenyl-acrylic acid)

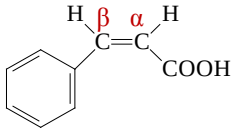


Figure 15.3
allo-cinnamic acid (*cis*, *Z*)

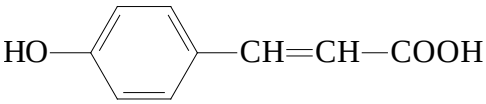


Figure 15.4
para-coumaric acid

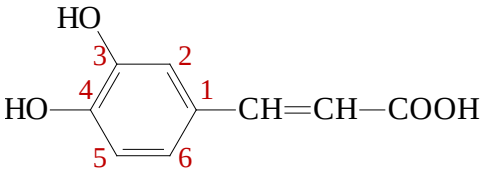


Figure 15.5
Caffeic acid. Its monomethylether is ferulic acid (3-methoxy-derivative).

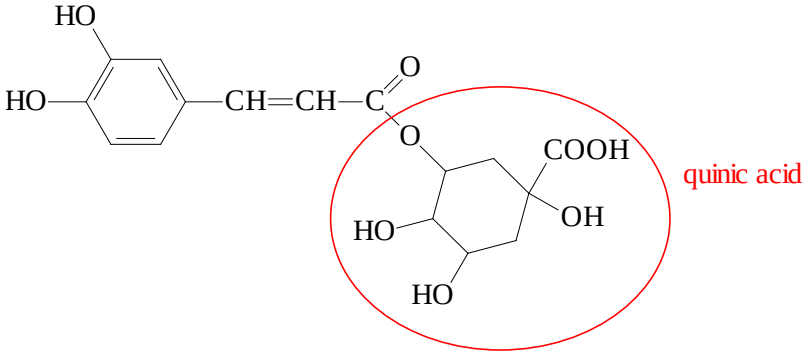
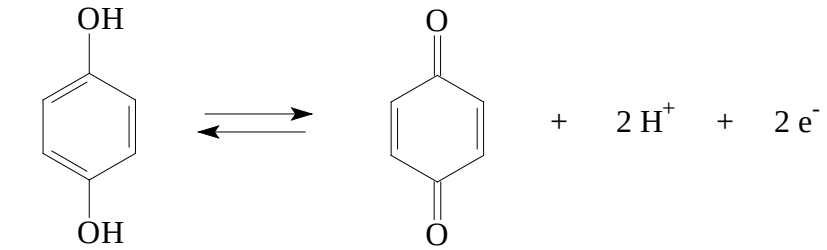


Figure 15.6
Chlorogenic acid

The unsaturation of the alkyl chain and the great number of the phenolic OH groups results in a huge variety of forms that occur in various plants. Phenoloids can be polymerized; they can form ethers and glycosides. The polymeric substances called tannins can be used as protein-precipitating agents. Phenolic compounds are effective substances of a great number of medicinal plants, and their role is important also in allelopathic reactions.

15.2 Phenols, phenolic acids, phenylpropanoid-derivatives

- the glycosides of hydroquinone (Figure 15.7) (e.g. arbutin) and its methylethers are characteristic in several species of the plant families Ericaceae and Rosaceae



hydroquinone *p*-benzoquinone (*p*-quinone, quinone)

Figure 15.7
Hydroquinone and *p*-benzoquinone

- Phenol-carboxylic acids, derived from benzoic acid (Figure 15.8), are very important in plants

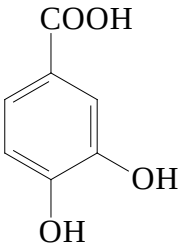


Figure 15.8
3,4-dihydroxybenzoic acid

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) (Figure 15.9) is a dihydroxybenzoic acid derivative, used as a flavoring agent. The highest amount of vanillin has been reported from the roots of *Angelica sinensis*, a herb used in Chinese traditional medicine.

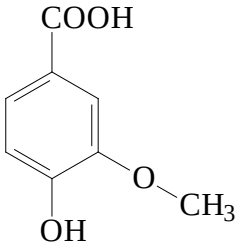


Figure 15.9
Vanillic acid

Gallic acid (Figure 15.10) is a trihydroxybenzoic acid, found in various plant species such as oaks (*Quercus* sp.) witch hazel (*Hamamelis* sp.), and tea (*Camellia sinensis*). Gallic acid is commonly used in the pharmaceutical industry, and can also be used as a starting material in the synthesis of the psychedelic alkaloid mescaline.

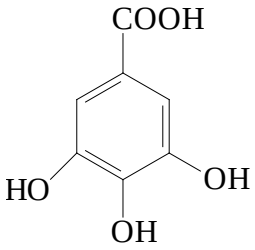


Figure 15.10
Gallic acid

Salicylic alcohol, and its glycoside formed with D-glucose, **salicin** (Figure 15.11), occur in high amounts in the bark of various willow (*Salix*) species (Figure 15.12), in the

catkin of poplar (*Populus*) species (Figure 15.13) and in meadowsweet (*Filipendula*) species (Figure 15.14).

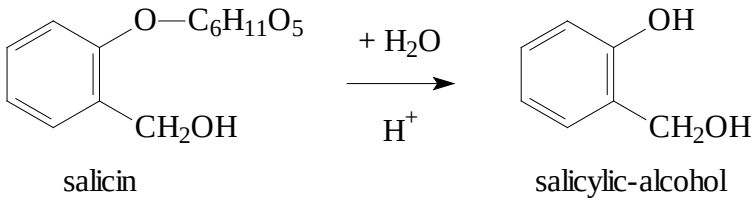


Figure 15.11
Formation of salicylic-alcohol from salicin

Figure 15.12
Salix sp.(willow)

Figure 15.13
Populus sp. (poplar)

Figure 15.14
Filipendula sp. (meadowsweet)

The discovery of salicin, salicyl-alcohol and salicylic acid (Figure 15.15) resulted in the production of **Aspirin** (acetylsalicylic acid) (Figure 15.16).

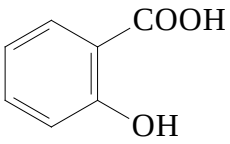


Figure 15.15
Salicylic acid (ortho-hydroxy-benzoic acid)

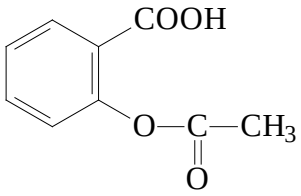


Figure 15.16
Aspirin (acetyl-salicylic acid)

Anisealcohol (4-methoxy-benzyalcohol or para-methoxy-benzylalcohol) and **anisealdehyde** [4(p)-methoxy-benzaldehyde] (Figure 15.17) are components of the volatile oil of vanilla (*Vanilla planifolia*).

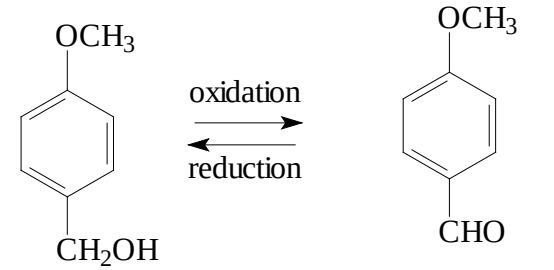


Figure 15.17
Anisealcohol and anisealdehyde as members of redox-system

15.3 Unsaturated phenolalcohols

Unsaturated phenolalcohols are the derivatives of a ***trans*(*E*)-cinnamyl alcohol** (Figure 15.18).The most important representatives include p-coumaryl-alcohol (Figure 15.19), coniferyl-alcohol (Figure 15.20) and sinapyl-alcohol (siringenine) (Figure 15.21), which are the direct precursors of lignins.

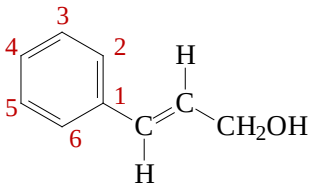


Figure 15.18
trans (E)-cynnamyl alcohol

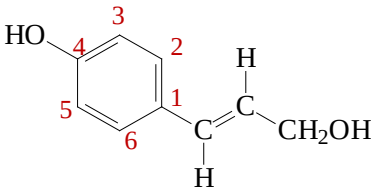


Figure 15.19
p-coumaryl-alcohol

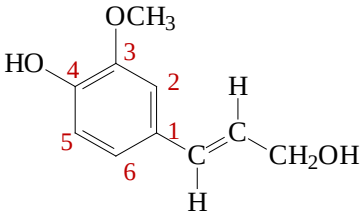


Figure 15.20
Coniferyl-alcohol

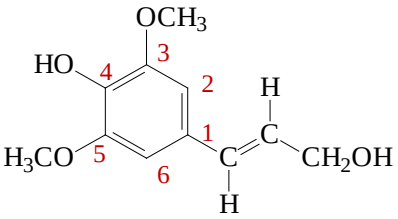


Figure 15.21
Sinapyl-alcohol

The above mentioned unsaturated phenolalcohols are stored in the form of their 4-O-β-D-glycosides in perennial plants, which are able to form woody (lignified) structures. As a result of an enzymatic hydrolysis their phenolic OH group becomes free, and then the enzyme phenol-oxydase starts the biosynthesis of different lignins. **Lignins** are complex chemical compounds, the organic biopolymers of phenolic compounds such as p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol.

15.4 Lignans

- complex compounds formed during the condensation of phenylpropanoid units
- important biologically active substances include **podophyllotoxine** and **α-peltatine,** e.g. in **american mandrake (mayapple)** (*Podophyllum peltatum*) (*Berberidaceae – Berberis* sp.)

Flavanolignans

- hinder lipid-peroxidation
- membrane stabilizers
- components of important liver-protective medicaments

e.g. in milk thistle (*Silybum marianum*) (Figure 15.22) (Asteraceae)

The effective substances of milk thistle include **silybin(in), silymarin, silychristin, and silydianin.** These flavanolignanes can be used in case of intoxication by deadly amanita (death cap, *Amanita phalloides*).

Figure 15.22
Silybum marianum (milk thistle)

15.5 Coumarins

- their fundamental compound is **coumarin** (Figure 15.23), which is the lactone of 2-hydroxy-allo-cinnamic acid (ortho-hydroxy-allo-cinnamic acid)
- **coumarin** (α-benz(o)-pyron) : colourless, crystalline compound with an odour reminiscent of hay

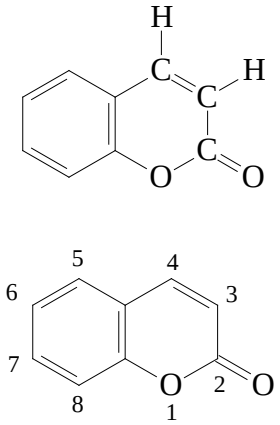


Figure 15.23
Coumarin

- they are wide-spread in plants, e.g.: woodruff (*Asperula odorata*) and Tonka bean (*Dipteryx odorata*)
- in most of plants coumarins are present not in their free form, but as glycosides of coumarinic acid (Figure 15.24):

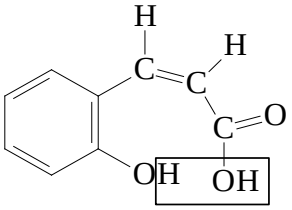


Figure 15.24
Coumarinic acid

- coumarin is formed from the glycosides of coumarinic acid during enzymatic degradation which starts by the etiolation of plant → smell of hay
- coumarin derivatives include e.g. **aesculin** (Figure 15.25) (glycoside of aesculetin) which occurs in horse-chestnut (*Aesculus hippocastanum*)

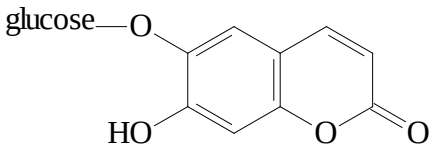


Figure 15.25
Aesculin

- simple coumarin derivatives are present in melilot (*Melilotus*) species (Fabaceae) (Figure 15.26), which contain **dicoumarol** (Figure 15.27), a compound with anticoagulant activity

Figure 15.26
Melilotus officinalis (yellow sweet clover)

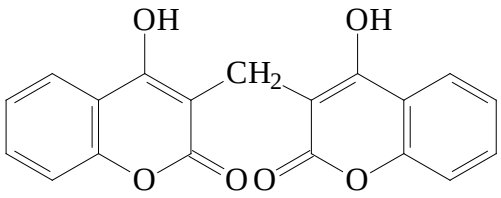


Figure 15.27
Dicoumarol

Furanocoumarins

Furanocoumarins are closely related to coumarins, and occur particularly in the Rutaceae (Figure 15.34-35) and Apiaceae (Figure 15.32-33). Psoralen (Figure 15.28) is the parent compound, structurally related to coumarin by the addition of a fused furan ring. Psoralen can be found naturally in the seeds of *Psoralea*, as well as in celery and parsley.

Further examples of furanocoumarins include e.g. angelicin (Figure 15.29).

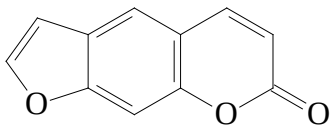


Figure 15.28
Psoralen

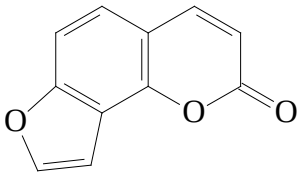


Figure 15.29
Angelicin

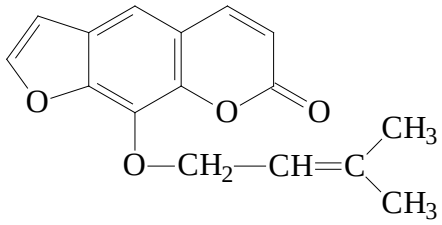


Figure 15.30
Imperatorin

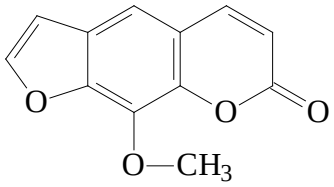


Figure 15.31
Xanthotoxin

- furanocoumarins are **phototoxic**
- they are responsible for the conservation of the capability of resistance in plants

Several members of the **Apiaceae (Carrot family)** contain furanocoumarins, including garden angelica (Figure 15.32), coriander, parsnip and hogweed (Figure 15.33). Representatives of the **Rutaceae (Rue family)** with furanocoumarins include common rue (Figure 15.34) and citrus species (Figure 15.35).

Figure 15.32
Angelica archangelica (garden angelica)

Figure 15.33
Heracleum sphondylium (hogweed) leaf

Figure 15.34
Ruta graveolens (common rue)

Figure 15.35
Citrus limon (lemon)

15.6 Flavonoids

- these compounds are the condensed derivatives of α- and γ-pyran (Figure 15.36-37), respectively of α- and γ-pyrone (Figure 15.38-39) with benzene:

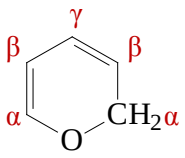


Figure 15.36
α-pyran

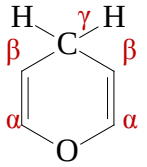


Figure 15.37
γ-pyran

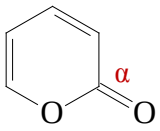


Figure 15.38
α-pyrone

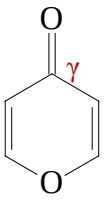
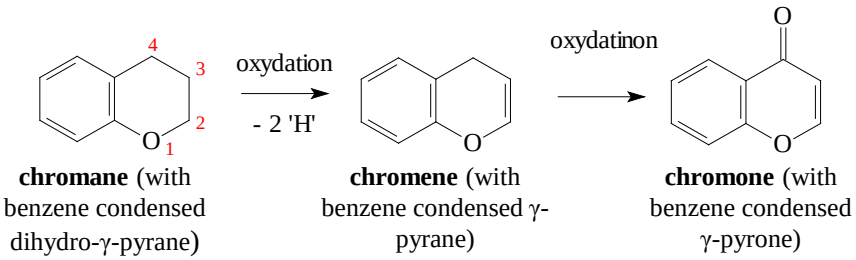


Figure 15.39
γ-pyrone

- Coumarin (Figure 15.23) is α-pyrone condensed with benzene.

- Pyranes and pyrones condensed with benzene form the **ring skeleton** of a great number of **natural plant pigments**, such as chromane, chromene and chromone (Figure 15.40).



chromane (with benzene condensed dihydro-γ-pyrane)

chromene (with benzene condensed γ-pyrane)

chromone (with benzene condensed γ-pyrone)

Figure 15.40
Skeletons of chromane, chromene and chromone

- The derivatives where the C-2 carbon atom is substituted with phenyl group include **flavan** (Figure 15.41)**, flaven** (Figure 15.42) and **flavon** (Figure 15.43).

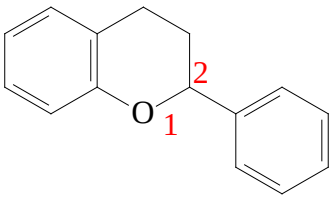


Figure 15.41
Flavan

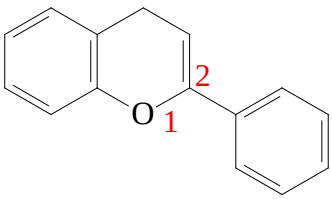


Figure 15.42
Flaven

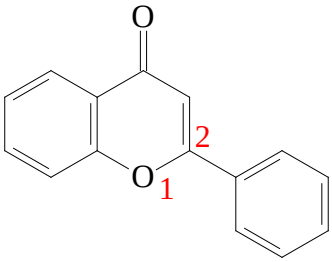


Figure 15.43
Flavon

- The glycosides of the hydroxy derivatives of the above compounds form one type of **yellow pigments** of different plants. The most important of them is **quercetin** (Figure 15.45), which is the 5,7,3',4'-tetrahydroxy-derivative of flavonol (3-hydroxy-flavon) (Figure 15.44).

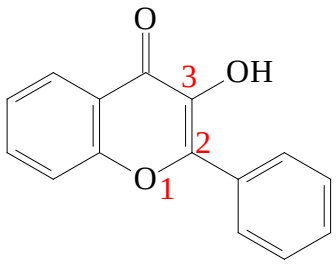


Figure 15.44
Flavonol (3-hydroxy-flavon)

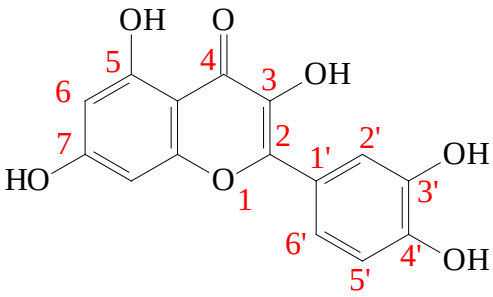


Figure 15.45
Quercetin (5,7,3',4'-tetrahydroxy-flavonol)

- **Rutin (Vitamin P)**, which is a glycoside of quercetin formed with rutinose on the C-3 OH group, regulates the permeability of capillary blood vessels. Rutin can be extracted by industrial method from the flowering shoots of buckwheat (*Fagopyrum esculentum*; *Fagopyrum tataricum*) (Polygonaceae), or from the flower buds of the common Japanese pagodatree (*Sophora japonica*, *Fabaceae*), where the rutin content reaches 15-20 %.
- Ginkgo (*Ginkgo biloba*; Ginkgoaceae) contains physiologically active bioflavonoids and proanthocyanidins
- ***isoflavonoids***: the phenyl group is connected to the C-3 carbon atom
- ***neoflavonoids + glycosides***: the phenyl group is connected to the C-4 carbon atom

Occurrence:

- **legumes** (*Fabaceae*)
- **devil's fig or prickly poppy (*Argemone*) sp.** (Clusiaceae)
- **madder sp.** (Rubiaceae)

15.7 Anthocyanins and anthocyanidins

- the **hydroxy derivatives of flaven (2-phenyl-chromen)** are wide-spread in plants as glycosides; their comprehensive name: **flavylium** or **anthocyan** (anthocyanin) pigments
- the alkaline hydrolysis of glycosides yields the corresponding coloured **aglycones** (**anthocyanidins**), which can be formed during acidic hydrolysis of **oxoniumsalts** (Figure 15.46).

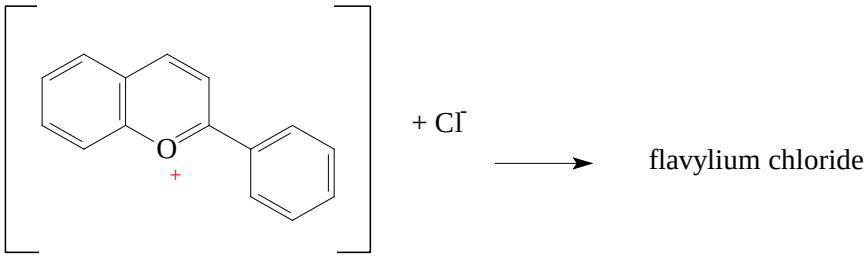
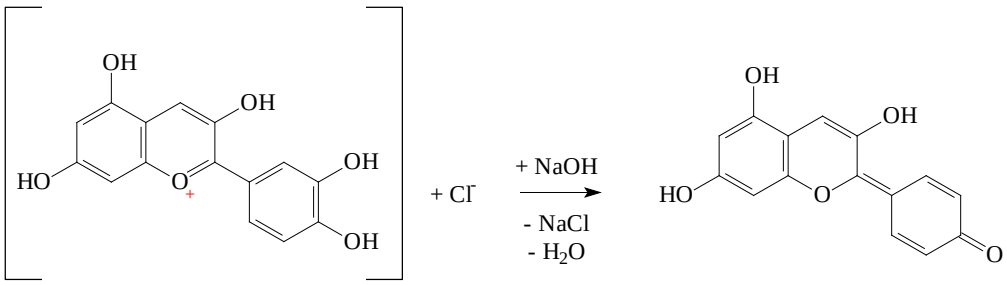


Figure 15.46
Formation of oxoniumsalts

- the glycosides formed with the 4-6 OH groups substituted derivatives of flavylium chloride are responsible for the **colour** of various petals
- On the effect of alkali the structure of the coloured salts will be rearranged; this process results a change of colour (Figure 15.47). This phenomenon is in the background of their indicator property:



pelargonidin(ium) chloride (red) — pelargonidin (blue-violet)

Figure 15.47
Colour change of pelargonidin chloride – pelargonidin

- The colour of the **anthocyan-pigments depends on the pH value of the plant saps**. This property explains the different shades of colour caused by anthocyanidins.
- In the petals of red rose and of blue cornflower (*Centaurea cyanus*) (Figure 15.48) the same cyanin-pigment is present and the pH values of their press-saps are identical, too:

Figure 15.48
Centaurea cyanus (cornflower)

- explanation of the difference in colour: → in the petals of cornflower (*Centaurea cyanus*) a coloured pigment-lac (pigment salt, formed with metal ions) is present
- flavonoids and anthocyanidins are antioxidants, inhibitors of lipid peroxidation, and scavengers → **antitumor effect**

Anthocyanins

- **anthocyanins having glycosidic character**
- water soluble
- the corresponding **aglycones are the anthocyanidins**
- occur mainly in the a corolla (petals) or perigonium (tepals) of angiosperms
- the most important anthocyanidins include *pelargonidin, cyanidin, peonidin, delphinidin, petunidin, malvidin*, with corresponding chloride salts (Figure 15.49-50).

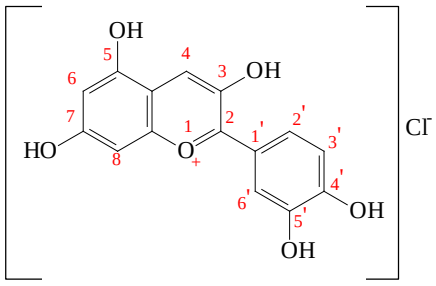


Figure 15.49
Cyanidin chloride (3,5,7,3',4'-pentahydroxyflavylium chloride)

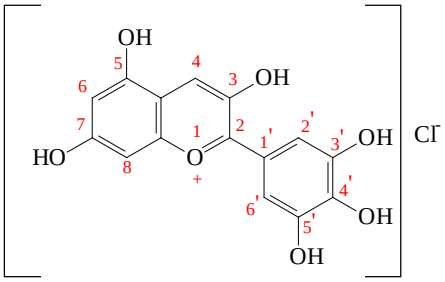


Figure 15.50
Delphinidin chloride (3,5,7,3',4'5'-hexahydroxyflavylium chloride)

- The most important agricultural plants containing anthocyanins are **grapes (*Vitis vinifera*)**; the anthocyanin-pigments are present in the red wine varieties.
- the following fruits containing anthocyanins in high quantity have pharmaceutical value:

Anthocyanin-pigments occur in a variety of plants and plant parts. Pharmaceutical value can be attributed to plant organs containing high levels of anthocyanins, such as the fruits of grape-vine (Figure 15.51), blueberry (Figure 2.48), black currant (Figure 15.52) and elderberry (Figure 15.53); the petals of Moorish mallow and cornflower; and the sepals of hibiscus or roselle (Figure 15.54).

Figure 15.51
Vitis vinifera (grape-vine) in bloom

Figure 15.52
Ribes nigrum (black currant)

Figure 15.53
Fruits of elderberry = *Sambuci fructus* (*Sambucus nigra*)

Figure 15.54
Roselle – calyx of *Hibiscus sabdariffa* (hibiscus)

15.8 Tannins

Characteristic features of tannins

- plant polyphenols, which precipitate proteins; tanning materials
- water-soluble
- if oxidized to quinones, can bind to proteins
- their quality and quantity determines the ability of plants against illnesses
- a great number of polyphenols are antibacterial, antimitotic and antiviral
- through their phenolic OH-groups their characteristic chemical reactions are:

condensation, ester- and glycoside-formation

- their functional effects are diversified
- according to their biogenesis they have two groups:

hydrolysable and condensed tannins

Condensed tannins or- proanthocyanidins: polymers of the flavan–3–ol

Hydrolysable tannins are called gallotannins; they are esters of gallic acid (Figure 15.10) or of digallic acid (Figure 15.55), formed with glucose. The main component of digallic acid is penta-meta-digalloyl-β-D-glucose (Figure 15.56).

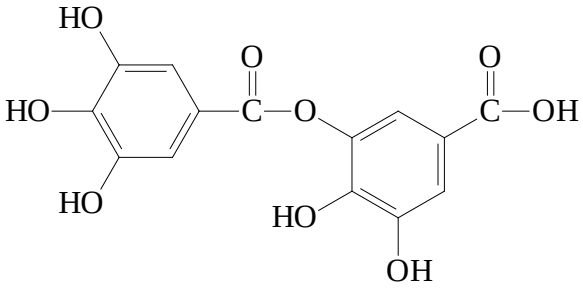


Figure 15.55
(meta)di-gallic acid (component of Chinese tannin)

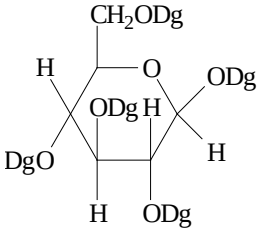


Figure 15.56
Penta-meta-digalloyl-β-D-glucose

- gallotanins occur mainly in the following plant families:
  - Ericaceae
  - Combretaceae
  - Anacardiaceae
  - Geraniaceae
  - Aceraceae
- ellagitannnins containing mainly dimer gallic acid occur in the plant families Rosaceae, Geraniaceae, Onagraceae and Lytharceae
- The bark of oak trees (Figure 15.57) and their galls contain hydrolysable tannins in high quantity. Further examples include common beech (Figure 15.58) and common hazel (Figure 15.59).

Figure 15.57
Bark and leaves of *Quercus* (oak) spp.

Figure 15.58
Fagus sylvatica (common beech)

Figure 15.59
Leaf and male catkin of *Corylus avellana* (common hazel)

15.9 Quinones

- Quinones include simple quinones, naphthoquinones, antraquinones and naphthodianthrones. Simple quinones rarely occur in nature, however, naphthoquinones are frequent in various plants. A well-known naphthoquinone derivative is juglone (Figure 15.60), occurring in walnut-tree (Juglandaceae), and having alleopathic effect.

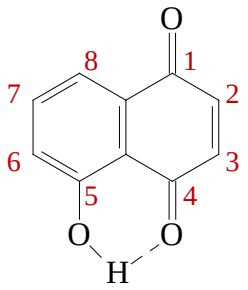


Figure 15.60
Juglone (5-hydroxy-1,4-naphthoquinone)

- juglone: occurs in all green parts of walnut-tree
- the structure of juglone is stabilized with H-bond (H-bridge) → new quasi six-membered ring
- forms with Cu^{2+} és Ni^{2+} - ions violet-coloured chelate-complexes (juglone : metal ion = 2 : 1)
- the occurrence of anthraquinone (Figure 15.61) derivatives is frequent in the following plant families:
  - Rubiaceae (madder sp.)
  - Fabaceae (legumes)
  - Polygonaceae (knotweeed or *Polygonum* sp.)
  - Rhamnaceae (buckthorn sp.)
  - Liliaceae
  - Scrophulariaceae

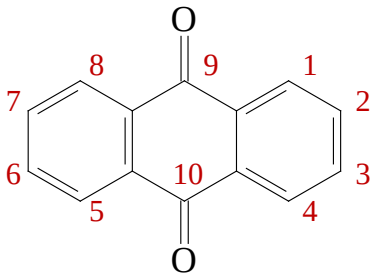


Figure 15.61
Anthraquinone (9,10-antraquinone)

- **glycosides of its hydroxy-derivatives are effective substances of medicinal plants having laxative effect.** Examples include senna (*Cassia*) species (Figure 15.62), aloes (Figure 15.63) and rhubarbs (Figure 15.64).

Figure 15.62
Cassia (senna) species

Figure 15.63
Aloe sp. (aloe)

Figure 15.64
Rheum rhabarbarum (rhubarb, pieplant)

- Hypericine is a compound with naphthodianthrone-skeleton (Figure 15.65), being the main active substance of St. John's wort (*Hypericum perforatum*, Hypericaceae) (Figure 15.66), used in the treatment of mild depression.
- Hypericine can exert photosensitizing effect, inducing allergic symptoms, particularly in sensitive individuals and at strong light

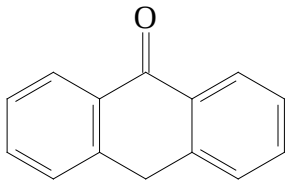


Figure 15.65
Anthrone

Figure 15.66
Hypericum perforatum (St. John's wort)

15.10 Terpenophenols

- The most well-known terpenophenols are cannabinoids, which are the effective substances of hemp (*Cannabis sativa*; Cannabinaceae) (Figure 15.67). The most significant one is tetrahydro-cannabinol (THC), which has hallucinogenic effect.
- In harle-hemp THC can be detected in lower quantities, whereas Indian hemp (*Cannabis sativa* subsp. *indica*) is characterised by higher amounts (> 1 %) of THC.
- drugs of *Cannabis sativa*:
  - hashish: THC-content: 5-20 %
  - marihuana: THC-content 2-6 %

Figure 15.67
Cannabis sativa (hemp)

- Hops (*Humulus lupulus*) (Figure 15.68) contain terpenophenol derivatives. The resin of hop strobile contains the bitter and sour substances humulone and lupulone, which are substituted derivatives of 4-cyclohexene-1,3-dione (Figure 15.69), with a complicated structure. Both compounds contain OH group (enolic OH group, acidic character). The other C-atoms bear substituents with complicated structures. Herbal preparations containing the extracts of hop strobile have moderate sedative effect.

Figure 15.68
Humulus lupulus (hop) and its strobili

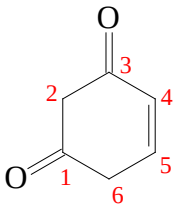


Figure 15.69
4-cyclohexene-1,3-dione

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