ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
FAKULTA TROPICKÉHO ZEMĚDĚLSTVÍ

Animal Chemical Ecology

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Animal Chemical Ecology

- **Chemical ecology - scope:**
- natural products of ecological importance, produced by organisms (animals, plants, microorganisms) and mediating communication between and among these organisms

- most natural products have more functions (effects)
- parsimony (Nature saves energy)
- dichotomy of effects (two or more functions of some natural products)
- different organisms use the same compound(s) for different functions, in different context
- biodiversity and chemodiversity

Contents

Ecology

relations among organisms or groups of organisms and their environment

Environmental science

pollution, factors influencing environment in a negative way

Chemical ecology

chemically mediated interactions between organisms,
communication between organisms

Types of interactions:

insect - insect (pheromones, allomones, kairomones)
insect - plant (host plant attractants, flower fragrance)
plant - plant ("SOS signals")
interactions between microorganisms
tritrophic and multitrophic interactions (plant-insect-insect; plant-insect-plant; plant-insect-pathogen)

Research in chemical ecology

- close cooperation of chemists and biologists
- good knowledge of the biology of organism whose signals are to be studied
- prepared bioassay (how the identified compounds will be tested)
- isolation of active compounds
- chemical analysis
- structure determination
- synthesis of active compounds
- bioassay – in the laboratory (behaviour)
- in the field

Communication

- exchange of information between and among organisms
- mediated by a set of signs or signals shared by both partners, the releaser and the receiver

Communication means of animals

optical

movements, mimesis, colour



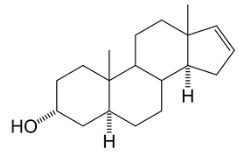

acoustic

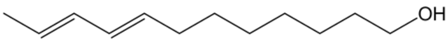
bird's singing and drumming,
insect stridulation

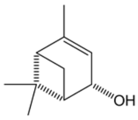

Communication means of animals

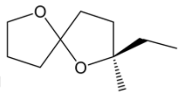
chemical

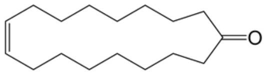
secretions of exocrine glands





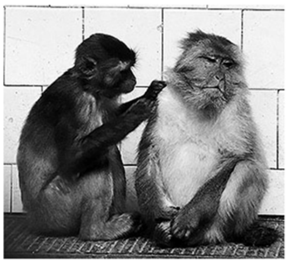






contact

contact signals in mating, daily hygiene

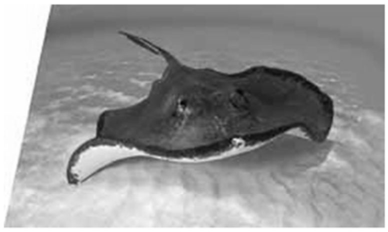


Contact – recognition in social insects (cuticular waxes of specific composition)

Communication means of animals

electrical

in some fish families



All organisms release chemical signals and react on odours of others

- chemical signals deliver life-important information quickly, simply, unambiguously


- lower importance in humans

- the youngest discipline in plants


Insect's senses of smell and taste

- search for food (prey)
- search for a partner, choice, mating
- choice of a spot/host for egglaying
- aggregation (before winter or to overcome resistance of a host)
- regulation of space and sufficient food
- alarm, defence, or attack
- organisation of social life

smell – transmitted by air (volatile compounds)
taste – contact (non-volatiles, water-soluble)

Semiochemicals
(chemical speech, information transfer

cells (immune response)

bakteria (chemotaxis)

algae (attraction of gametes)

plants (attraction of pollinators)

insects (sexual behaviour, regulation of social life)

vertebrates (dominance, territory marking)

humans (immunity, sexual behaviour)

Pheromones
pherein (to carry) horman (to excite)
(Karlson a Lüscher, 1959)
within a species

Allelochemicals
kairomones, allomones
synomones
between species

Infochemicals – modern equivalent to semiochemicals

Types of pheromones according to biological function

- Sex
- Aggregation
- Trail
- Alarm
- Marking (space)
- Identification (social)
- Dispersion

Types of pheromones

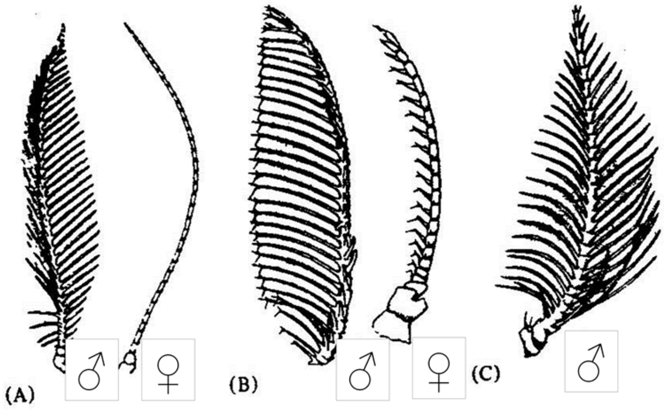
- **Sex pheromones – attraction of mates, stimulation of copulation behaviour.**
- **Aggregation pheromone** – attracts more individuals of the same species. Purpose: aggregation for overwintering, gathering at food source, or mass attack and overcome the host resistance (bark beetles).
- **Trail pheromones** – used by social insects to mark trails to food sources.
- **Alarm pheromones** – used by social insects in case of attack by predators.
- **Marking pheromones** – host etiketation to avoid multiple parasitizing, or territory marking.
- **Identification pheromones** distinguish separate colonies of social insects ("home odour").
- **Dispersion pheromones** are a signal for running in different directions.

Sex pheromone – attracts the opposite sex for mating

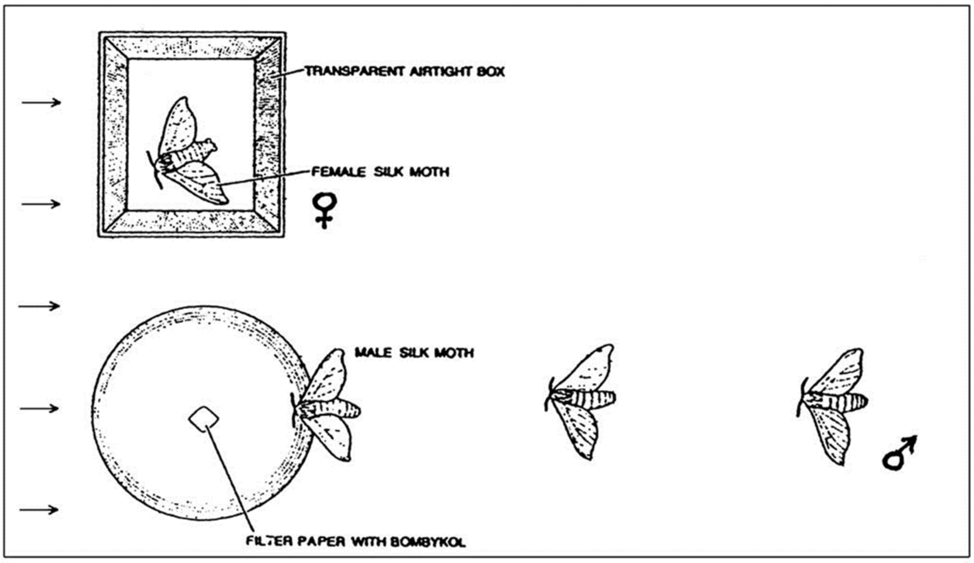
In moth, the sex pheromone producer is mostly the female.

Sexual dimorfism in moths, different shape of antennae

gipsy moth, male



Prove of existence and function of sex pheromone



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Aggregation pheromone – attracts individuals of both sexes at a long distance (bark beetles)

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Aggregation pheromone is a signal for a suitable site for food, mating, and development of brood.

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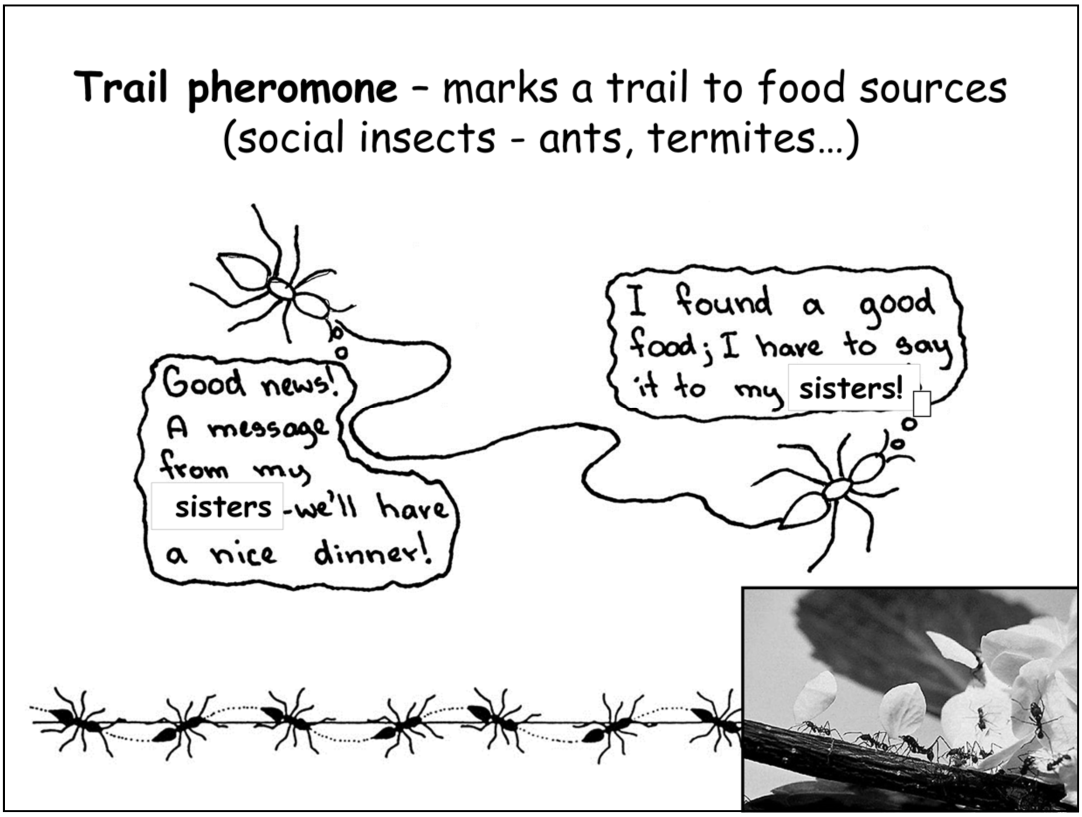

The concentration gradient tells directions (towards food source or towards home). Principle – volatility and stepwise evaporation of the pheromone.

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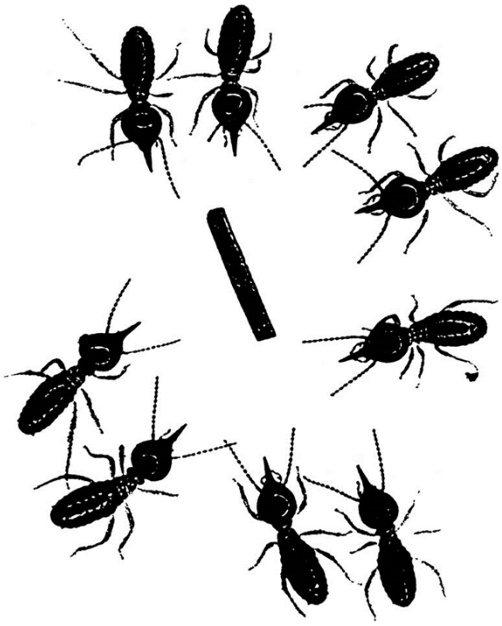
Alarm pheromone - releases defence behaviour in other colony members, specifically soldiers (mostly in social insects - ants, termites, but also in species living socially in some periods of their life – aphids, bugs)

Prove of existence and function of alarm pheromone (termite soldiers)



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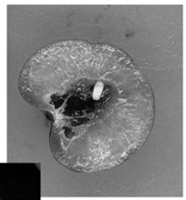
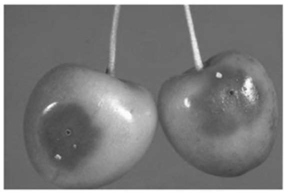
Marking (space) pheromone – marks an occupied territory (space), assuring enough food for the next generation

cherry fruit fly





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The term “marking pheromone” is also used for territory-marking signals (e.g. bumblebees)

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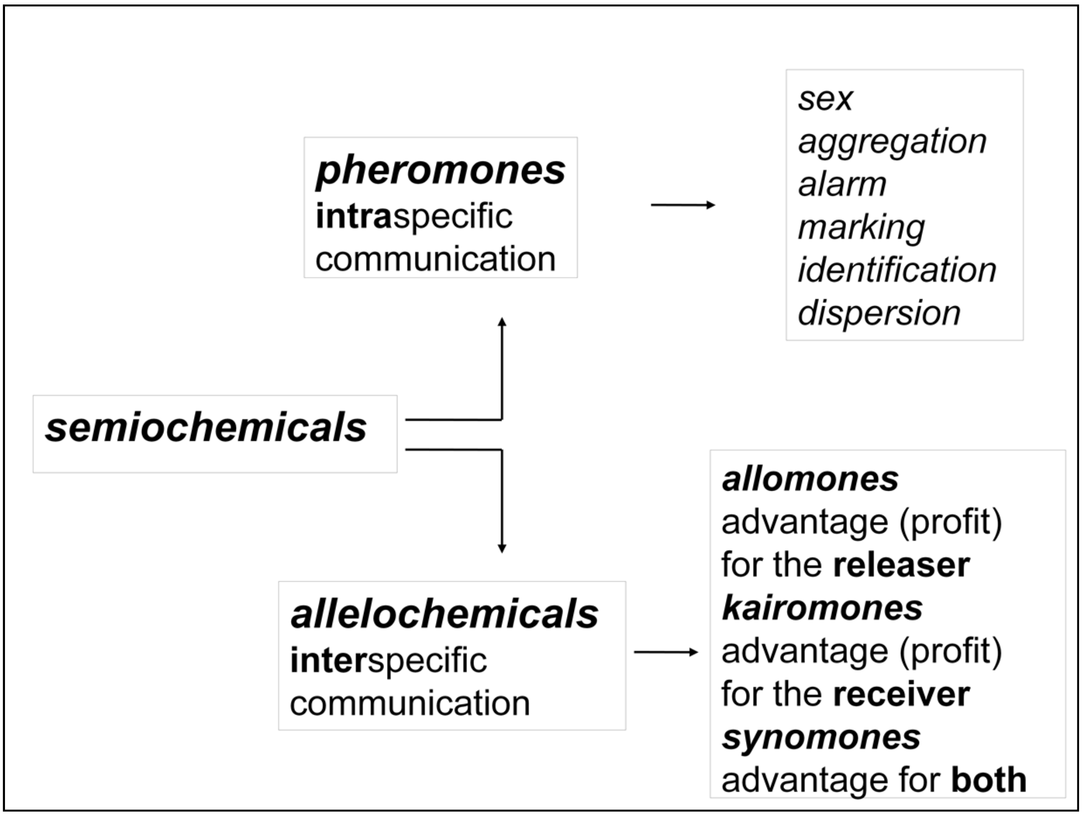
Alarm pheromone is sometimes a signal for dispersion, too.


pheromones
intraspecific communication
sex
aggregation
alarm
marking
identification
dispersion
semiochemicals
allelochemicals
interspecific communication
allomones
advantage (profit) for the releaser
kairomones
advantage (profit) for the receiver
synomones
advantage for both

ALLOMONE

chemical signal advantageous for producer (releaser)

Spider ***Mastophora cornigera***

produces a mixture of *Z*9-14:Ald, *Z*11-16:Ald, *Z*9-14:Ac, and *Z*11-16:Ac

predator of ~ 15 moth species that use these compounds as sex pheromone (attracts males)

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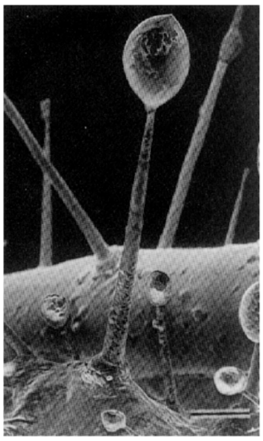
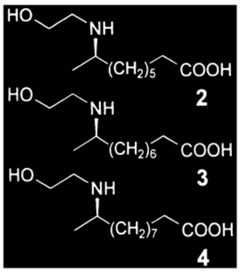
All defense compounds belong to allomones.

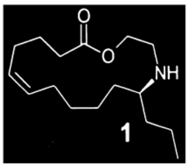
Chemical defence
(common, well studied)

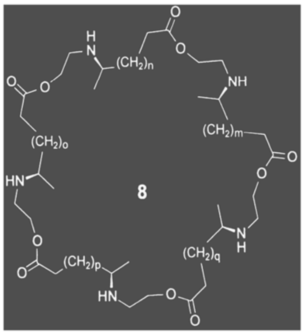
Ladybird pupa *Epilachna borealis*
covered by hollow hairs that secrete a sticky exudate containing a “combinatorial” mixture of alkaloids



Schröder, Science 1998







As s result, predators (birds, ants) avoid the bad-tasting pupa.

KAIROMONE

chemical signal advantageous for receiver

Parasitic wasp *Microplitis croceipes*
Caterpillar *Spodoptera exigua*

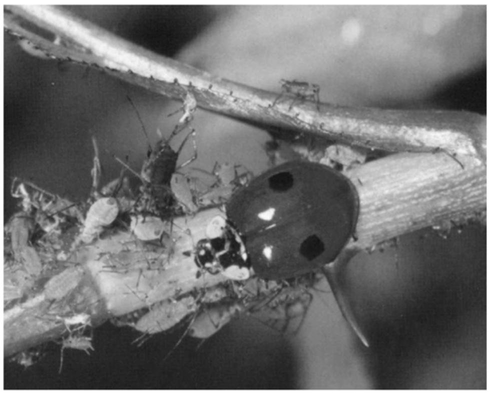
Caterpillar produces chemicals that make it "visible"

wasp localises the caterpillar based on chemicals from its faeces

parasitoids of insect eggs – a similar principle

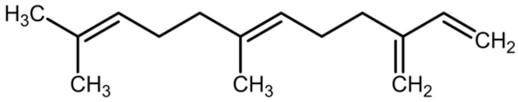
parasitoids of insect eggs register sexual pheromones of female moths and thus they easily localize the laid eggs of their prey

KAIROMONE
chemical signal advantageous for receiver



aphids × ladybirds

aphids produce alarm pheromone (*E*-β-farnesene) that attracts predators, ladybirds



(E)- β-Farnesene

SYNOMONE

chemical signal advantageous for both sides

typical example – flower fragrance

plant offers nectar
in flowers, insects pollinate
its flowers

BUT:

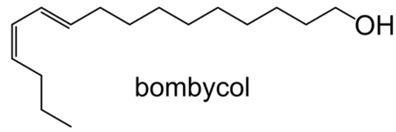
orchids (*Ophrys*) attract males of solitary bees by a mixture of compounds resembling the sex pheromone of con-specific females, but males are not awarded with nectar (pollination by deceit)

History of pheromones

- 1914 Fabre J.H. — males of moths are able to localise females for a long distance
- 1925-1939 — biological evidence of species-specifity of female attractants
- 1939 Butenant A. — first attempt to isolate attractant of the silk moth
- 1950-1961 — isolation and identification of bombycol
- 1961 — synthesis of all 4 possible isomers
- 1959 — Karlson+Lüscher introduced the term „pheromone" (Nature 183, 55)

silk moth

Isolation of first sex pheromone



- 10^6 pupae of silk moth
- glands of 5×10^5 females dissected
- obtained 280 g of lipidic extract
- neutral fraction 125 g
- repeated extraction, transesterification, precipitation, and recrystallisation gave a crude fraction of 4'-nitrobenzen-4-carboxylic acid esters (9 steps), obtained 5,6 g
- more separation steps and recrystallisation led to 6 mg of nitroazobenzencarboxylate of bombycol
- spectral analysis (IR, UV) and derivatisation led to the structure determination
- synthesis of all 4 isomers

Pheromones of moths

- **Molecular weight 100-300** represents a suitable volatility, but also a sufficient number of structural variants (10-18 carbon atoms)
- **Most common types of compounds** – aliphatic alcohols, esters, aldehydes, ketones, acids
- **Less common** – epoxides, ketals, acetals, phenols
- **High species specificity** – different chain length, number of double bonds, regioisomers, stereoisomers; more components in specific ratio
- Related species usually have similar pheromones (similar biosynthetic pathways)

Pheromones of moths

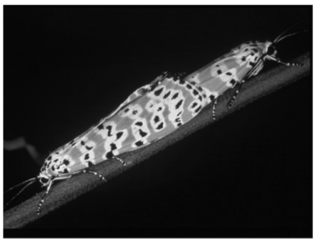
	Females	**Males**
Purpose	Reproduction	Attraction, competition
Localisation	Abdominal tip	Various
Strukture	Aliphatic comp.	Often heterocykles
Amount / gland	< 1 μg	> 1 μg
Biosynthesis	Fatty acids	Food precursors
Duration	Short to long	Very short
Receptors	Male antennae	Antennae of both sexes
Specificity of receptors	All components	Main components only

Biosynthesis is controlled hormonally (PBAN)

Chemistry in the life of the rattlebox moth, *Utetheisa ornatrix*

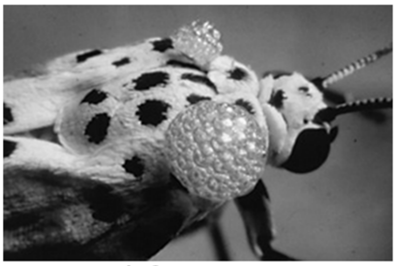
T. Eisner & J. Meinwald, Cornell University, USA

adult



mating

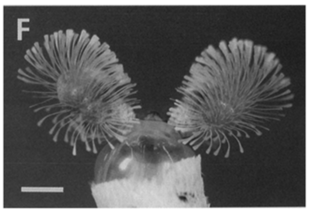
larva
on the host plant
Crotalaria spectabilis



defence

Female pheromone of *U. ornatrix*

coruship



male coremata

- Female calls at the sunset, emitting a pheromone in 1,5 sec pulses.
- Attracted male touches the female with his abdominal hairs (coremata). Acceptance results in mating.

components of female pheromone

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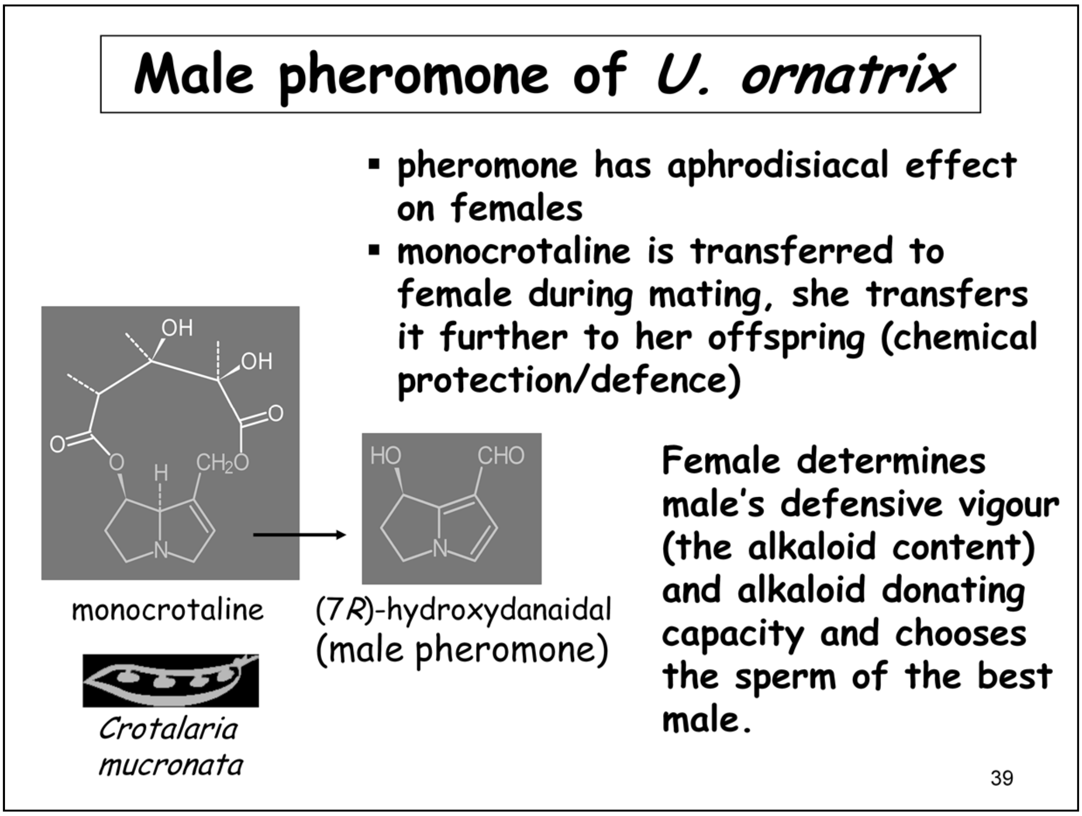
Eisner T. & Meinwald J.: The chemistry of sexual selection. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 50-55.



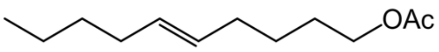
Utetheisa

Females mate more than once, they store sperms that are combined with the accumulated alkaloid. According to the quality of the male pheromone, she decides which sperm to use for fertilization of eggs.

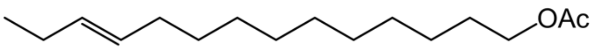
She uses alkaloid for protection of laid eggs.

Types of compounds in pheromones of different animals

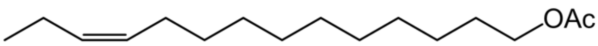
Moths (Lepidoptera)



peach twig borer
Anarsia lineatella



European corn borer
Ostrinia nubilalis



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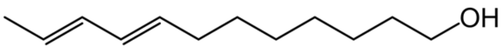
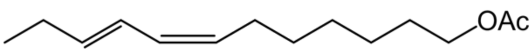
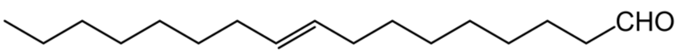


codling moth
Cydia pomonella

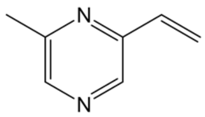


European grapevine moth
Lobesia botrana

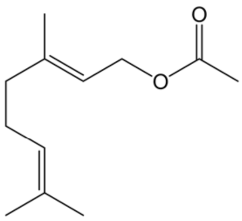


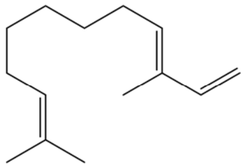
cotton bollworm
Helicoverpa armigera

Flies (Diptera; fruit fly = vrtule)

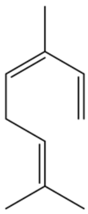


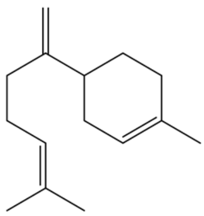
Papaya fruit fly



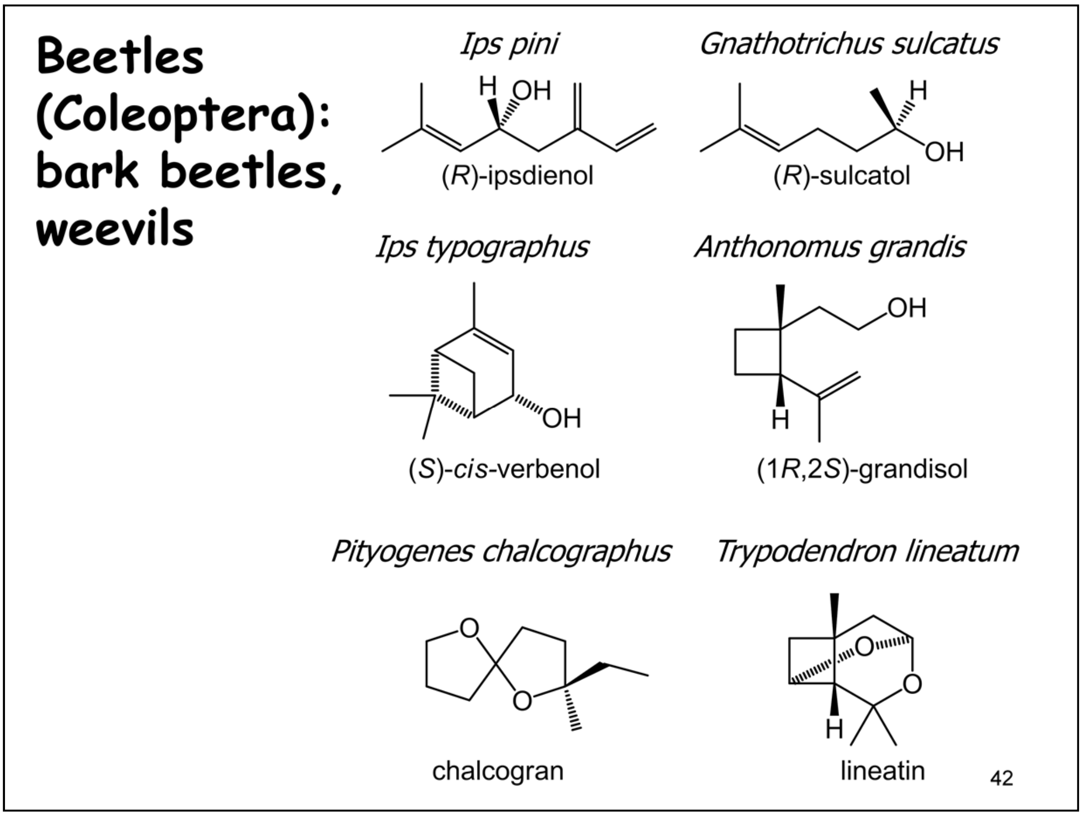


Mediterranean fruit fly



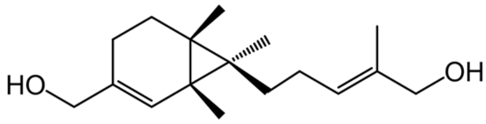


Caribbean fruit fly



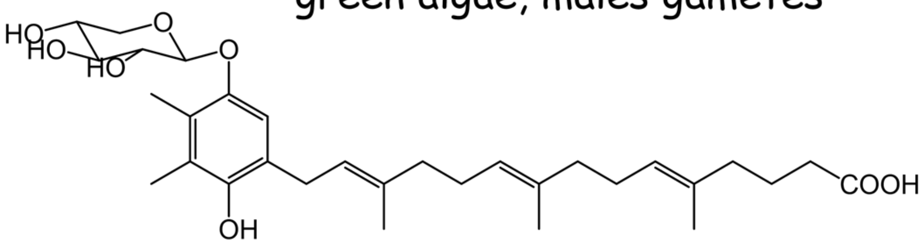
Anthonomus – pest on cotton

Algae and seaweed



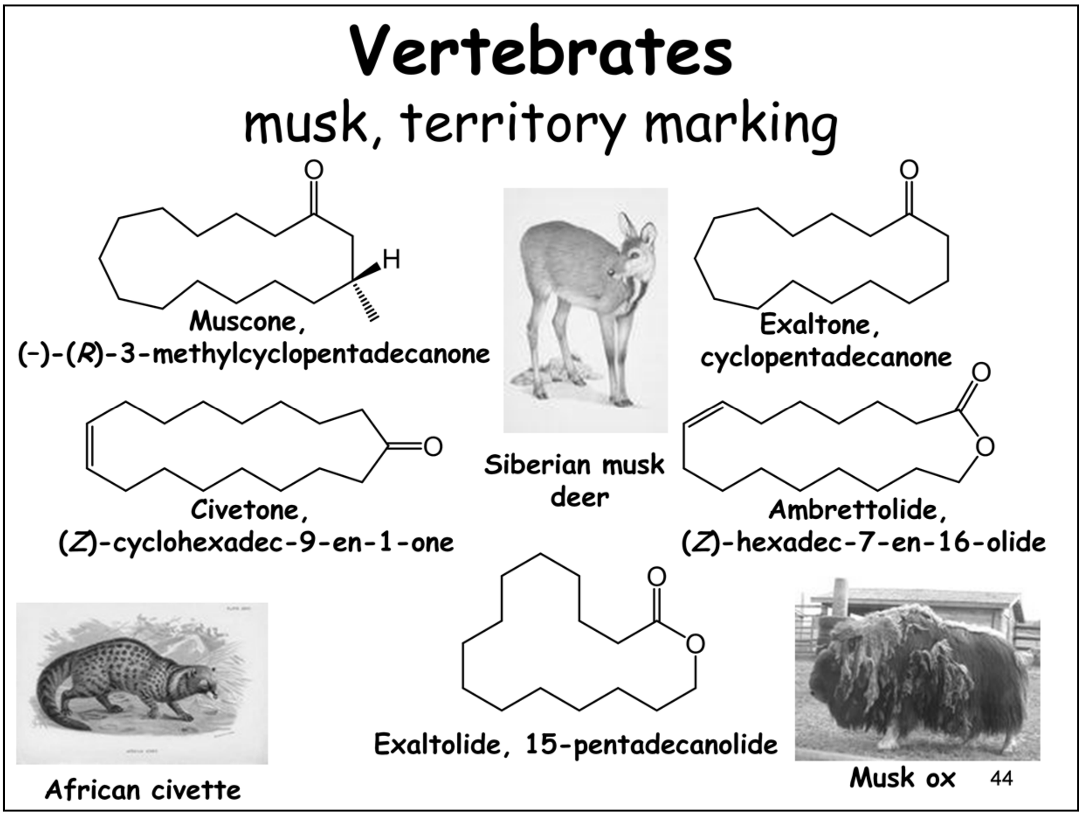
sirenin, *Allomyces*

green algae, males gametes



lurlene, *Chlamydomonas eugametos*

seaweed



muscon – Moschus moschiferus L., kabar pižmový, žije v Himalajích, velikost srnce

civetone – Viverra zibetha L., civetka africká

exaltone – Viverricula indica Desmarest, civetka indická

ambrettolid, exaltolid – pižmové vůně z rostlin (ibišek, angelika)

Family Moschidae

Himalayan Musk Deer, *Moschus chrysogaster*

Siberian Musk Deer, *Moschus moschiferus*

Dwarf Musk Deer, *Moschus brezovskii*

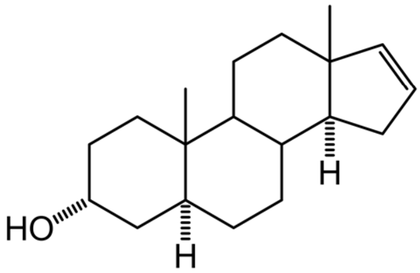
Black Musk Deer, *Moschus fuscus*

The variety which appears in commerce is a secretion of the musk deer; but the odor is also emitted by the musk ox and muskrat of India and Europe, by the Musk Duck (*Biziura lobata*) of southern Australia, the musk shrew, the musk beetle (*Calichroma moschata*), the alligator of Central America, and by several other animals.

In the vegetable kingdom it is present in the musk flower (*Mimulus moschatus*), the musk wood of the Guianas and West Indies, and in the seeds of *Abelmoschus moschatus* (musk seeds).

To obtain the perfume from the musk deer, the animal is killed and the gland completely removed and dried, either in the sun, on a hot stone, or by immersion in hot oil. It appears in commerce as "musk in pod" (i.e. the glands are entire) or as "musk in grain" (in which the perfume has been extracted from its receptacle).

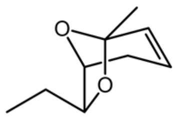
Humans, steroid compounds



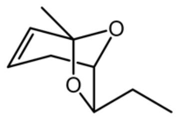
vomeroferine

Structure similarities

mouse, *Mus domesticus*

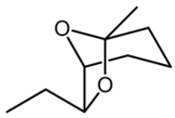


(*R,R*)-3,4-dehydro-
exo-brevicomine

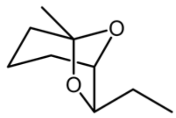


(*S,S*)-3,4-dehydro-
exo-brevicomine

bark beetles, *Dendroctonus spp.*

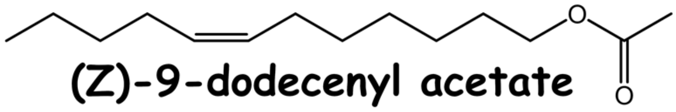


(*R,R*)-*exo*-brevicomine



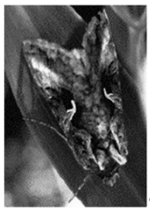
(*S,S*)-*exo*-brevicomine

Parsimony
(dichotomy of effects)



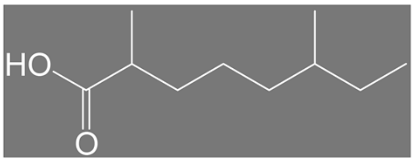
(Z)-9-dodecenyl acetate

Female sex pheromone
of many moth species
and the female sex signal
of African elephant.



Parsimony

(thriftiness, economy)

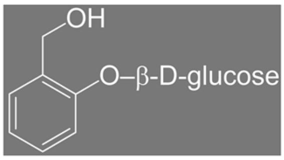


2,6-dimethyloctanoic acid

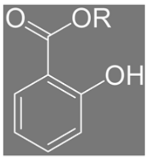
component of wax in some birds

- feathers impregnation
- disinfection (strong antibiotics)

Salicylic acid and its derivatives



salicine



salicylic acid
methyl salicylate

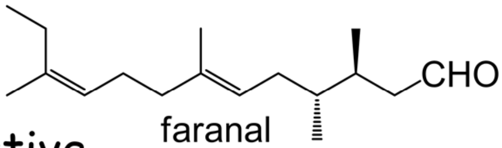
bark, leaves, and flowers of willow (*Salix*),
bark and leaves of evergreen (non-deciduous) birch

- antipyretic effect in humans
- pyretic effect in flowers (heat production)
- „*SOS*“ signalling in plants

Chiral compounds as pheromones

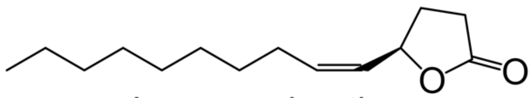
A. Only one enantiomer is produced

1. antipode is less active or inactive
   most cases



faranal

2. antipode is equally active
   several cases
3. antipode is inhibitor
   2 cases

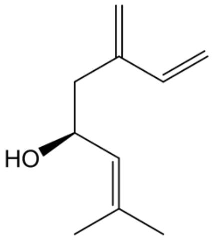


Japanese beetle

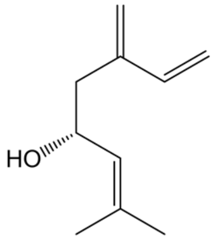
1 – *Monomorium pharaonis*, ant

3 – *Popillia japonica*, Japanese beetle

Different species of one genus use opposite enantiomers



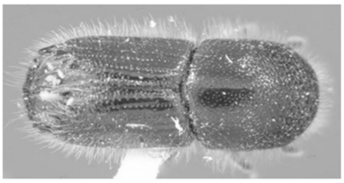
(+)



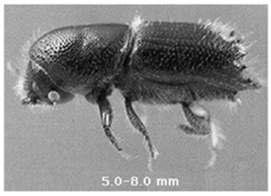
(–)

ipsdienol

Ips paraconfusus



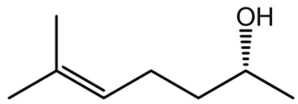
Ips calligraphus



Chiral compounds as pheromones

B. Both enantiomers are produced

1. optimal response to natural ratio of enantiomers
   most cases



sulcatol

2. response to one of them is stronger than to the natural ratio
   one case
3. one enantiomer inhibits
   not known

sulcatol – bark beetle *Gnathotrichus sulcatus*

One enantiomer attracts males, the other one females

(*R*)-(–)-oleane
males

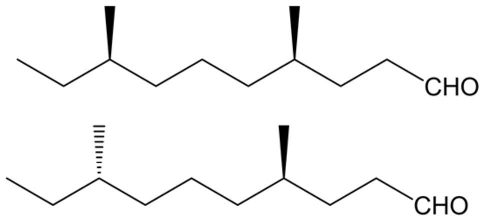
(*S*)-(+)-oleane
females

fruit fly *Dacus oleae*,
pest on olives

53

Dacus oleae, olive fly

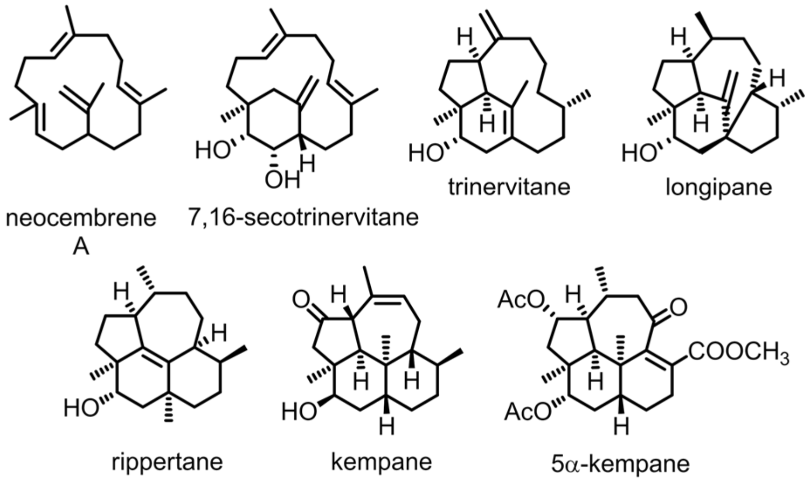
One isomer is active, another one (unnatural) increases activity



Tribolium castaneum,
rust red flour beetle

Defence substances

structure examples

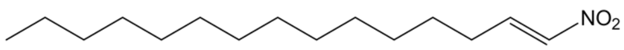


termite soldiers use chemical weapons (Nasutitermitinae)

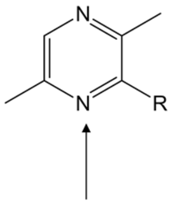
55

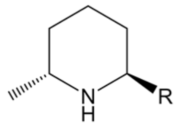
Šobotník J, et al.: Chemical warfare in termites. *J. Insect Physiol.* **2010,** *56,* 1012-1021.

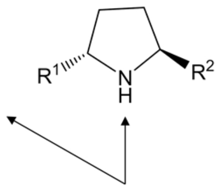
Other social insects



(E)-1-nitropentadec-1-ene
termites, *Prorhinotermes simplex*







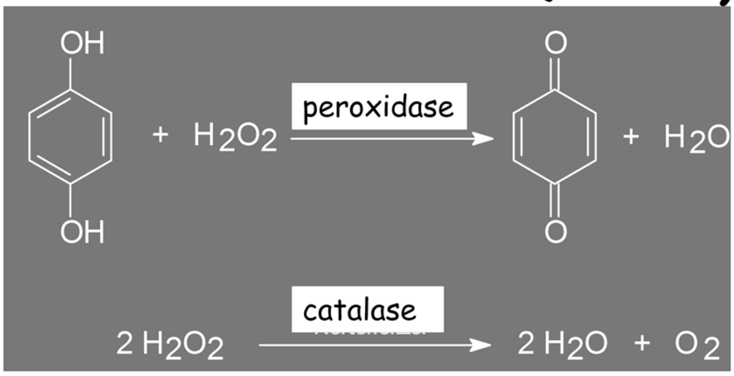
ants, families:

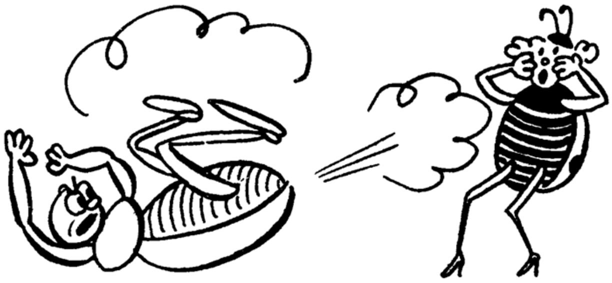
Poneridae

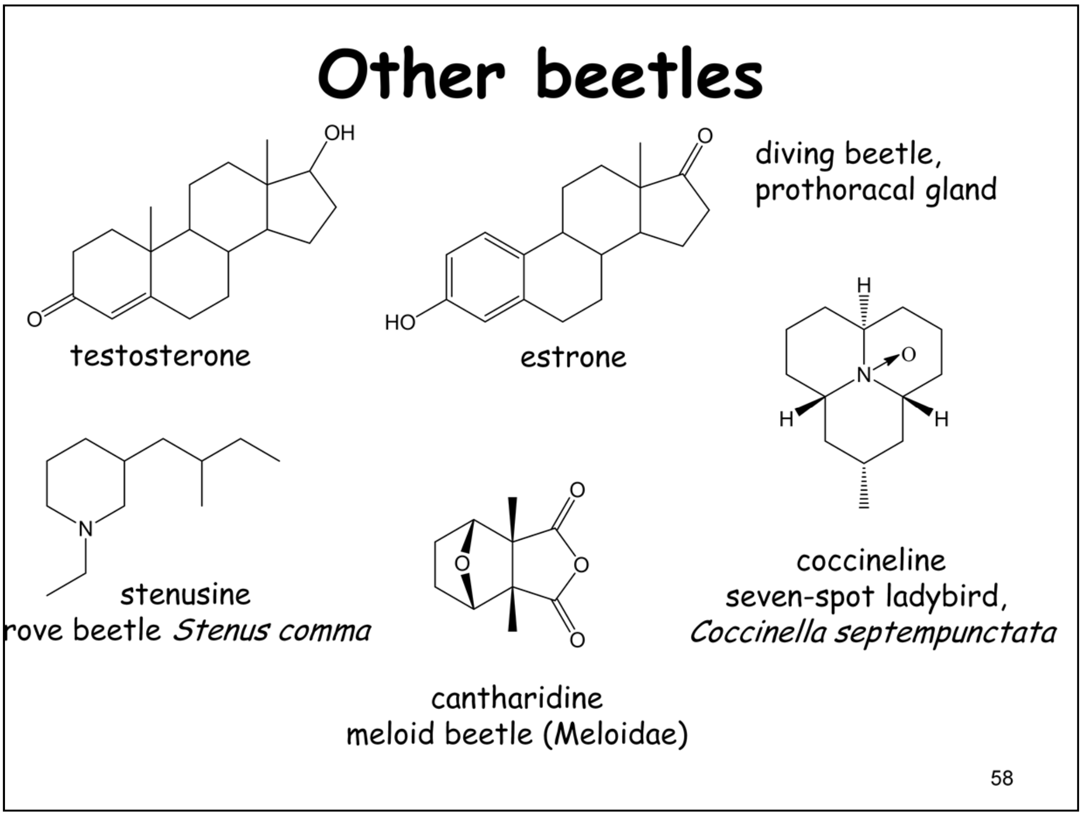
Myrmicidae

Šobotník J, et al.: Chemical warfare in termites. *J. Insect Physiol.* **2010,** *56,* 1012-1021.

Bombardier beetles (*Brachynus*)

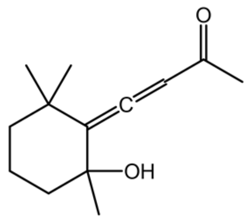






Cases of intoxication of children with cantharidine are reported.

Structural curiosities as insect defence substances



romallenone
grasshopper *Romalea microptera*

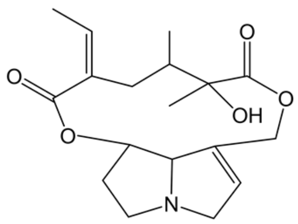
NH_3, 4,5 %

burying beetle,
Nicrophorus vespilio

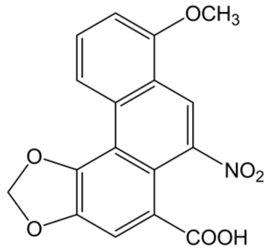
HCN

millipedes,
Chilopoda and Diplopoda

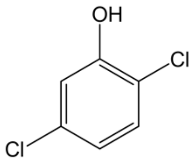
Sequestration of food



senecionine
moth *Callimorpha jacobaeae*



aristolochic acid
butterfly *Pachlioptera aristolochiae*



grasshopper *Romalea microptera*

Why to study pheromones?

- many insect species are serious pests, causing great damage on crops :
- cereals
- fruits and vegetables
- stored food (flour)
- forests and wood
- fabrics and clothes (wool, furs)

- **Insecticides** – chemicals killing insects (non-selectively)
- Environment-friendly methods of plant protection are needed
- **I**ntegrated **P**est **C**ontrol *or* **I**ntegrated **P**est **M**anagement)

Disadvantages of the use of insecticides

- application of large amounts of synthetic chemicals that stay in the environment unchanged
- toxicity, non-specific to target pest
- repeated application is necessary
- development of resistance
- killing of beneficial insects

Advantage of the use of pheromones

- low concentrations
- specific to the target insect
- non-toxic

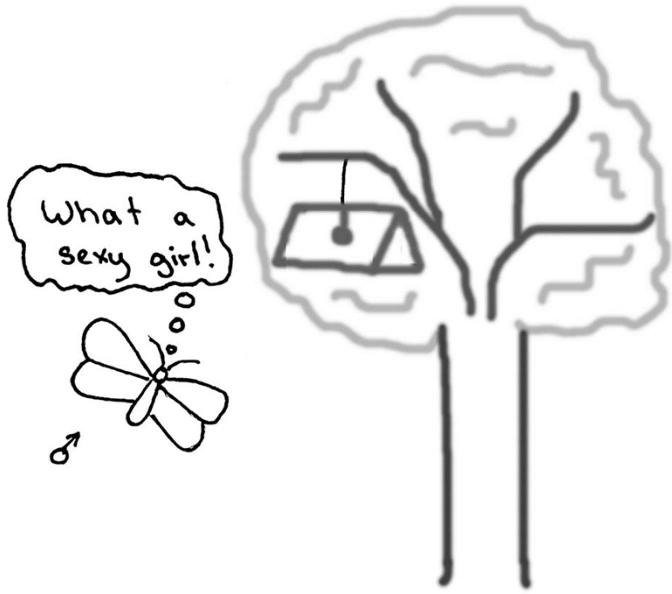
Disadvantage of the use of pheromones

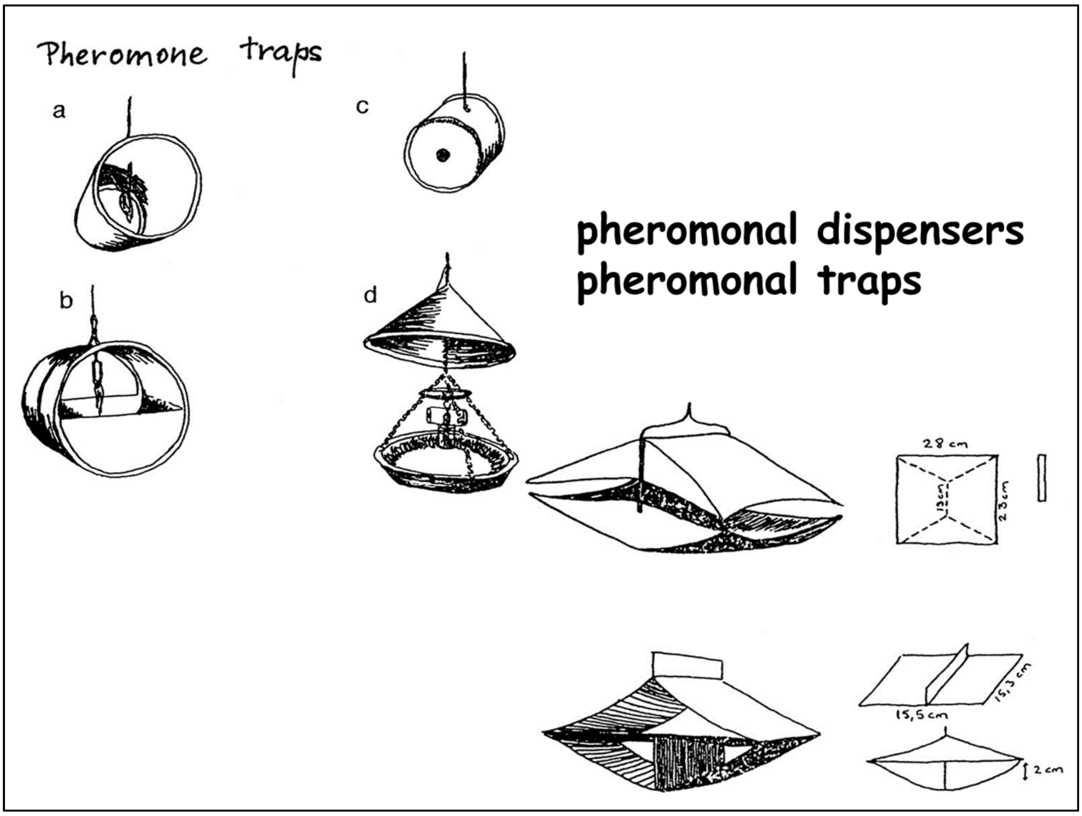
- expensive production
- demands on formulation, traps checking, evaluation (qualified management)

Practical use of pheromones

- **Monitoring** of the flight and population density of pest insect; pheromone trap replaces a female (in moths) and lures males; pheromonal dispensers and traps.
- **Mass trapping** – use of aggregation pheromone in case of a high population density; lures both sexes (*attract and kill*).
- **Mating disruption** – “high” concentration of sex pheromone; males are unable to localize a female and thus, no mating occurs.

Monitoring of the flight and population density of insects; pheromone trap replaces a female (in moths) and lures males; pheromonal dispensers and traps.

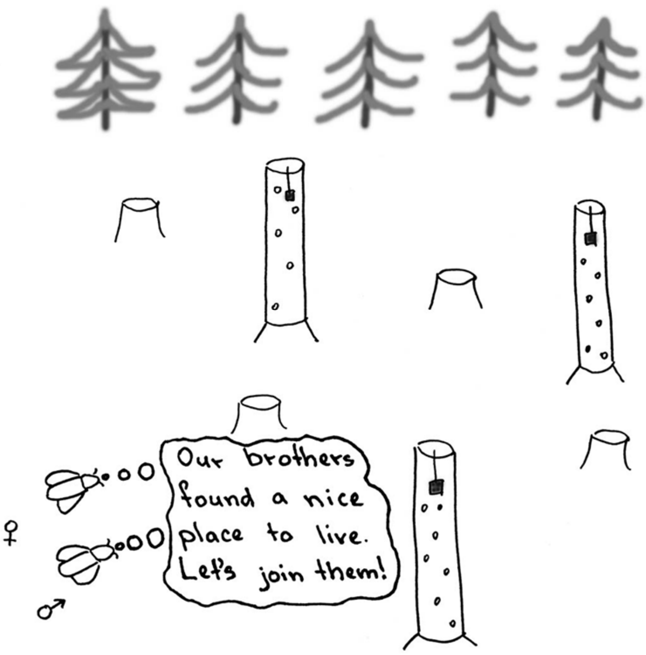




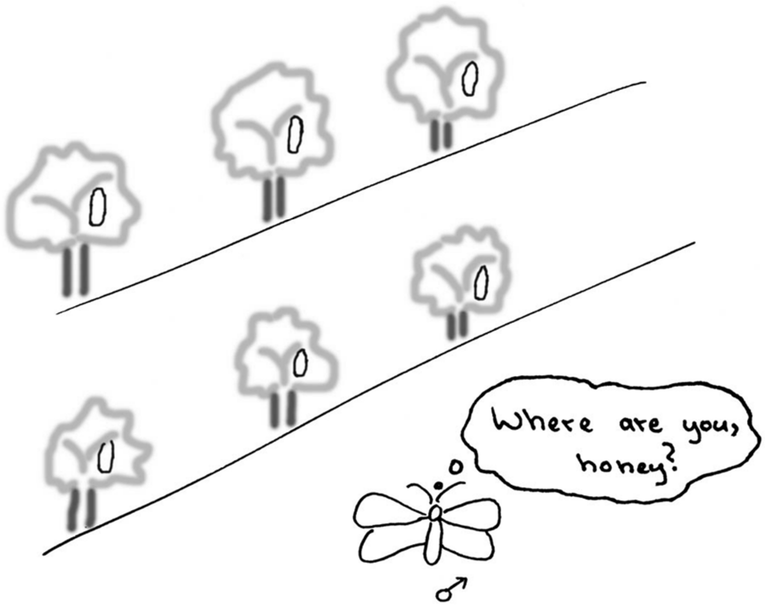
Pheromone traps
a
b
c
d
pheromonal dispensers
pheromonal traps
28 cm
23 cm
13 cm
15,5 cm
15,3 cm
2 cm

Mass trapping – use of aggregation pheromone in case of a high population density; lures both sexes

Attract and kill – combination with insecticide



Mating disruption – "high" concentration of sex pheromone; males are unable to localize a female and thus, no mating occurs.



Used extensively to protect apples in orchards (pest: *Cydia pomonella*) and grapes in vineyards (pest: *Lobesia botrana*).

World use of IPM

Monitoring	32.1 %
Mass trapping	23.3 %
Attract-and-kill	2.2 %
Mating disruption	42.4 %

Data from nineties, 25 countries. Pheromone-treated areas represented 1 % of all fields.

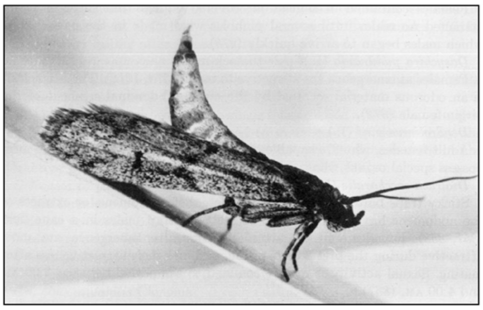
Techniques of isolation and analysis in the natural product research

- Sample preparation, isolation techniques
- Identification methods, structure elucidation
- Determination of absolute configuration (stereochemistry) of natural products in microscale
- Biological methods of testing activity of isolated natural products

Methods in Chemical Ecology. Vol. 1. Chemical Methods. J. Millar & K. F. Haynes, Kluwer Academic Publishers, London 1998, second printing 2000.

Good knowledge of the life cycle of the selected organism

- does it produce chemical signals?
- when does the production reach its maximum?
- which organ/tissue/gland produces the signal?



female moth calling

Sample preparation

- hydrodistillation (essential oils)
- solvent extraction (universal)
- „head-space“ techniques (volatile compounds)
- solid-phase microextraction, SPME (volatile compounds)
- solid sample injection (insect glands)

Hydrodistillation, steam distillation

- Clevenger apparatus
- plant material cut in pieces, boils in water
- essential oil distil off together with steam, forming the upper layer

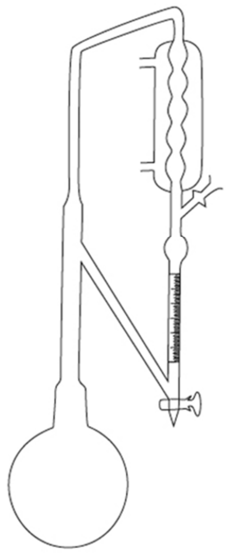


Figure 1. Clevenger-type apparatus — *Appareil de type Clevenger.*

Hydrodistillation, steam distillation

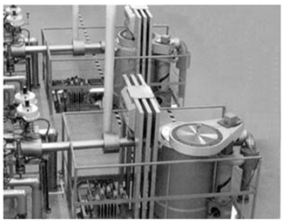
Advantage	Disadvantage
large scale possible	danger of artefacts (oxidation)
	suitable for plants, not insects

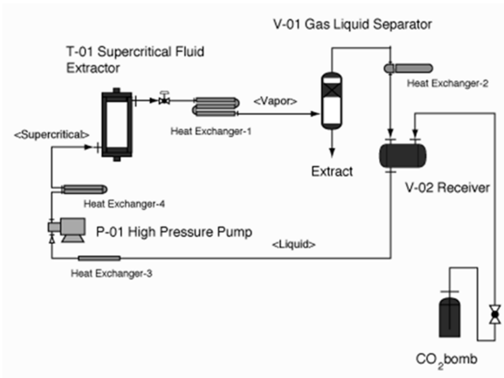
Solvent extraction

Advantage	Disadvantage
simple	presence of balast compounds
possible to repeat	pure solvents needed
analysis	sometimes a low concentration
	(amounts produced in the moment
	of extraction)

Supercritical fluid extraction

- fluid, which has the ability of dissolution at the supercritical pressure and the supercritical temperature
- most often used CO_2
- higher dissolving capability for various substances
- extraction is fast





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Supercritical fluid extraction technique is a new separation technique, which is developed by use of the fluid, which has the ability of dissolution at the supercritical pressure and the supercritical temperature. The supercritical fluid extraction technique has many characteristics, such as high extraction efficient, simply separation technology, no need solvent recovery equipment, easy operation condition, widely used future, etc. Supercritical fluid extraction technique is always completed in the room temperature. Since it is no poisonous, no residual, and green manufacture, there are more and more studies and applications about it home and abroad in recent years.

Physical properties of supercritical fluid carbon dioxide

The density is similar to that of a liquid, and offers higher dissolving capability for various substances.

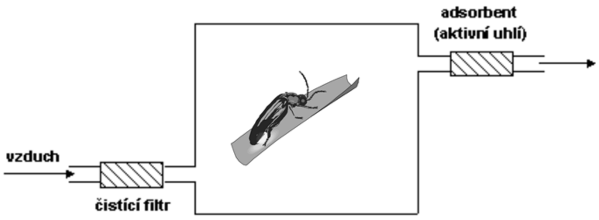
Because the viscosity is similar to that of a gas and the diffusion coefficient is larger than that of a liquid, substance extraction is faster.

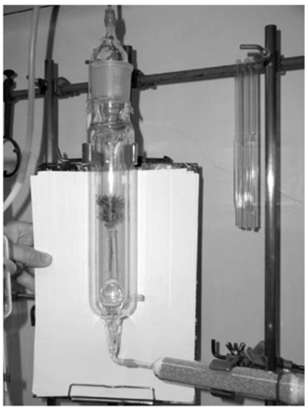
Supercritical fluid extraction

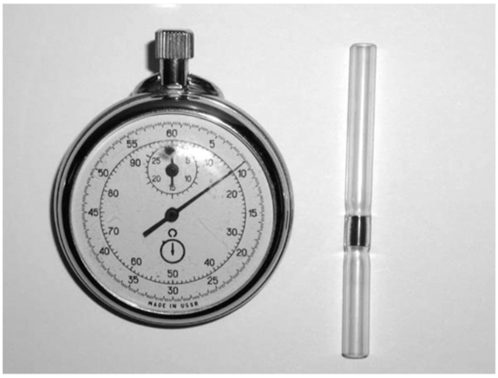
Advantage	Disadvantage
good extraction potential	expensive apparatus
room temperature	CO_2 is a greenhouse gas
reuse of solvent	
CO_2 - green chemistry	
safe in food processing	
cheap and easy to handle	
large scale possible	

„Head-space“ techniques

- static
- dynamic





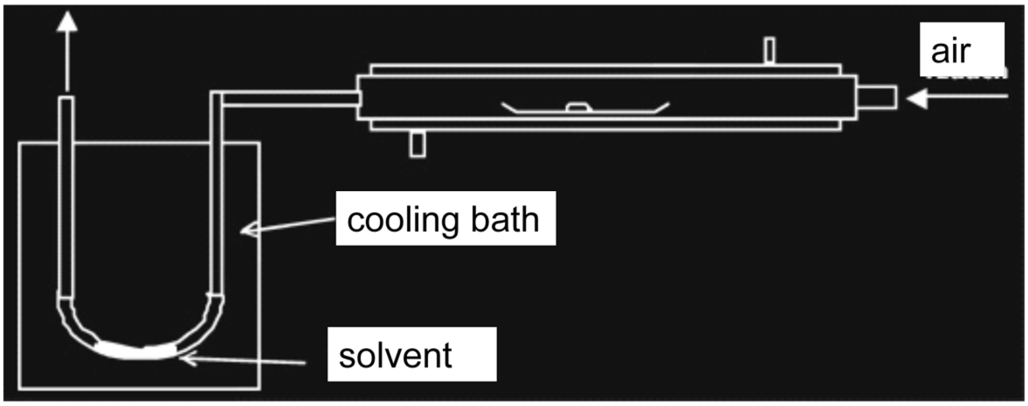


◆ **Trapping volatiles:**

◆ **sorbents:**
◆ charcoal
◆ Porapak Q
◆ Tenax

◆ elution with solvent
◆ freezing out

„Head-space“ techniques



„Head-space“ techniques

Advantage	Disadvantage
accurate composition	apparatus needed
higher concentration	pure solvents needed
compared to the extraction	danger of „break-through“
possible to repeat	danger of contamination
analysis	of the loop
closed loop possible	

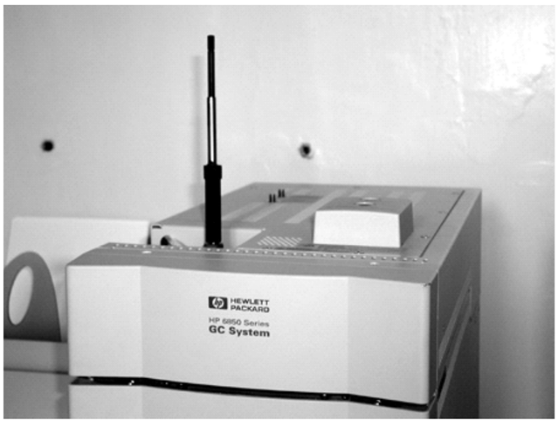
Codling moth (*Cydia pomonella*)

gland	head-space
10:OH	10:OH
12:OH	12:OH
E9-12:OH	E9-12:OH
E8E10-12:Ald	-
E8E10-12:Ac	-
E8E10-12:OH	E8E10-12:OH
Z8E10-12:OH	Z8E10-12:OH
E8Z10-12:OH	E8Z10-12:OH
14:OH	14:OH
16:OH	16:OH
18:OH	18:OH
18:Ac	-
20:Ac	-

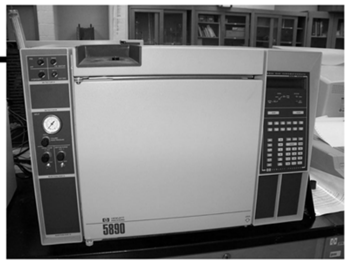
Solid phase microextraction

- SPME
- developed originally for trace analysis of organic compounds in water
- adsorption on a thin film of polysiloxane
- thermal desorption in GC injector

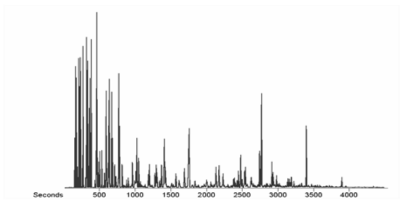
SPME

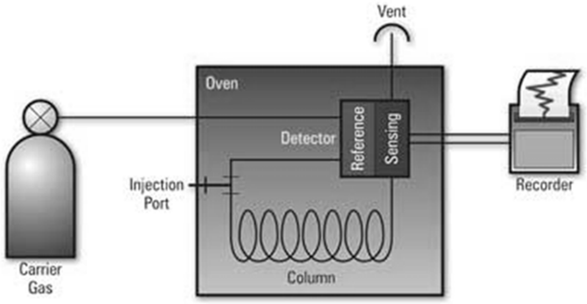


Gas chromatography



- sensitive analytical technique
- potent in separation complex mixtures
- evaporation of the sample in a heated injector
- capillary silica chromatographic column (standard 30 m x 0.25 mm)
- siloxane bound to the column walls
- carrier gas – He, H_2, N_2
- flame ionisation detector or selective detectors





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Gas chromatography (GC) is one of the most widely used techniques in modern analytical chemistry. In its basic form, GC is used to separate complex mixtures of different molecules based on their physical properties, such as polarity and boiling point. It is an ideal tool to analyze gas and liquid samples containing many hundreds or even thousands of different molecules, allowing the analyst to identify both the types of molecular species present and their concentrations.

Gas chromatography is a very sensitive method for the separation and quantification of chemicals. Like in any other chromatographic technique, separation of compounds depends on their partition between a stationary and a mobile phase. _In gas chromatography, the mobile phase is a gas that is moved through the column, while the stationary phase is a liquid film that coats the column filling (in packed columns) or the column wall (in capillary columns). Hence, the correct name for gas chromatography is "Gas Liquid Chromatography", abbreviated GLC. Compounds are injected onto the column and carried through it by the mobile phase; depending on their partition into the stationary phase, they move slower or faster. A sensitive detector is required at the end of the column to detect and quantify the compounds as they leave the column.

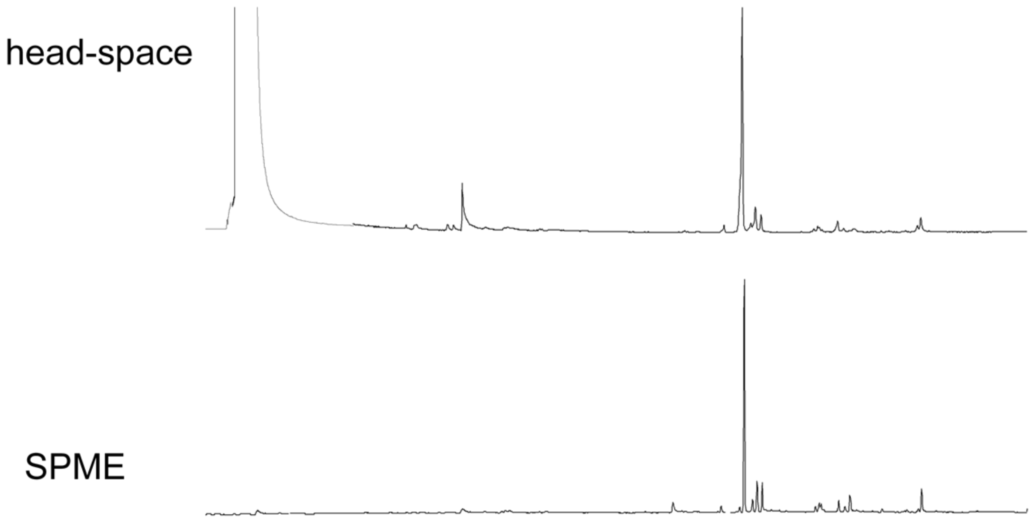
Compounds must be present in the gas phase so that partition between the gaseous mobile phase and the liquid stationary phase is possible. Thus, GLC must be carried out at temperatures above the boiling point of the compounds to be separated.

The mobile phase in GLC is an inert gas (nitrogen, helium, hydrogen). If one is interested in achieving good separation of mixtures, is is advantageous to use a temperature program: For the first part of the run, the column temperature is low; after the short fatty acids have passed the column, the temperature is gradually increased until all components have left.

SPME

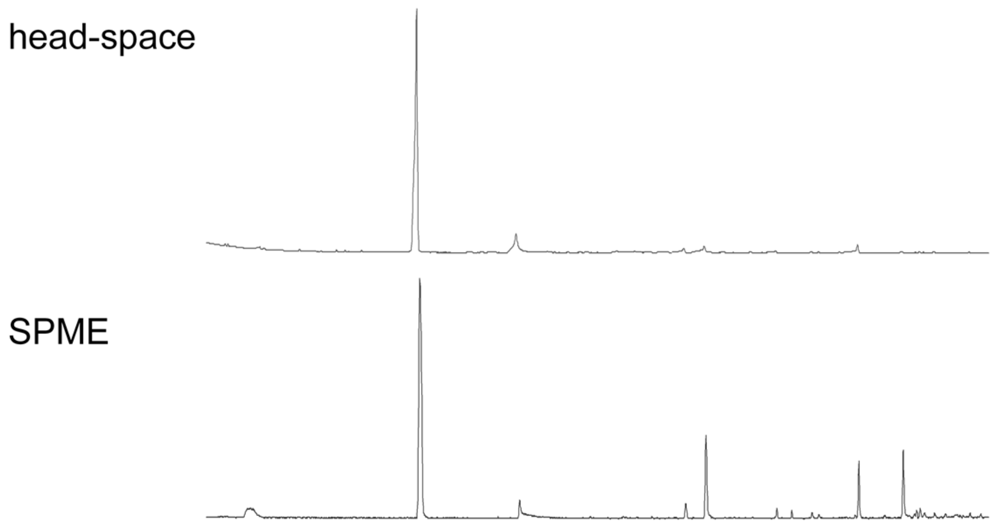
Advantage	Disadvantage
without solvent	only one analysis
high sensitivity	equipment needed
simple	
use in the field possible	

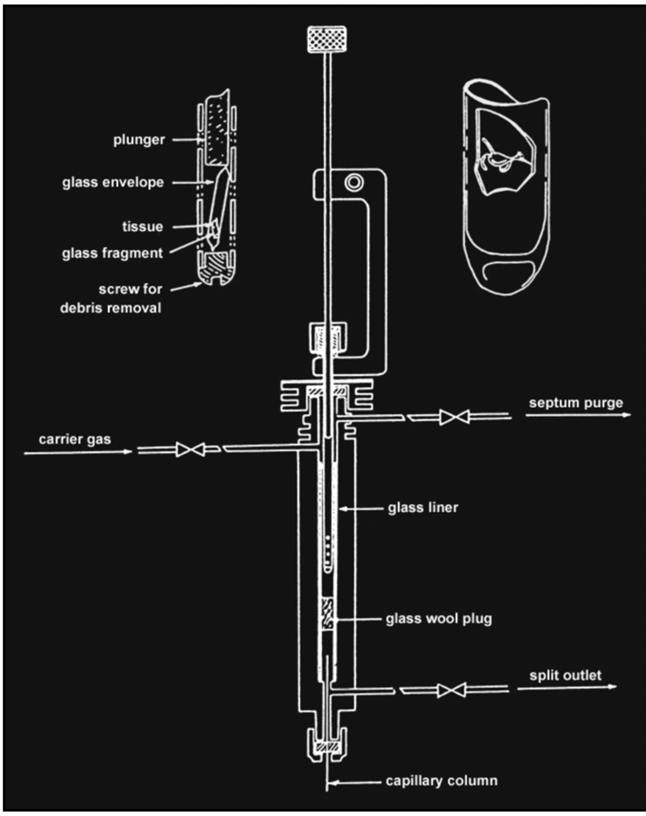
Chromatograms - DHS and SPME



SPME without solvent, enables to analyze the most volatile components

Small („light“) molecules discriminated (preference of „havier“molecules)





Solid sample injection

- biological material (gland) sealed in a capillary
- injection port adapted
- vaporisation of volatiles in the injector directly

Solid sample injection

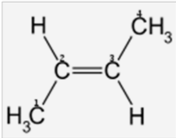
Advantage	Disadvantage
without solvent	only one analysis
no loss of compounds	injector port specially adapted
	cleaning of injector needed

Structure elucidation

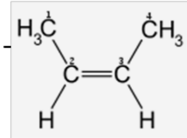
- classical spectral methods used in organic chemistry (IR, NMR, MS, UV, CD) – larger amount of sample (mg)
- derivatisation, degradation, X-ray
- „hyphenated techniques“ (GC-MS, LC-MS, GC-IR) – small amount of sample (µg)
- GCxGC-MS (2D-GC-MS, latest technique)
- 2D-GC determination of absolute configuration (standards)

Stereochemistry

- double bond configuration (geometry)

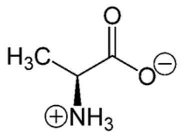


(*E*)-But-2-ene (*trans*)

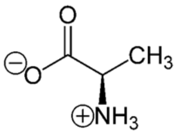


(*Z*)-But-2-ene (*cis*)

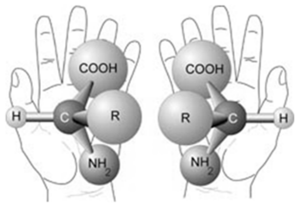
- absolute configuration (chirality, enantiomer, antipode)



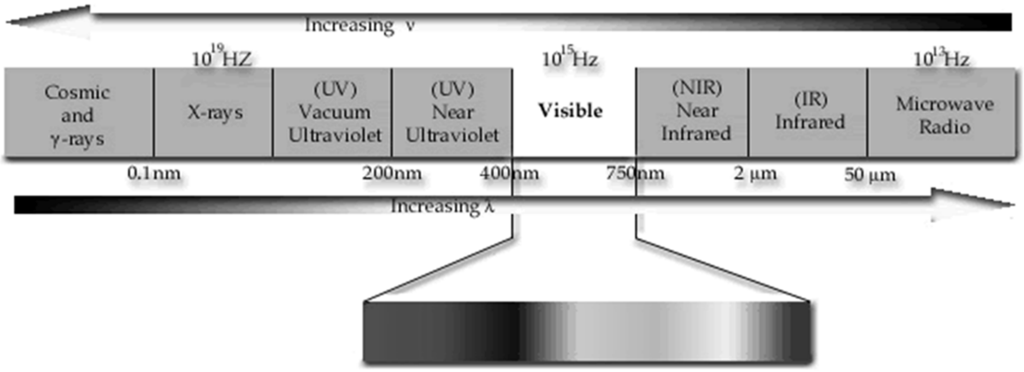
(*S*)-Alanine



(*R*)-alanine

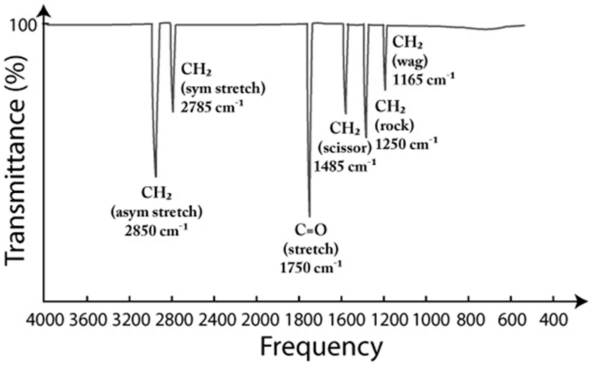


Spectroscopy and wavelength



Infrared (IR) spectroscopy

- wavelength range from 2,500 to 16,000 nm
- vibrational excitation of covalently bonded atoms and groups
- each functional group has a characteristic frequency



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IR spectrum of formaldehyde

http://www.mymcat.com/wiki/IR_Spectroscopy

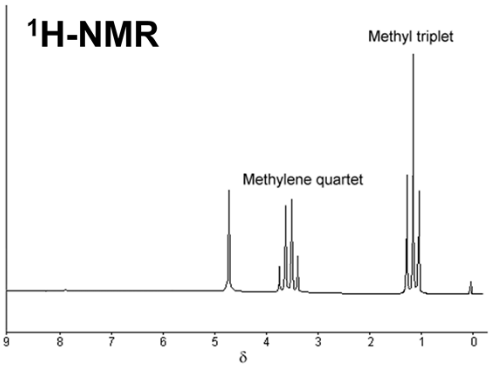
IR, or infared, spectroscopy is a vital tool to organic chemists wishing to identify what functional groups exist in unknown samples. The light our eyes see is but a small part of a broad spectrum of electromagnetic radiation. On the immediate high energy side of the visible spectrum lies the ultraviolet, and on the low energy side is the infrared. Having a wavelength range from 2,500 to 16,000 nm, the infared region is capable of revealing information not easily uncovered through basic means.

Photon energies associated with the infrared region are not large enough to excite electrons but they are still strong enough to induce vibrational excitation of covalently bonded atoms and groups. Covalent bonds are not rigid sticks or rods but are more like stiff springs that can be rotated (single bonds only), stretched, and bent. This wide variety of vibrational motions in turn is characteristic to a molecules component atoms. Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations and infrared spectrometers permit chemists to obtain absorption spectra of compounds that are a unique reflection of their molecular structure.

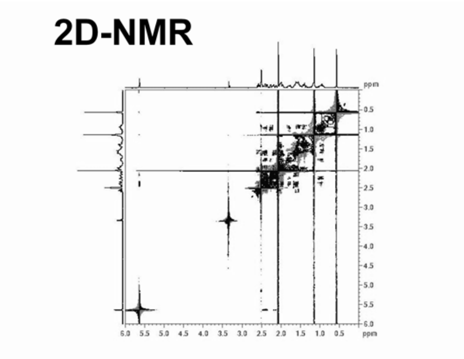
Nuclear magnetic resonance spectroscopy (NMR)

- excitation in magnetic field
- elements with odd number of protons + neutrons (^{1}H, ^{13}C, ^{31}P, ^{19}F)
- each type has a characteristic frequency (chemical shift)

^{1}H-NMR



2D-NMR

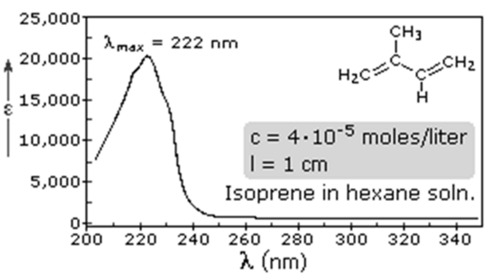


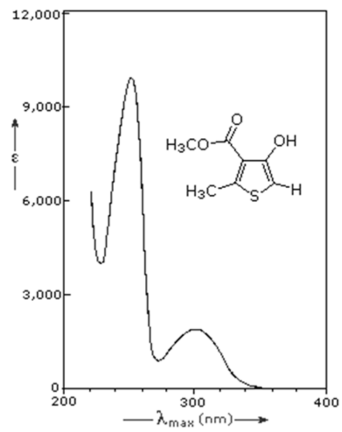
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/nmr/nmr1.htm

http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/nmr1.htm

Ultraviolet (UV) spectroscopy

- range 200 to 800 nm
- unsaturated compounds absorb UV light
- typical absorption of funcional groups

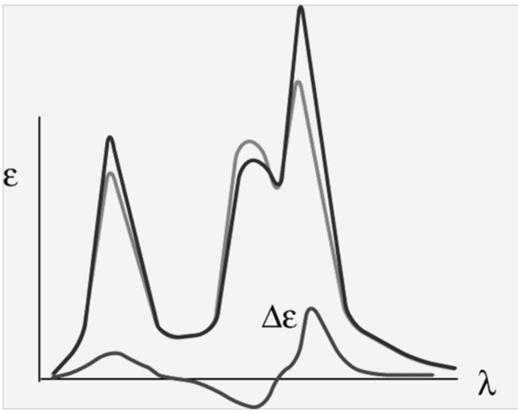


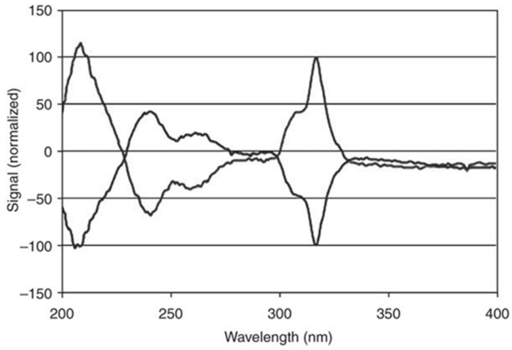


http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/UV-Vis/spectrum.htm

Optical rotation, circular dichroism

- chiral compounds, polarised light
- CD = absorption in UV + optical rotation

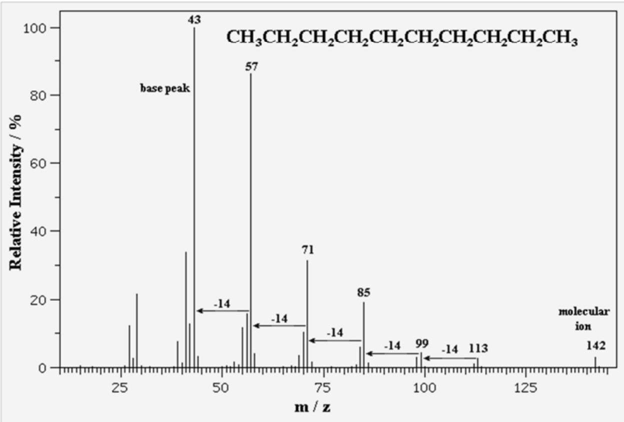




http://www.nsm.buffalo.edu/~jochena/research/opticalactivity.html

Mass spectrometry

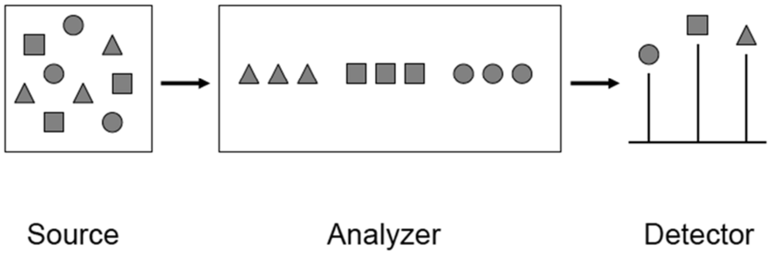
- Molecules in high vacuum
- Ion source, Electron ionisation
- Typical fragmentation of a molecule



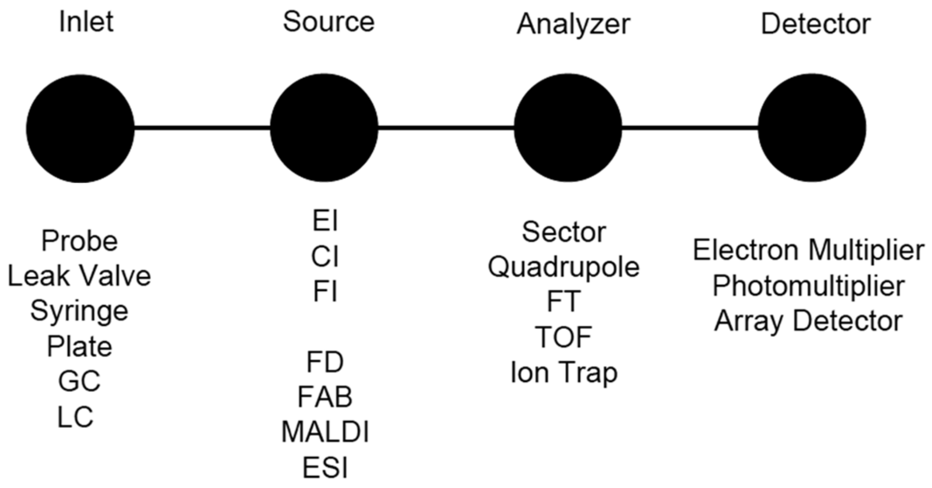
mass spectrum of *n*-decane

http://www.mhhe.com/physsci/chemistry/carey/student/olc/ch13ms.html

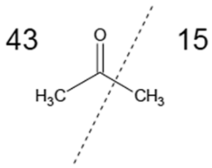
How a Mass Spectrometer Works



Parts of a Mass Spectrometer

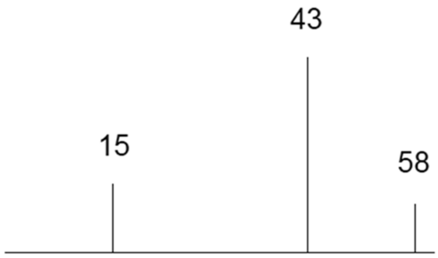


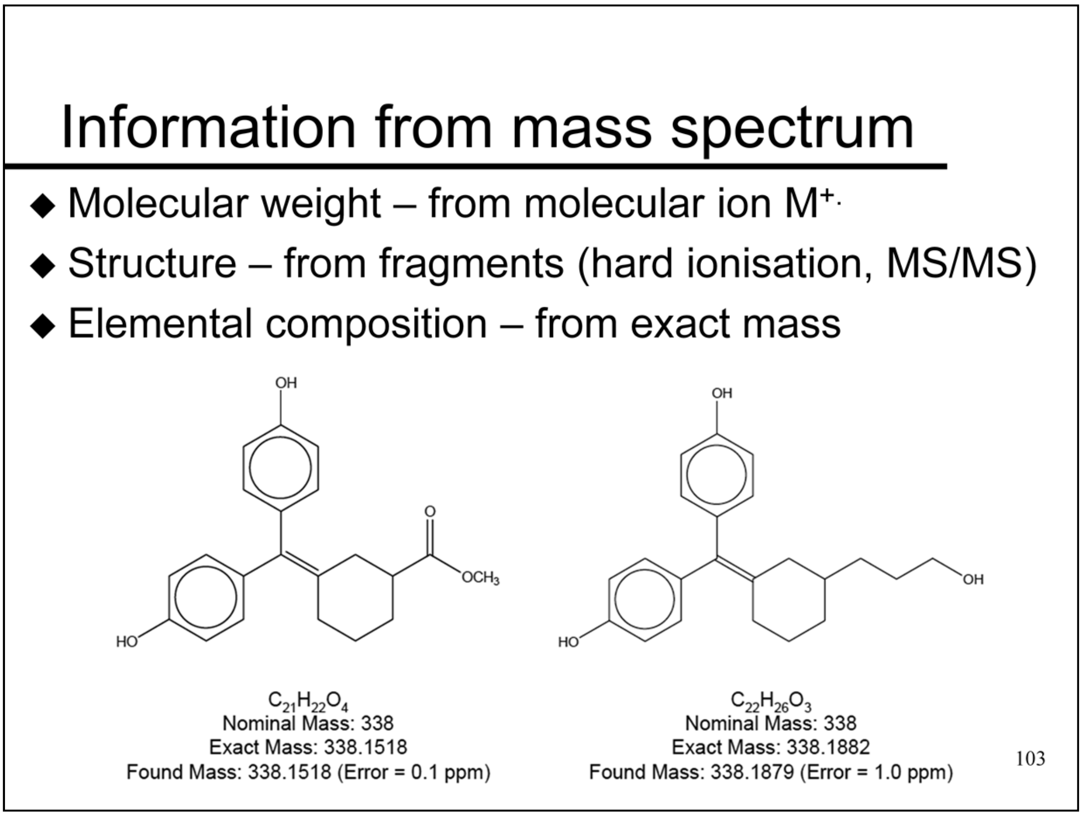
Interpreting a Mass Spectrum



Acetone

MW=58



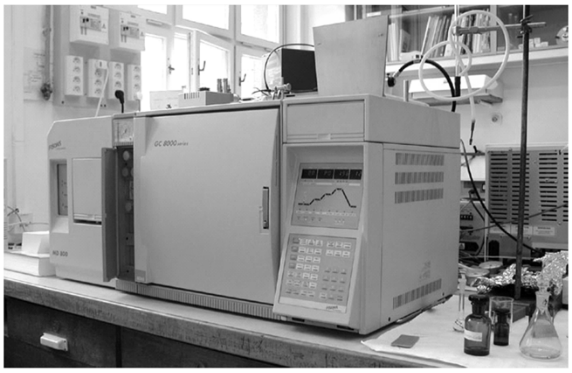


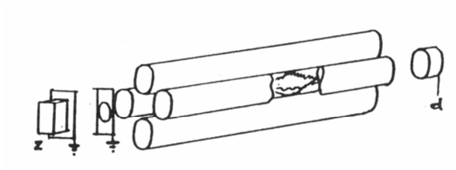
Exact Mass. In most mass spectrometry experiments the nominal mass is used and the mass to charge ratio of an ion is rounded to the nearest whole number. High resolution instruments, including double focusing and FT-ICR mass spectrometers, are capable of determining the "exact mass" of an ion. This is useful for interpretation because each element has a slightly different mass defect. This "mass defect" is the difference between the mass of the isotope and the nominal mass (which is equivalent to the number of protons and neutrons). Recall that the atomic mass scale is defined by carbon-12 with a mass of exactly 12.0000 u. The exact mass of a specific isotope is determined relative to C by high resolution mass 12 spectrometry (see Table 3). High resolution mass spectrometry can distinguish compounds with the same nominal mass but different exact mass caused by different elemental composition. For example, C H , CH O, and NO all have a nominal mass of 30 u. Because they have 2 6 2 the same nominal mass, a mass spectrometer with unit mass resolution can not distinguish these three ions. However, the exact masses for C_2H_6(30.04695039), CH_2O (30.01056487) and NO (29.99798882) are different and a high resolution mass spectrometer can distinguish these three compounds.

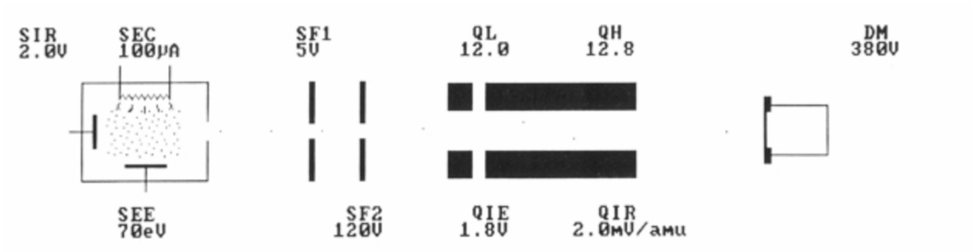
Gas chromatography - mass spectrometry (GC-MS)

- benchtop instrument, usually without direct inlet
- silica capillary column, inner diameter 0,25 mm, carrier gas helium
- end of the column introduced into the ion source
- 2 classical types - quadrupole and ion trap
- new type –**T**ime **O**f **F**light (TOF)

Quadrupole GC-MS

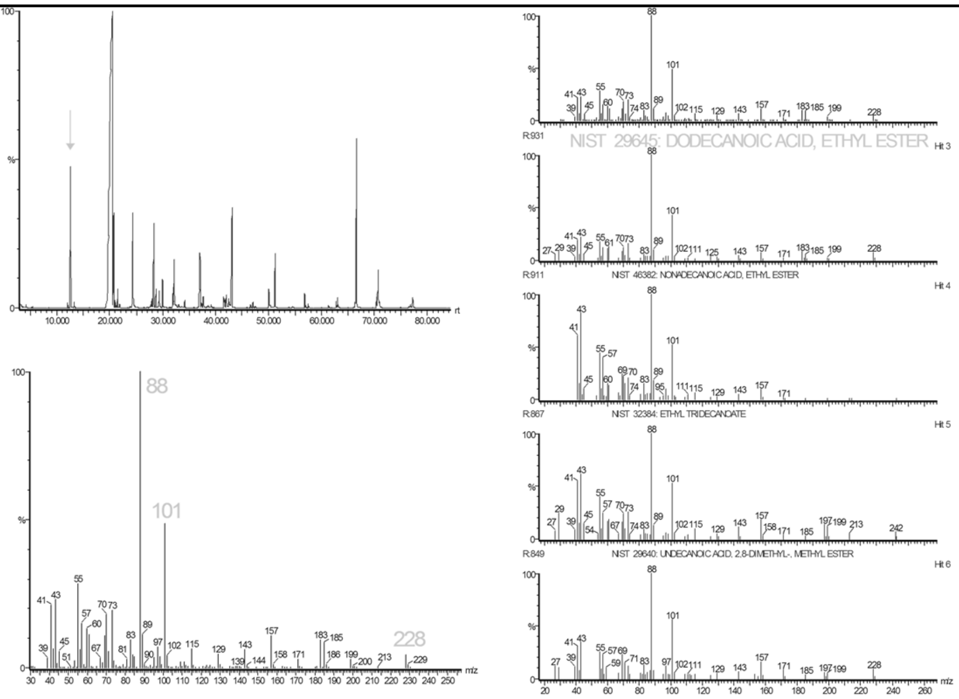






Quadrupole GC-MS

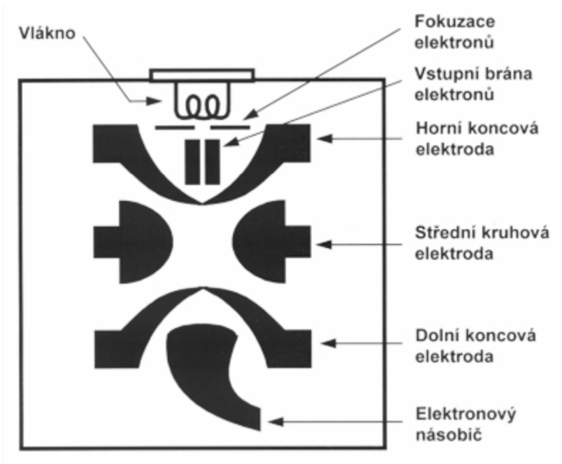
- ions arise in ion source
- electron ionisation (EI)
- positive and negative ions possible to record
- chemical ionisation (CI) – determination of molecular weight (reaction gas methane, ammonia, isobutane)
- change from EI to CI is time-demanding in some instruments


NIST 29645: DODECANOIC ACID, ETHYL ESTER
R:931
Hit 3
R:911
NIST 46382: NONADECANOIC ACID, ETHYL ESTER
Hit 4
R:867
NIST 32384: ETHYL TRIDECANOATE
Hit 5
R:849
NIST 29640: UNDECANOIC ACID, 2,8-DIMETHYL-, METHYL ESTER
Hit 6
rt
m/z

Quadrupole GC-MS

- spectra comparable with big sector spectrometers
- comparison with databases **N**ational **I**nstitute of **S**tandards and **T**echnology (over 60 000 spectra) and Wiley Library (230 000 spectra)
- sensitivity can be increased using **S**elective **I**on **M**onitoring (registeration of one fragment only)

Ion trap


Vlákno
Fokuzace elektronů
Vstupní brána elektronů
Horní koncová elektroda
Střední kruhová elektroda
Dolní koncová elektroda
Elektronový násobič

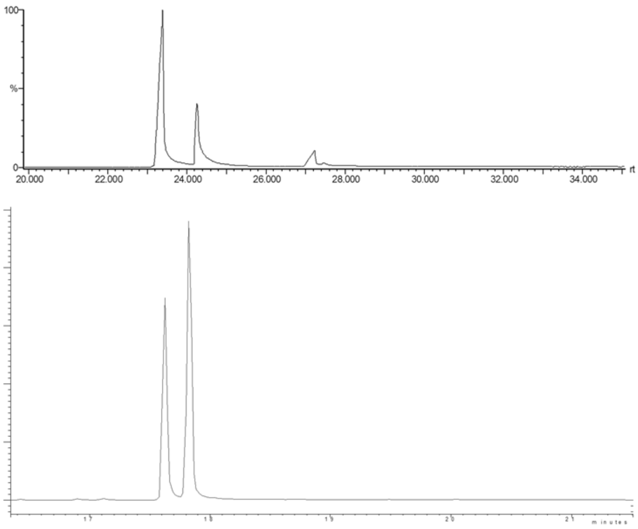
Ion trap

- classical - internal ionisation
- ions arise in the ion trap where they are stored and analysed
- higher sensitivity than quadrupole GC-MS
- recorded spectra sometimes different from sector spectrometers
- EI and CI possible in one injection
- tandem technique MS/MS and $MS^{(n)}$

◆Comparison quadrupole – - ion trap (selectivity of detection)

quadrupole GC-MS

ion trap



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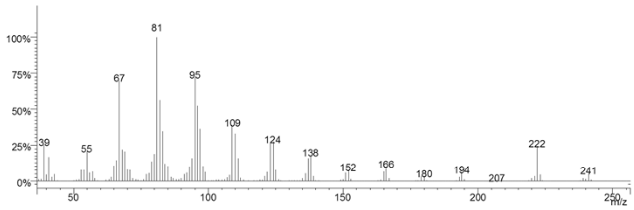
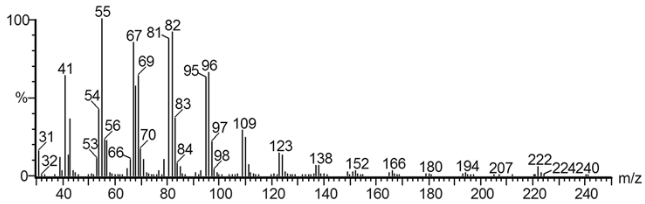
Extract of the pheromonal gland (marking pheromone) of *Bombus lapidarius,* red-tailed bumblebee (hexadecenol, hexadecanol, hexadecenoic acid)

Spectrum of hexadec-9-en-1-ol

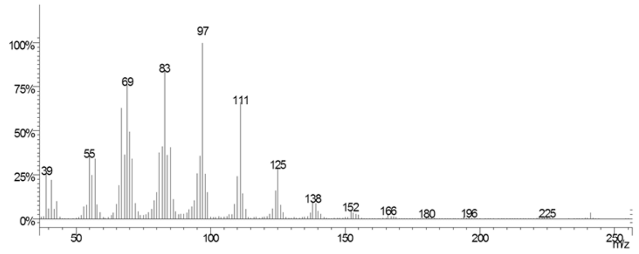
ion trap



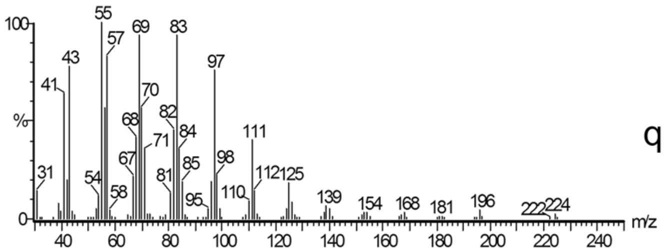


quadrupole GC-MS

Spectrum of hexadecan-1-ol

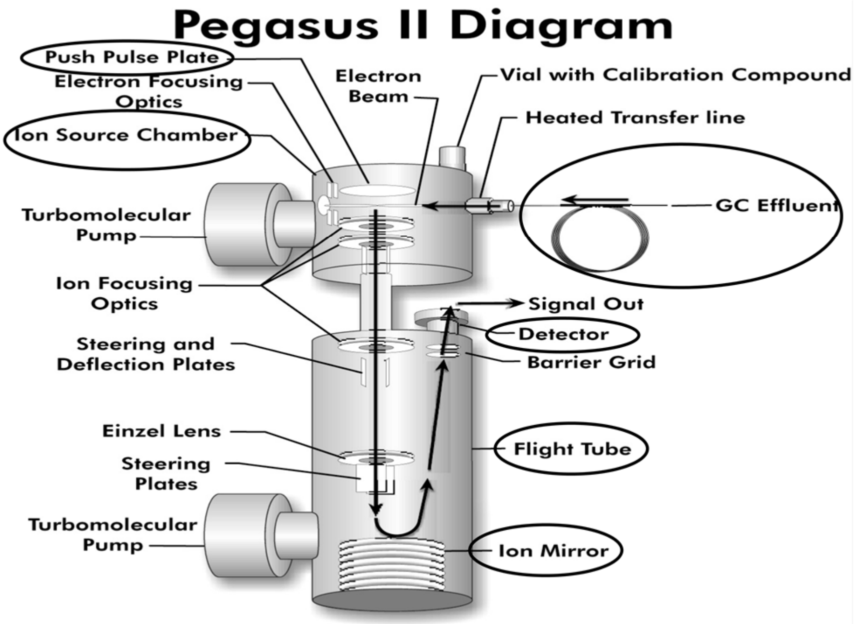


ion trap



quadrupole GC-MS

GC-TOF



Pegasus II Diagram
Push Pulse Plate
Electron Focusing Optics
Electron Beam
Vial with Calibration Compound
Heated Transfer line
Ion Source Chamber
Turbomolecular Pump
GC Effluent
Ion Focusing Optics
Signal Out
Detector
Steering and Deflection Plates
Barrier Grid
Einzel Lens
Flight Tube
Steering Plates
Turbomolecular Pump
Ion Mirror

Principles of TOF MS

- principle: Kinetic Energy $KE=1/2mv^2$
- measurement of time of ion flight
- first published GC-MS data: Gohlke, Anal. Chem. (1959)
- potential for recording of MS data
- development of analytical instrumentation in last decade with powerful computers

The objective of this slide is to establish that TOF is tried, tested, and proven MS technology. The reason for its resurgence is the development of high speed electronics and innovative ways of using these electronics to rapidly collect spectral data.

The unspoken objection you are attempting to address is that TOF is new and therefore may not generate classical spectra. TOF has been around longer than any other MS technique and has been in use constantly since its inception although it was used primarily or larger molecule analysis requiring higher mass ranges during the 1980's and 1990's.

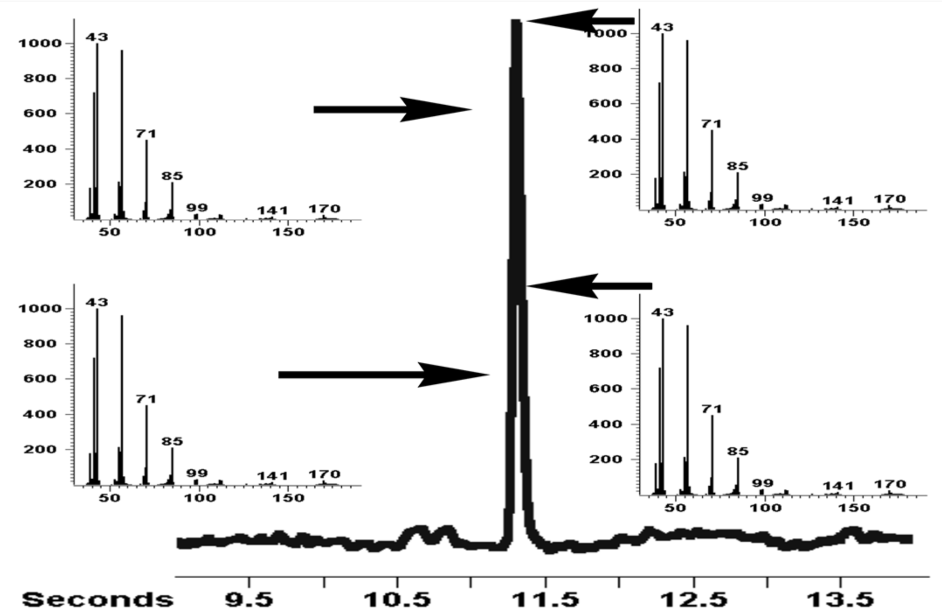
Advantage of GC-TOF

- mass determined at high resolution (elemental composition of ions, slow data collection)

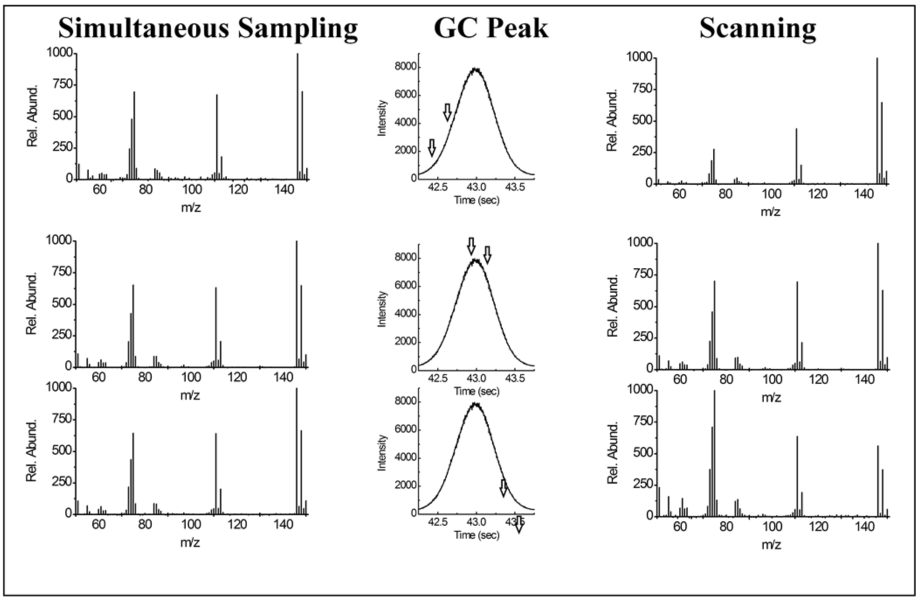
OR

- two-dimensional chromatography possible, GCxGCxTOF (quick data collection, but nominal mass only)

Mass spectrum does not differ at different parts of chromatograhic peak



Difference between scanning techniques and TOF



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The GC peak is for m/z 146 and the arrows show the points at which the adjacent spectra were taken. For simultaneous sampling the spectrum is from the first arrow since whole spectra can be obtained at any point along the chromatographic peak. The simulated scanning is based on scanning from the first to second arrow. For illustration the scanning was done over 100 mass units which would be equiv. to 5 spectra/sec. Each m/z values corresponds to an increment of time along the chromatographic peak.

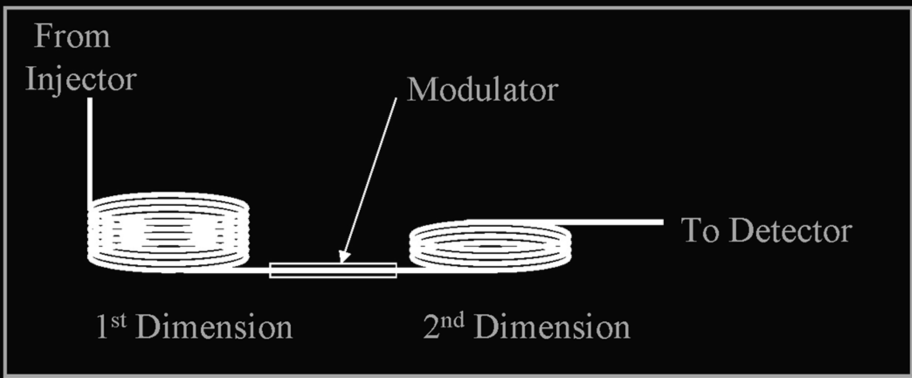
With simultaneous sampling the spectra are consistent over the chromatographic peak, concentration at any point along the peak has no effect. In contrast, sampling for scanning moves along the concentration profile of the peak producing skewed spectra. On the leading edge concentration is increasing and intensity increases with m/z value when scanning from high to low. While the top is reasonably flat the spectrum is similar to simultaneous sampling. And on the trailing edge the spectrum decreases from low to high as concentration decreases.

This difference is important in accurate spectra for library searching and for deconvoluting overlapping chromatographic peaks. In order to deconvolute mass spectra of coelutions the spectra must be consistent across the peak in order to attribute changes in the measured spectrum to different components rather than skewing.

Thus, simultaneous sampling has both quantitative and qualitative advantages over scanning.

GCxGC Principle

I) Two columns of DIFFERENT SELECTIVITY are coupled via a modulator



Chromatographic system

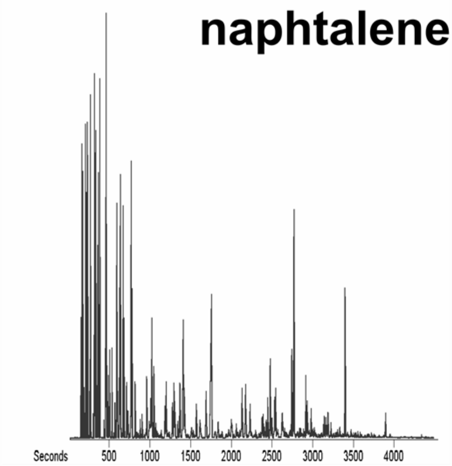
- first dimension – classical column, non-polar, 30 m x 0.25 mm
- secon dimension – short and narrow column (1-2 m x 0,1 mm), polar phase
- separation on the second column is very fast, therefore data collection must be fast, too

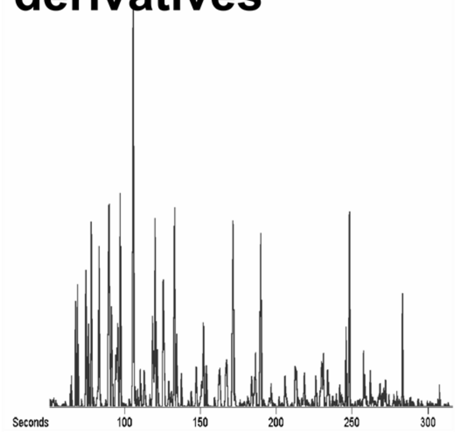
Fast GC: short time of analysis

Conventional GC *Fast GC*

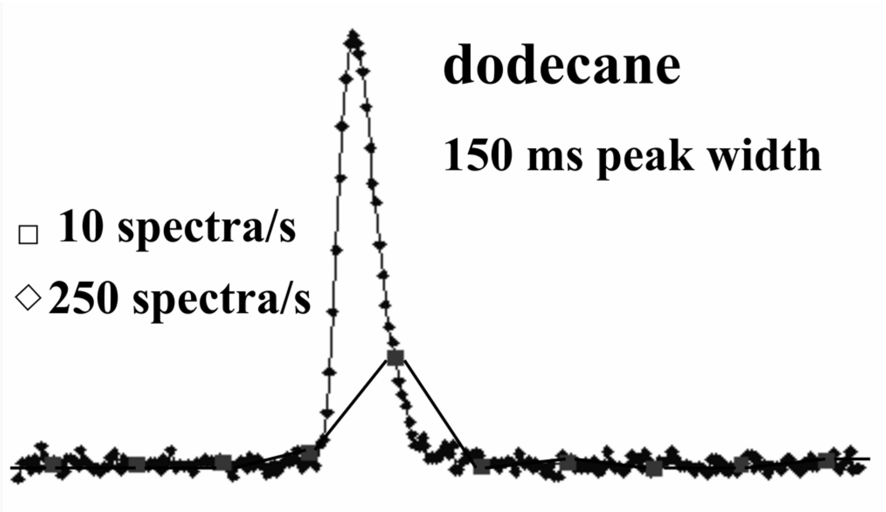
187 compounds in 75 min **187 compounds in 5 min**

naphtalene derivatives





TOF - fast data collection, very narrow peaks can be recorded



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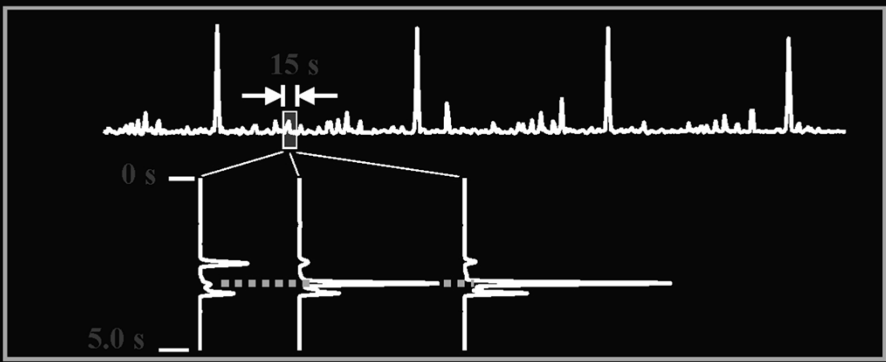
With quadrupole GC/MS, the usual scanning frequency is 1 spectrum per second.

GCxGC Principle

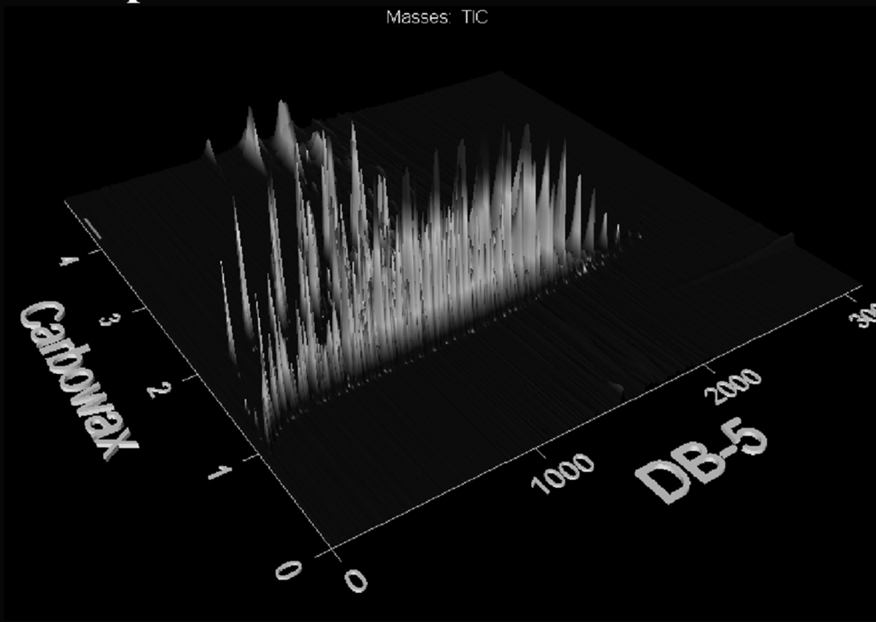
II) Modulator cuts slices of first column efluent and samples them onto second column. Each first dimension peak is modulated several times. On second column flash separation occurs



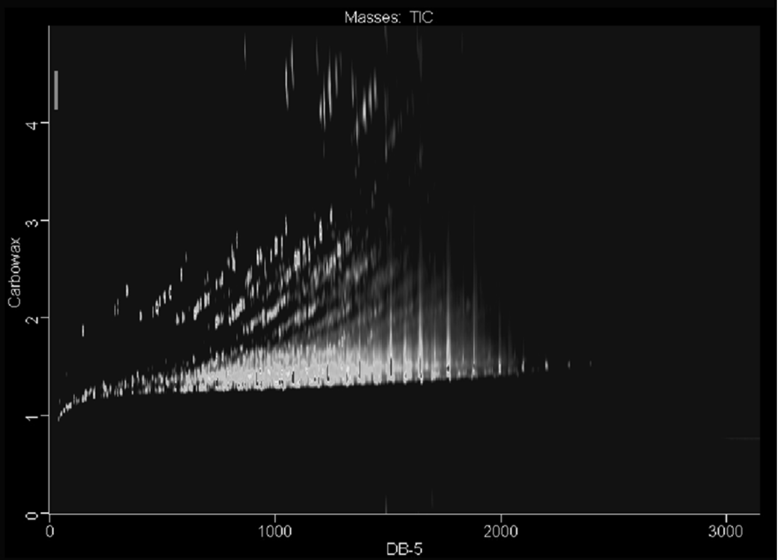
Pegasus 4D® description

- Gas chromatograph Agilent 6890N
- Secondary oven
- Dual stage jet cryo modulator (licensed from ZOEX)
- TOF-MS LECO Pegasus III
- Software ChromaTOF

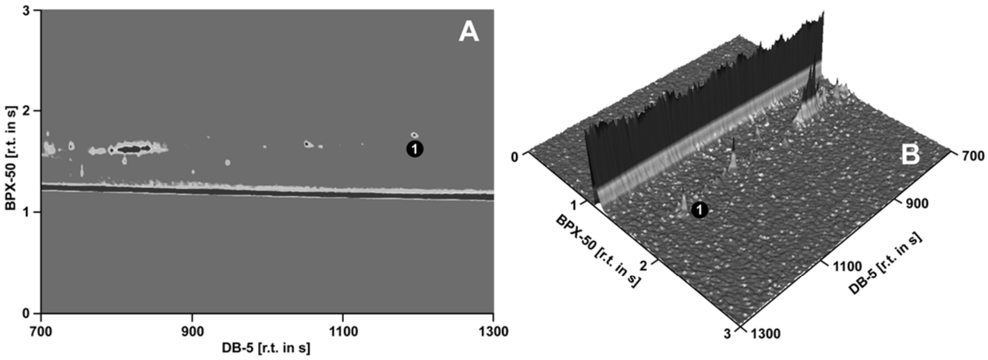
Visualisation of GCxGC data: Surface plots

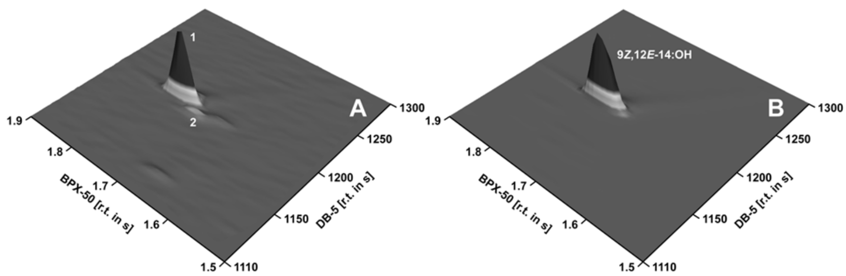


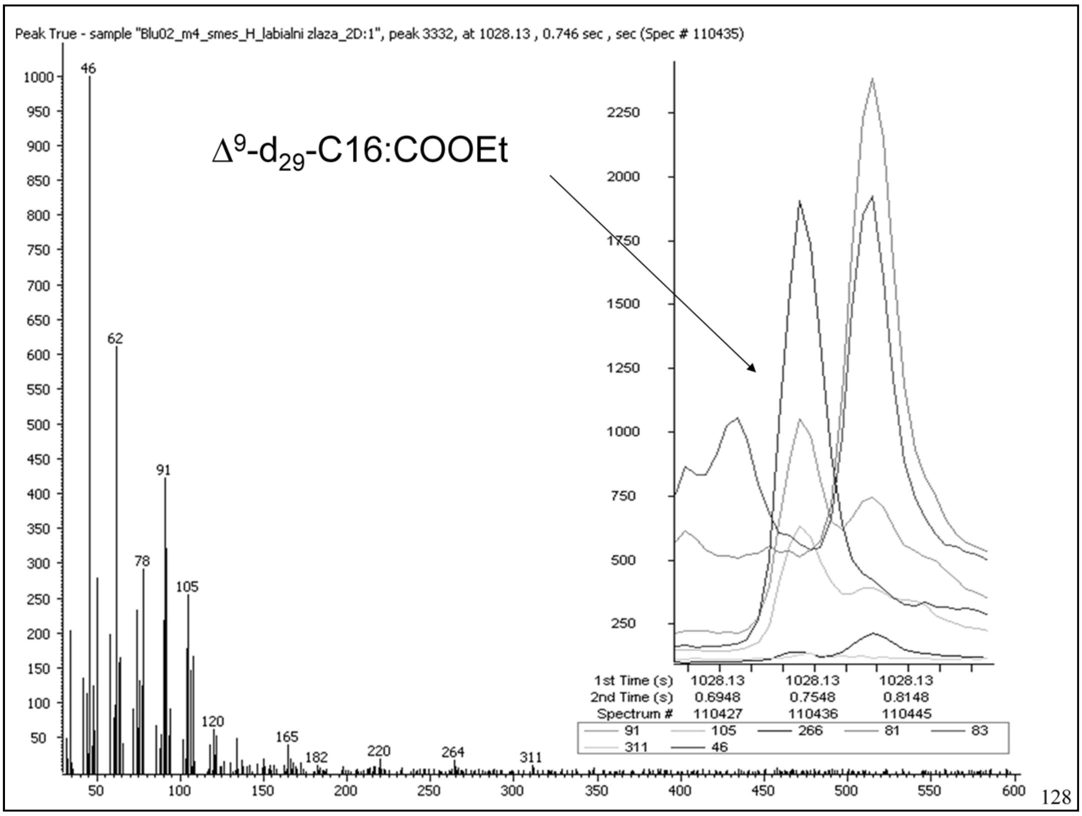
Visualisation of GCxGC data: Contour plots



2D-Technique removes the chemical noise and increases the sensitivity

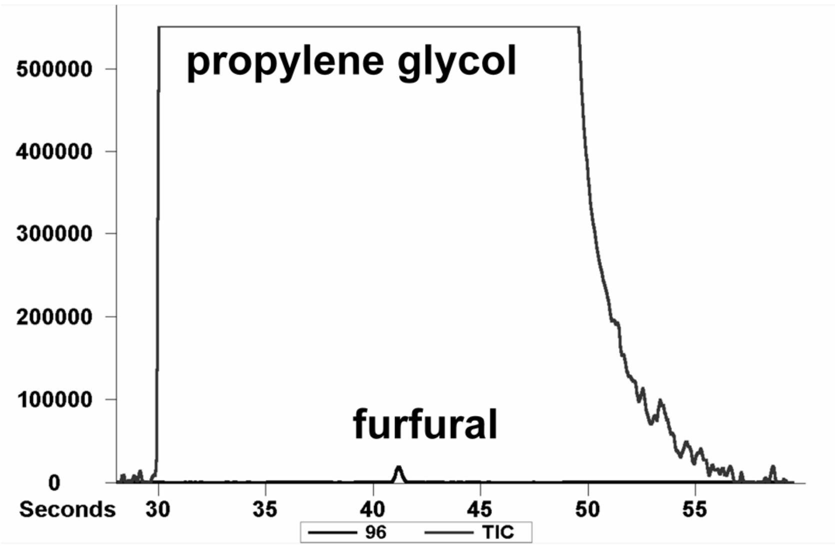






Deuterium-labeled compounds elute before the native ones on both, polar and non-polar columns.

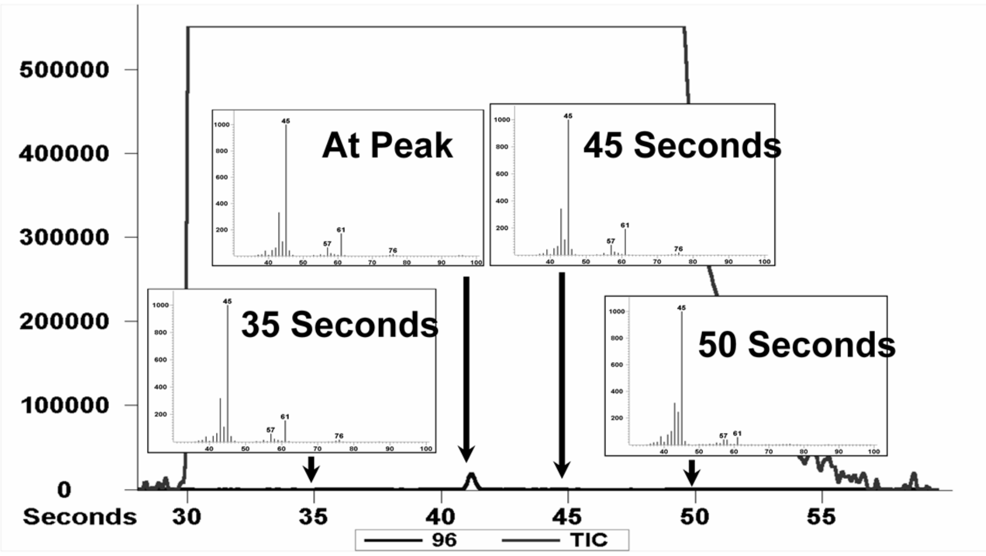
Coelution of analytes of very different concentration



peak area of furfural is 0.001 % of the glycol peak area

The automated peak location algorithm detects an unknown peak later identified as furfural buried beneath the propylene glycol. The peak area of this analyte is 0.001% of the peak area for the propylene glycol.

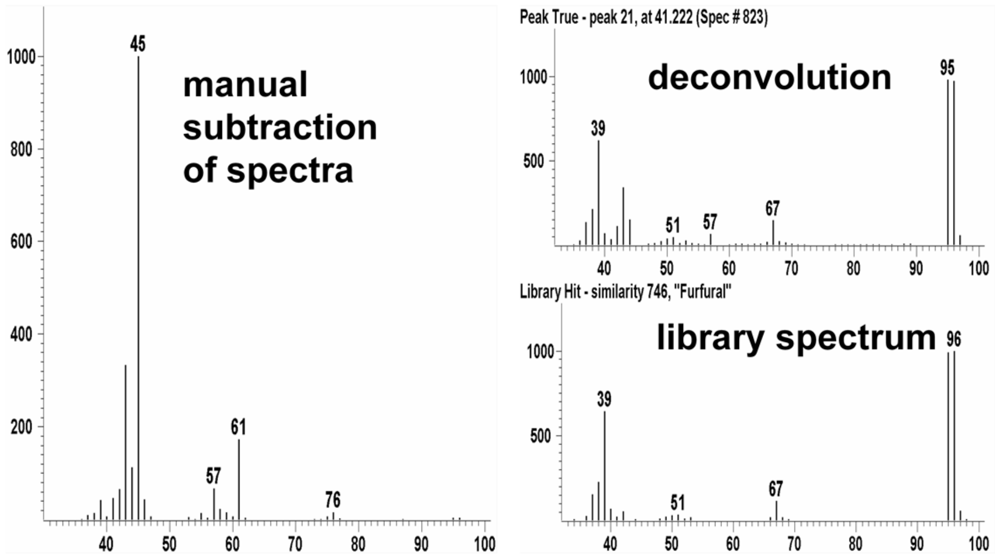
Spectra recorded at different parts of the peak of propylene glycol



without deconvolution it is impossible to determine furfural

Without deconvolution, it is impossible to detect this peak. Looking at mass spectra across the propylene glycol peak indicates that the analyte is completely obscured by the significantly higher concentration of propylene glycol. Unless one knew to specifically look for furfural, the analyte would not be detected.

Comparison of spectra



Comparing the background subtracted spectrum at the apex of the furfural peak to the NIST spectrum, it is obvious that there is no match for furfural. However, if the deconvoluted spectrum is compared a very good match of 75% is obtained.

Liquid chromatography – mass spectrometry (LC-MS)

- large amounts of mobile phase has to be removed
- particle beam interface
- thermospray (TSP)
- electrospray (ESI)
- chemical ionisation in atmospheric pressure (APCI)

Ions evaporate into gas phase from charged droplets.

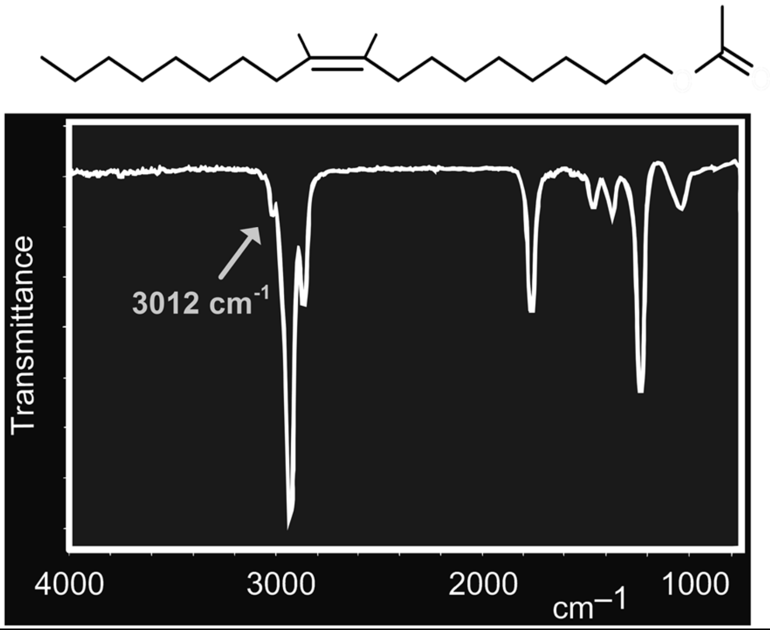
LC-MS

- only the technique of „particle beam interface“ gives spectra comparable with sector spectrometers
- other techniques give quasimolecular ions (addition or elimination of particle from molecular ion)

GC-FTIR (Fourier transform infrared spectroscopy)

- flow detection cell covered with a layer of gold („light pipe“)
- less sensitive compared to GC-MS (~ 100x)
- GC columns of a larger inner diameter (0,32-0,5 mm)
- carrier gas helium
- spectra in gas phase (no intermolecular interactions)

Double bond configuration (*E,Z*)

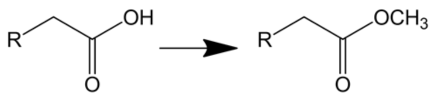


Derivatisation in microscale

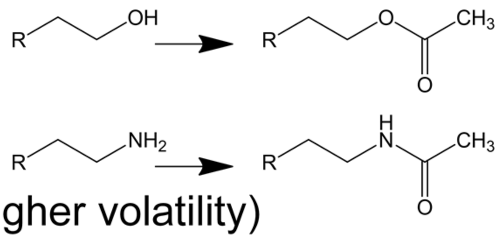
- characterisation of functional groups in the molecule
- spectra easier to interpret
- better separation
- increased volatility or thermal stability of compounds for GC analysis
- enantiomeric composition
- improvement of detection properties

Derivatisation reactions

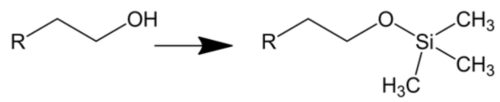
- methylation of acids with diazomethane CH_2N_2 (for GC)



- acetylation of alcohols and amines (for GC)

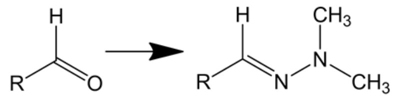


- silylation of alcohols (for higher volatility)

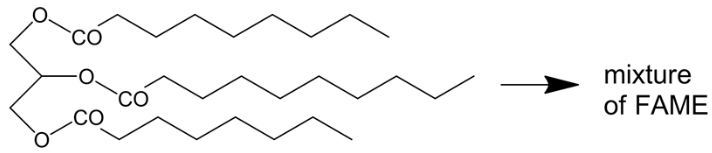


Derivatisation reactions

- dimethylhydrazones $NH_2N(CH_3)_2$ (CO, CHO)



- transesterification of triacylglycerols (for GC)

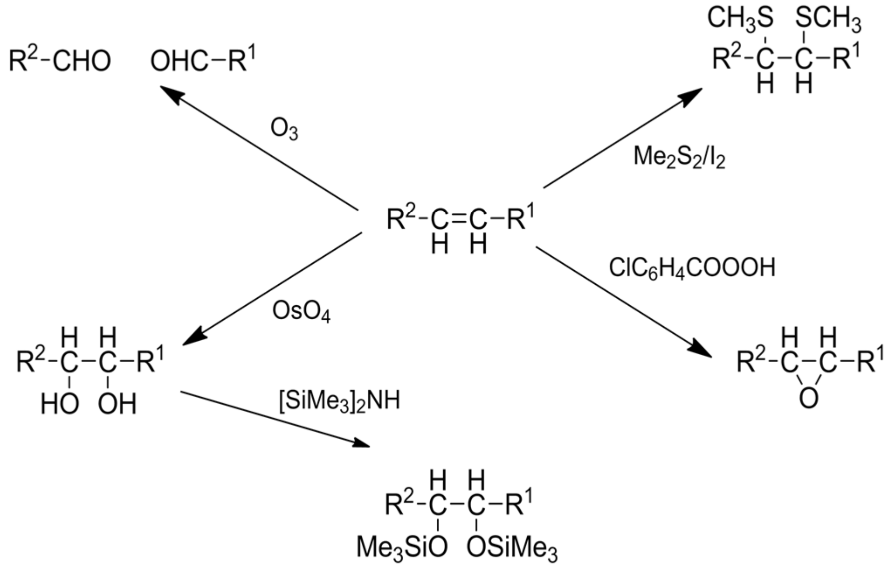


- catalytic hydrogenation (carbon skeleton chromatography)

Double bond position

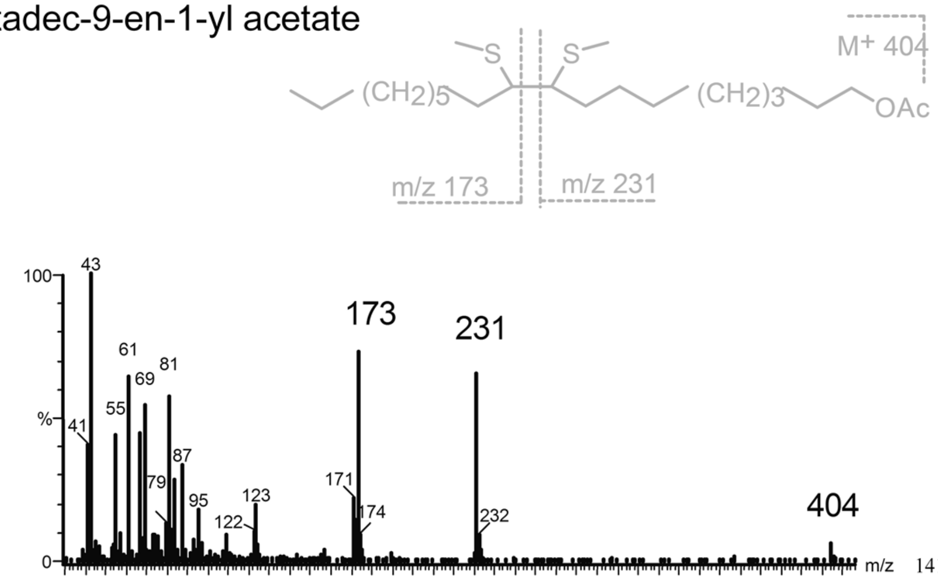
- ozonolysis - pure compound only, formation of aldehydes
- other oxidative cleavage of C=C bond (RuO_4) – formation of acids
- oxidation with OsO_4 – formation of a diol
- methylthiolation of double bond (reaction with dimethyl disulfide, DMDS, $(CH_3)_2S_2$) – possible in mixtures
- epoxidation with *m*-chlorperoxybenzoic acid (MCPBA) and following reactions

Double bond position

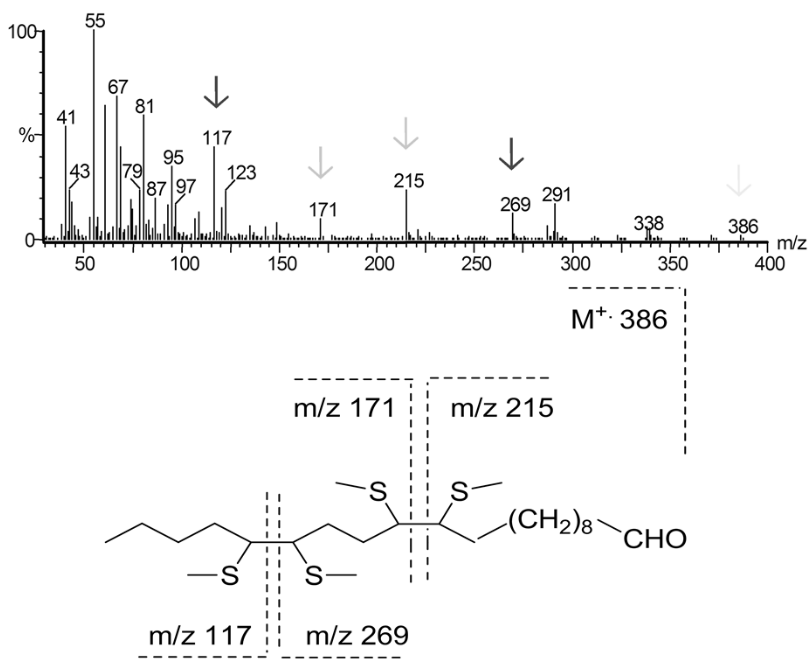


Fragmentation of DMDS adducts

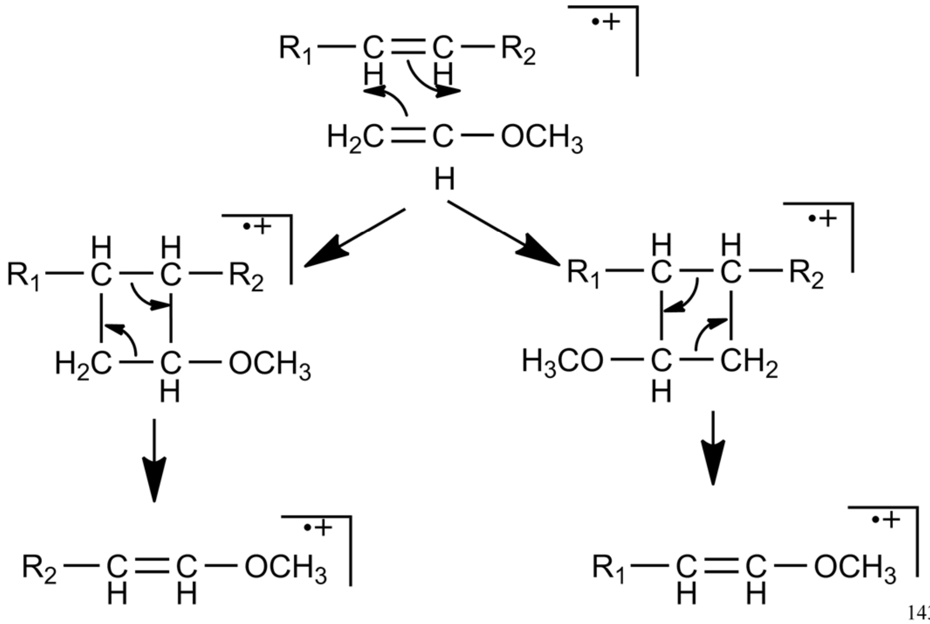
octadec-9-en-1-yl acetate



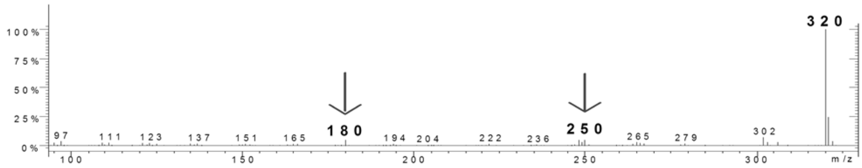
spectrum DMDS adducts of icosa-11,15-dienal



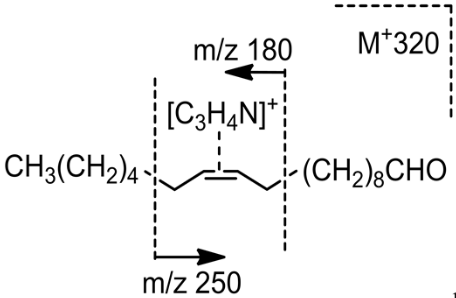
Chemical ionisation - methylvinylether



Chemical ionisation with acetonitrile in ion trap MS



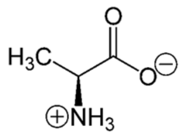
CI spectrum octadec-11-enal



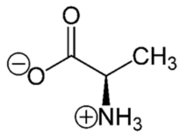
active particle **$[C_3H_4N]^+$**
m/z 54

Absolute configuration of natural products

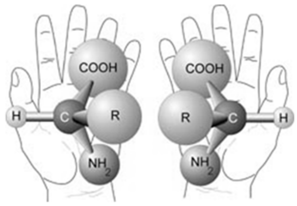
- enantiomers may have different ecological functions
- enantiomers may have different physiological effects
- determination of enantiomeric purity of natural products is important



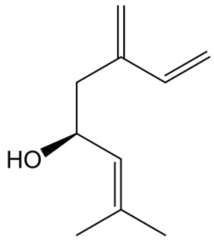
(*S*)-Alanine



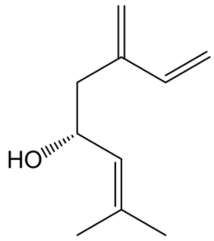
(*R*)-alanine



Different species of one genus use opposite enantiomers

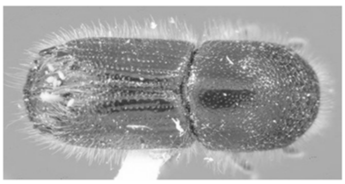


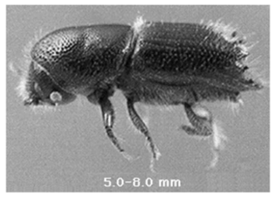
(+)



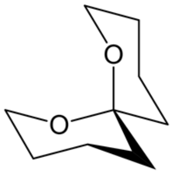
(–)

Ips paraconfusus ipsdienol *Ips calligraphus*

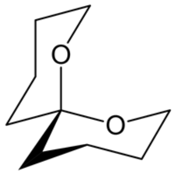




One enantiomer attracts males, the other one females



(*R*)-(−)-oleane
males

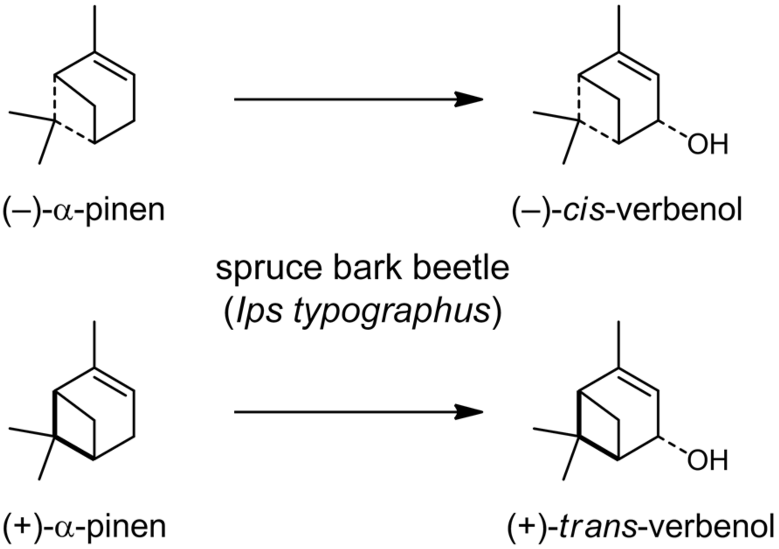


(*S*)-(+)-oleane
females

fruit fly *Dacus oleae*,
pest on olives

Dacus oleae, olive fly

Biotransformation of resin components to the aggregation pheromone of bark beetles



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Lindström M. et al.: Variation of enantiomeric composition of α-pinene in Norway spruce, *Picea abies*, and its influence on production of verbenol isomers by *Ips typographus* in the field. *J. Chem. Ecol*. **1989**, *15*, 541-48.

Determination of the absolute configuration

- separation and measurement of optical rotation
- chiroptical methods
- NMR with shift reagents
- preparation of diastereoisomers
- enantioselective chromatographic separation

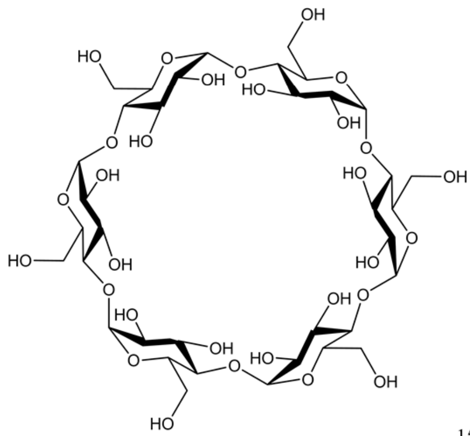
Classical methods

- large amount of pure natural product needed
- separation of the enantiomeric pair from other sample components is difficult
- measurement of optical rotation – inaccurate

Enantioselective chromatographic separations

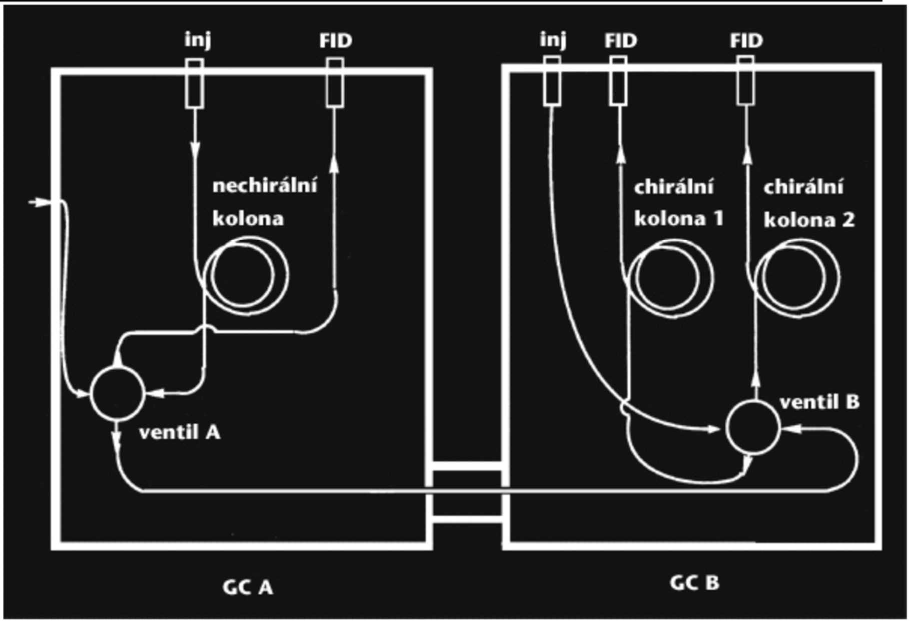
- columns based on cyklodextrin, hydroxy groups substituted with different groups

α-cyclodextrin, 6 units
β-cyclodextrin, 7 units
γ-cyclodextrin, 8 units



α-Cyclodextrin

Two-dimensional GC



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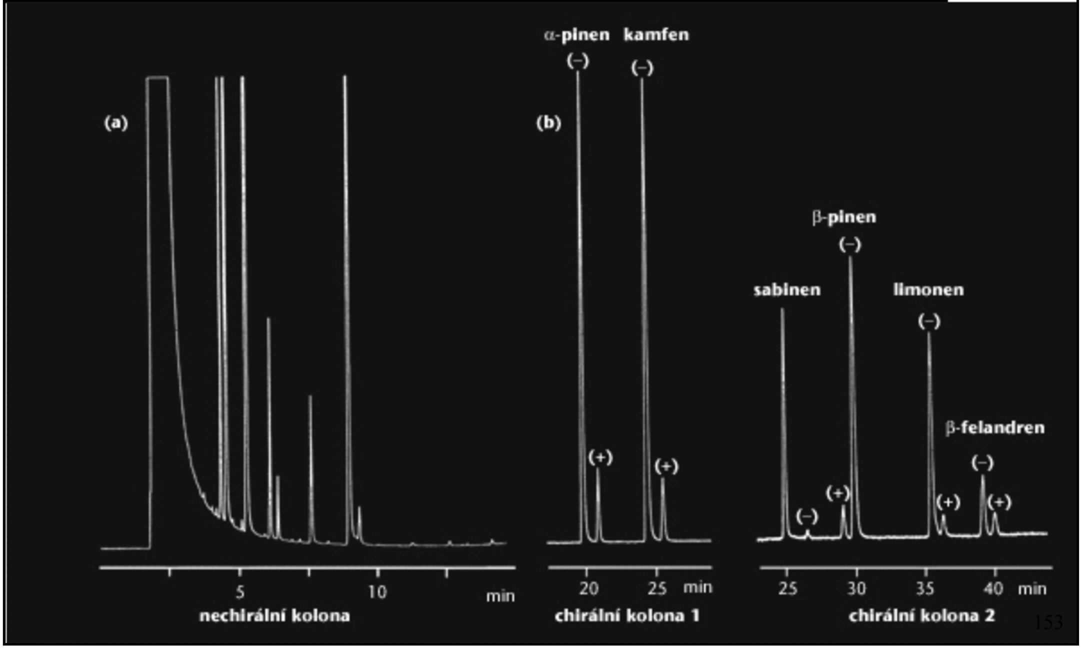
Borg-Karlson A.-K. et al.: Enantiomeric composition of monoterpene hydrocarbons in different tissues of Norway spruce, *Picea abies* (L.) Karst. A Multidimensional gas chromatography study. *Acta Chem. Scand*. **1993**, *47*, 138-144.

2D-GC, separation examples



Advantages of 2D-GC

- possible in minute quantities
- presaparation of components no needed
- high accuracy provided good (base-line) separation of enantiomeric pairs
- high sensitivity, detection of minor impurities of the opposite enantiomer
- information on enantiomeric purity of several components in one analysis

standards needed!

Bioassays in invertebrates

Electrophysiological methods

Classical electroantennography on insect antennae

- general method
- responses not specific
- dose-dependent responses
- very useful for analytical purposes

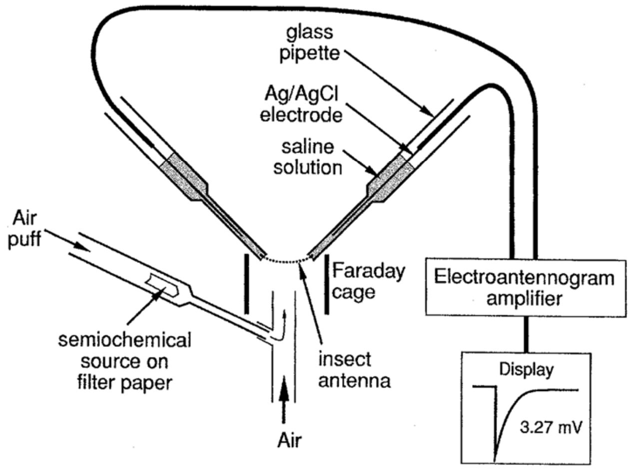
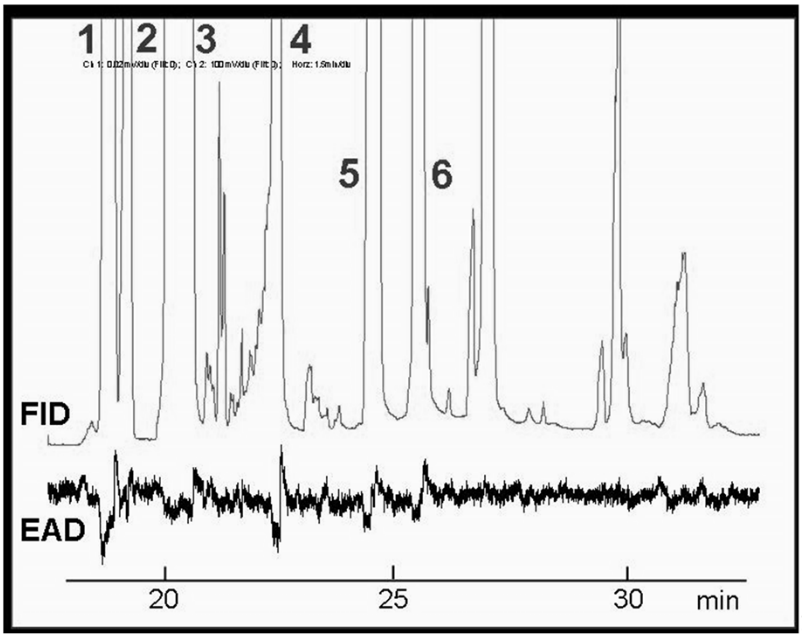


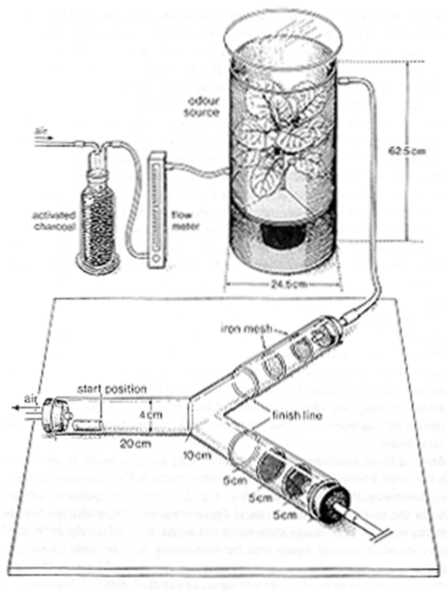
Figure 9.1. Electroantennogram system.

Connected gas chromatography – electroantennography (GC-EAG, GC-EAD)

insect antenna
as selective detector



Bioassays in invertebrates



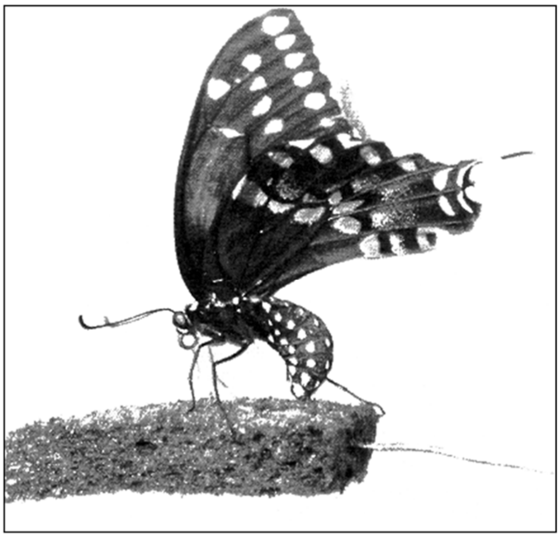
Olfactometer is used for testing volatile stimuli. In choice-test we observe responses to different stimuli (control, neutral, positive, negative). We observe movement of tested animal in the direction of tested compounds.

Y- olfactometr

Bioassays in invertebrates

Use of dummies of different shape

- artificial leaves and other plant parts for studies of interactions of plants and insects
- conspecific and heterospecific individuals for studies of social behaviour
- artificial females for studies of sexual behaviour



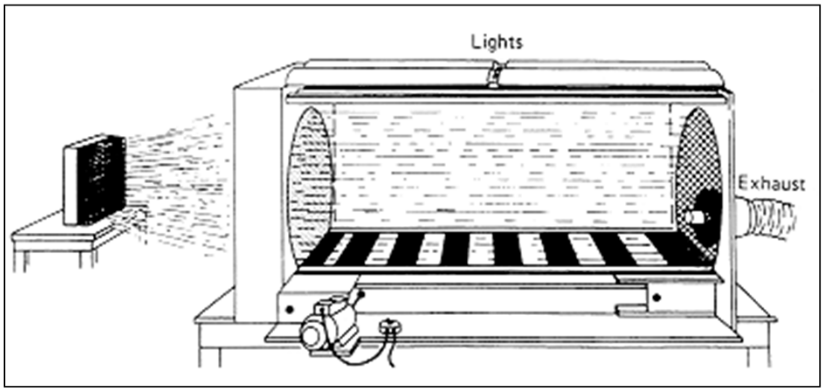
Oviposition test on an artificial leave (polyurethane impregnated with plant leave extract)

Bioassays in invertebrates

Wind tunnel

Observation and evaluation of behaviour in response to volatiles, e.g. sexual behaviour (sexual pheromone): activation, oriented flight, finding of odour source, copulation attempts

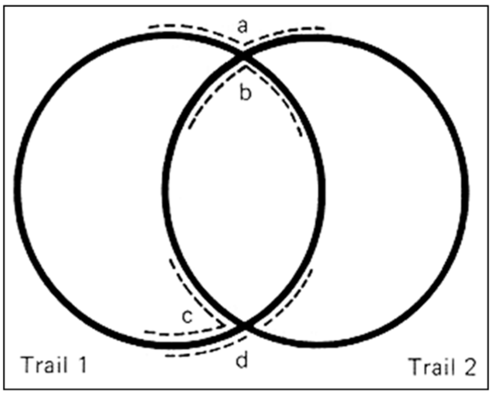
Original equipment by Carde et al. : Tested compound is adsorbed on a filter paper (dispenser), placed on left, insect introduced on right.



Bioassays in invertebrates

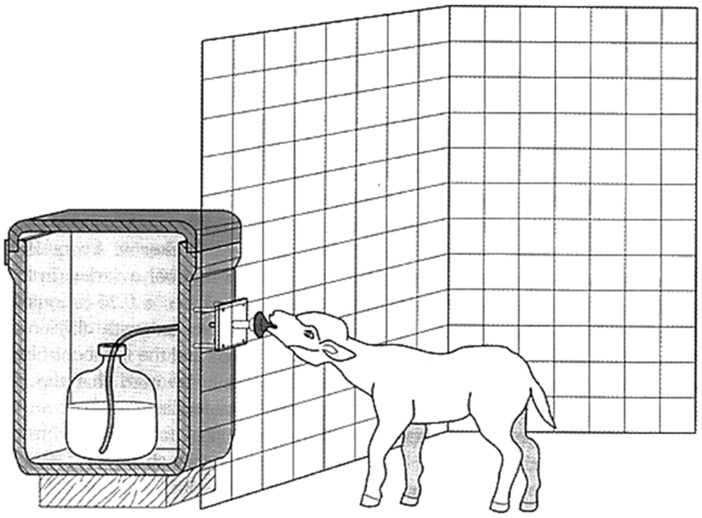
Test of trail pheromones:

Traces 1 and 2 are “drawn” by tested compound and movements of ants on the drawing are followed



Bioassays in mammals

Very complicated and demanding, problems with learning, responses influenced by the researchers (primates), costly in humans



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Design and evaluation of bioassays

Design a planning of a bioassays is crucial

- asking clear questions
- simple design, controlled environmental conditions
- sufficient number of experimental animals in the same physiological state
- sufficient number of repetitions
- independent values!
- recording, documentation, digitalisation, later evaluation

- negative and positive controls necessary
- avoid contamination
- elimination of animals in different physiological
- repetition, choice of suitable statistical method

In the literature we often find mistakes and misinterpretation.

Bumblebees

- primitive social structure
- one-year cycle
- fertilised queen overwinters
- starting colonies in spring
- workers take care of the brood
- reproductive individuals emerge in summer
- mating in the open air
- old queen, workers, and males die in the autumn

Chemical signals in bumblebees

females' signals:
· **sex pheromone** of virgin queens (premating phase)
· **queen's pheromone** (queen is the only egg layer)

males' signals:
· **marking (sex) pheromones** (premating behaviour)

workers' signals:
· **dominant signals**
· **orientation in the nest**

queen

Bombus terrestris

male

Males' premating behaviour

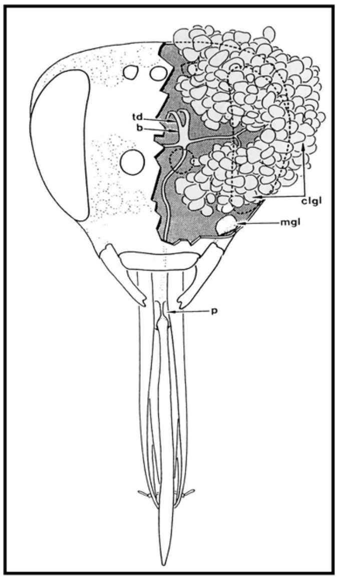
SPECIES USING PHEROMONE

Waiting near the nest | Perching species | Patrolling species

SPECIES NOT USING PHEROMONE

Male marking (sex) pheromones

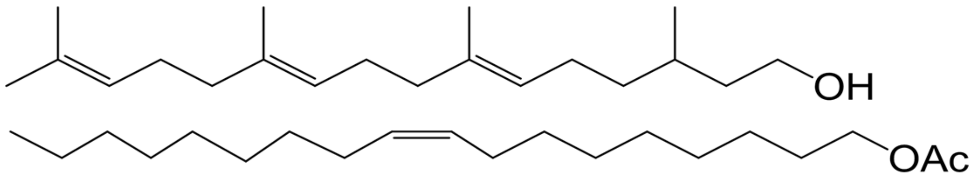
- production in the cephalic part of the labial gland
- cephalic part consists of numerous acini and the ducts connecting particular acini
- multicomponent mixtures



Kullenberg et al. 1973; Ågren et al. 1979, Bergström et al. 1981

Usual types of compounds

- aliphatic alcohols and aldehydes
- ethyl esters of fatty acids
- terpenic alcohols and their esters (mono-, sesqui-, and diterpenes)



- compositions are species - specific
- compositions may be used as a tool for chemotaxonomy

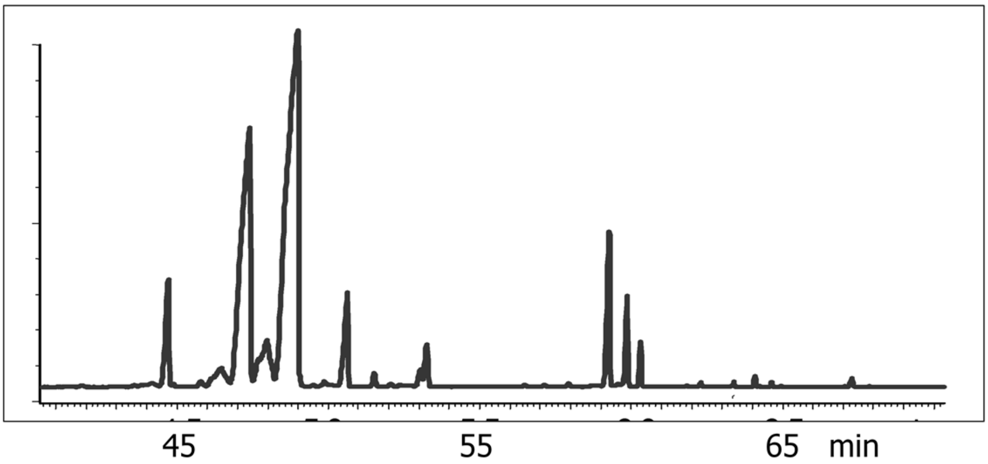
Bombus confusus - Locality of occurrence

Bombus confusus

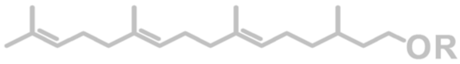
- premating strategy - perching (elevated perch)
- morphological adaptation – big eyes
- literature – species oriented optically in search for mate

male *B. confusus* on a perch

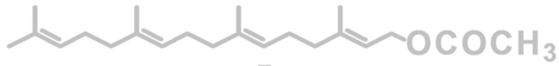
Labial gland extract



Main components of the secretion



1, R = H
2, R = $CO(CH_2)_4CH_3$
3, R = $CO(CH_2)_6CH_3$
4, R = $CO(CH_2)_8CH_3$



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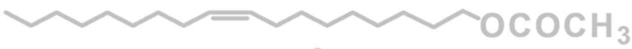

6

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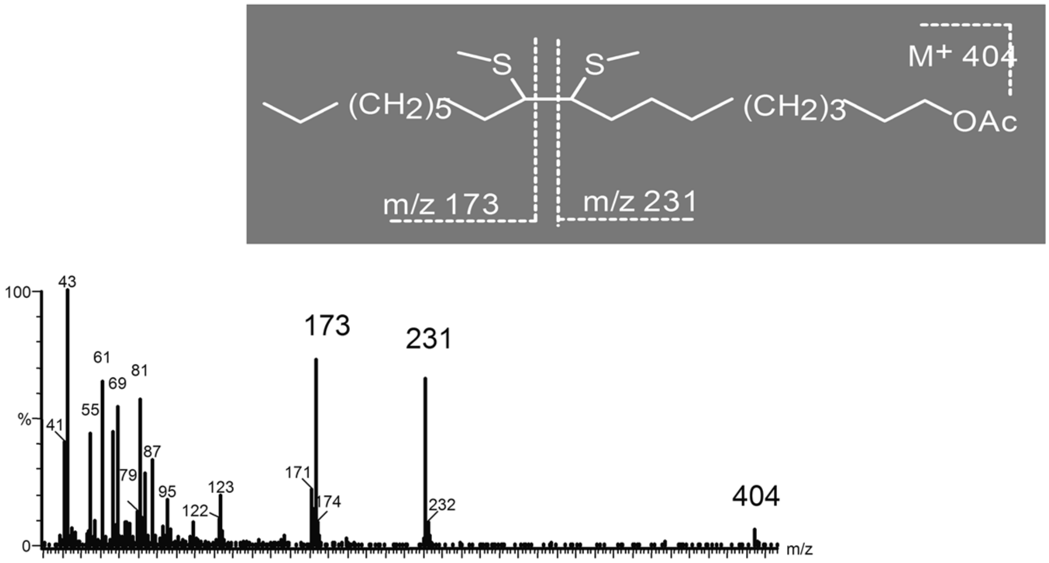
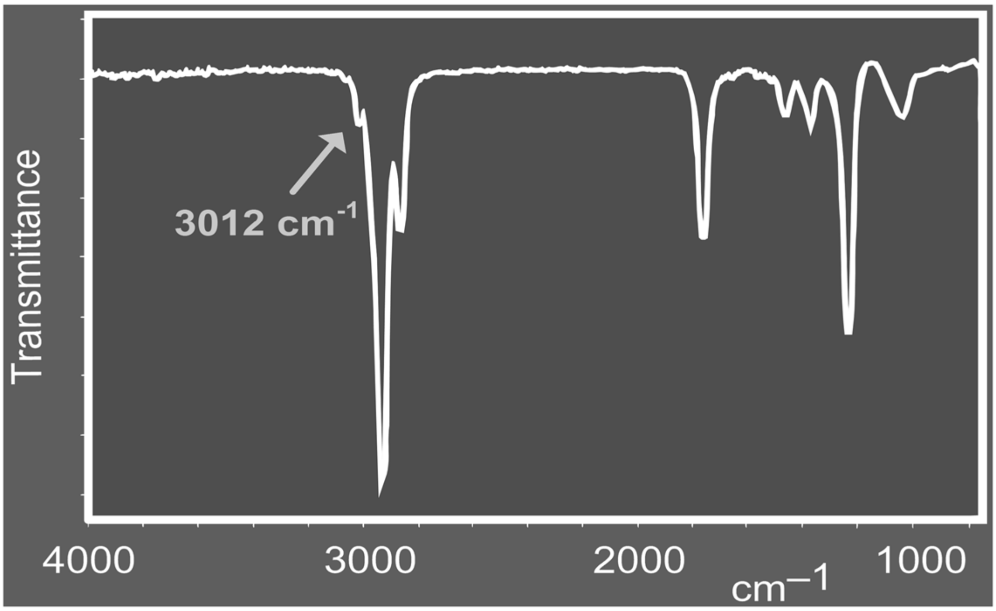
Hovorka O. et al.: Premating behavior of *Bombus confusus* males and analysis of their labial gland secretion. *J. Chem. Ecol.* **1998**, *24*, 183-193.

Double bond position

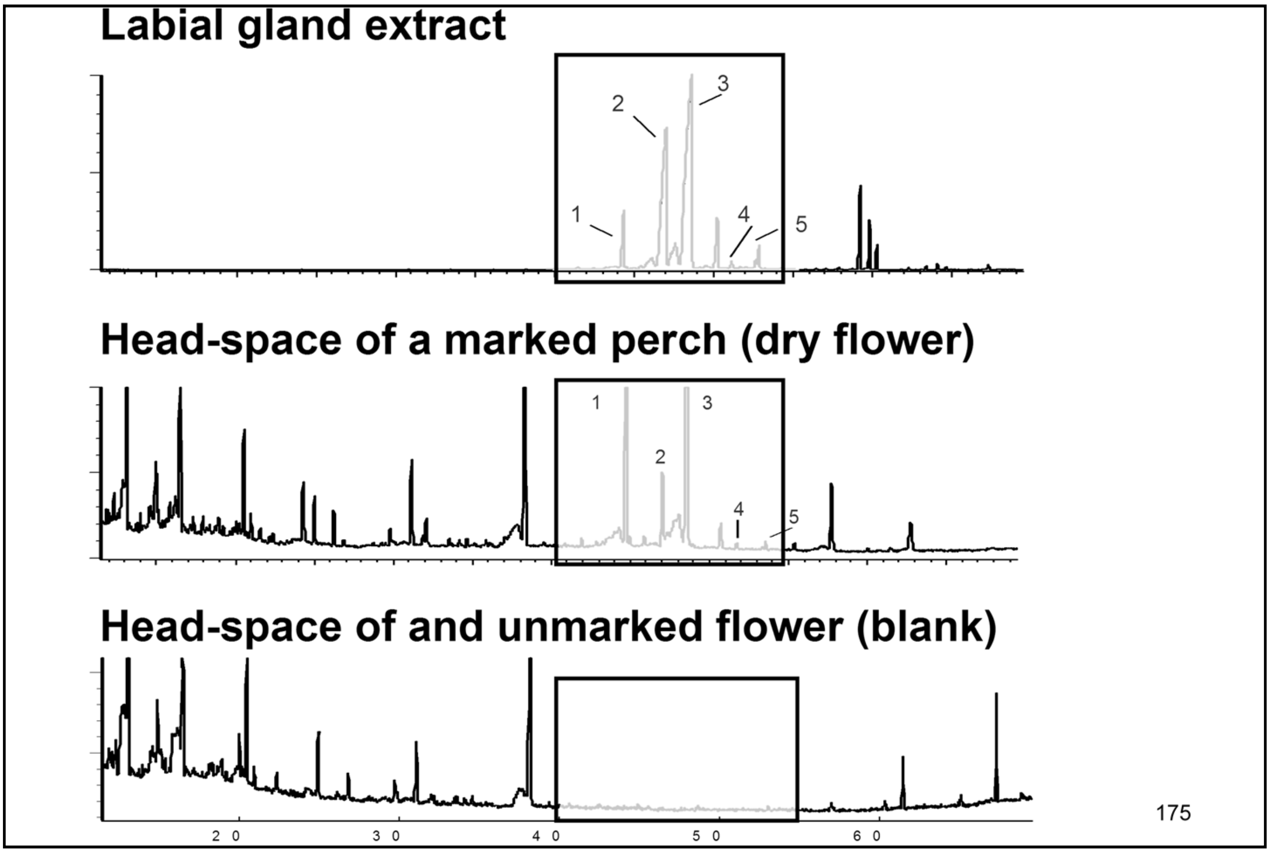


Z-Configuration of double bond (FT-IR)



Function of the labial gland secretion

- **Comparison of components:**
- of the labial gland extract
- of head-space sample of a marked perch
- of washing of a marked perch
- of head-space sample and washing of unmarked plants in the locality


Labial gland extract
1
2
3
4
5
Head-space of a marked perch (dry flower)
1
2
3
4
5
Head-space of and unmarked flower (blank)
20
30
40
50
60

Males' marking behaviour

- 42 males marked individually
- *Marking* – perches and vegetation in the vicinity (straws)
- *Perches:* dry flowers
- *Marking time:* morning, duration 18 min
- *Number of marks* of one male: 32-95

marking by *B. confusus* male

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Kindl J. et al.: Scent marking in male premating behavior of *Bombus confusus*. *J. Chem. Ecol.* **1999**, *25*, 1489-1500.

Territories of two *B. confusus* males

Conclusion

- *B. confusus* is not a species oriented optically only
- Males produce a secretion in their labial gland
- Secretion is used for marking perches and surroundings
- *B. confusus* is not exceptional among other perching species

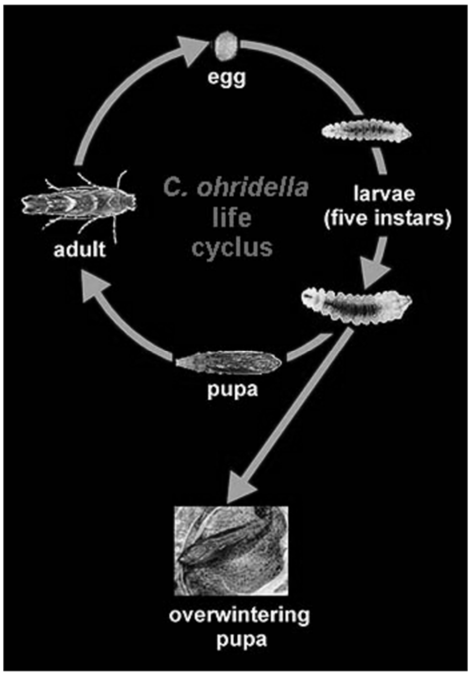
- Methods used for the research: extraction, head-space, GC-MS, derivatisation with DMDS, GC-FTIR, biological observations.

Use of bioassays for localisation of active components in mixtures

Identification of sex pheromone of horse chestnut leafminer (*Cameraria ohridella*) and its possible use for protection of chestnut trees

Cameraria ohridella **Deschka et Dimić 1986 (Lepidoptera: Gracillariidae) originates from Macedonia;**
it is a dangerous pest feeding on horse chestnut, ***Aesculus hippocastanum*** **(L).**

First record in the Czech Republic: 1994 (South Moravia). Now it is spread in the whole country.

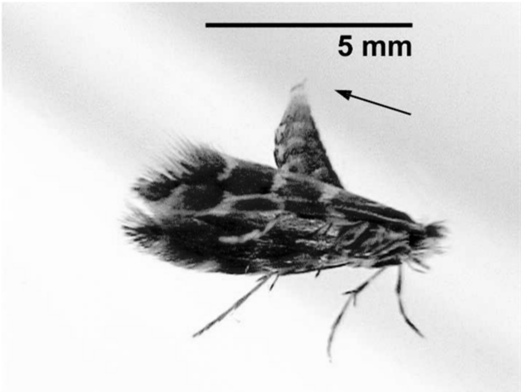


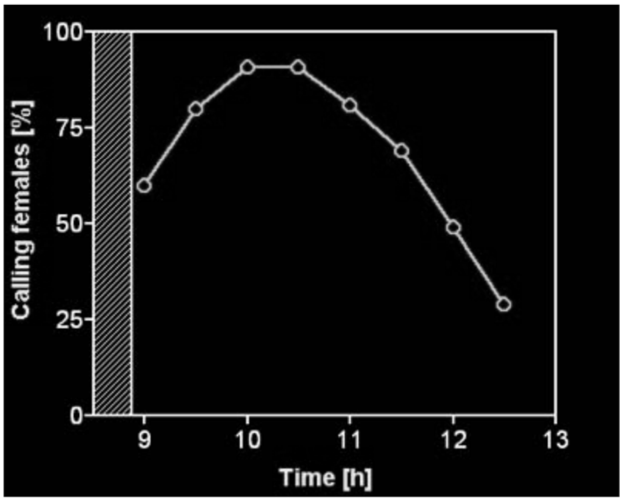
Biology (life cycle):

4 generations / year
overwintering in pupae
first generation in March / April

Typical damage of leaves

Time of females' calling activity



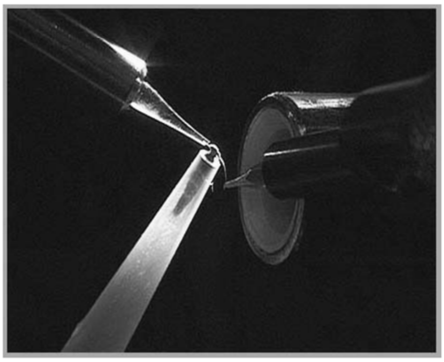


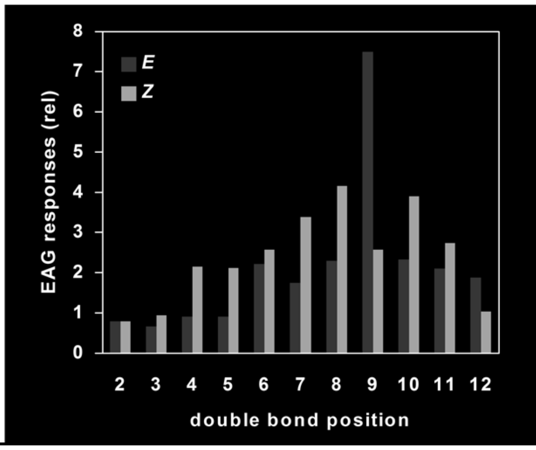
Identification of sex pheromone

A) Using EAG: Abdomens of calling females were dissected and extracted with hexane. EAG responses to saturated compounds (standards):

R-OH < R-Ac < 12:Ald < 14:Ald

EAG map of 14:Ald monoenes:



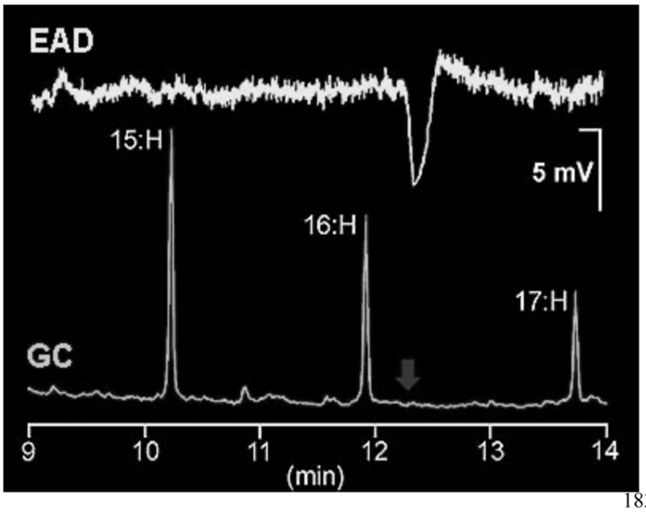


Identification of sex pheromone

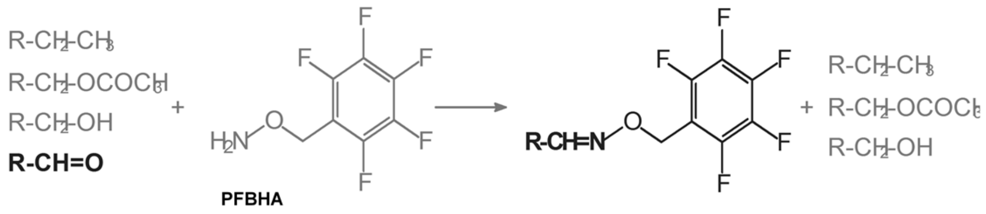
B) Chemical approach:

Extract from 150 females didn't give any good mass spectrum.

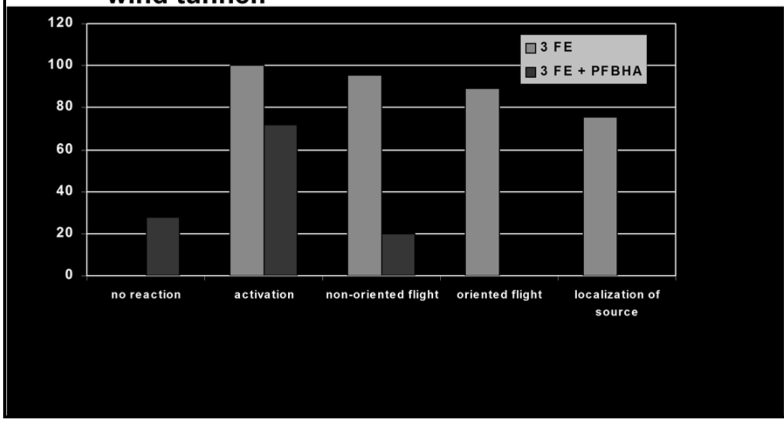
Strong EAG and GC-EAD signal on male antenna



Prove of aldehydic group



wind tunnel:



No response to standards of alcohols or acetates

PFBHA in MeOH was added to extract of females' abdomens

EAG activity disappeared after derivatisation

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pentafluorobenzylhydroxylamin

Identification of sex pheromone

C) Kováts index (KI):

$$I = 100\left[n + (N-n)\frac{\log t'_r(\text{unknown}) - \log t'_r(n)}{\log t'_r(N) - \log t'_r(n)}\right]$$

where n is the number of carbon atoms in the *smaller* alkane

N is the number of carbon atoms in the *larger* alkane

$t'_r(n)$ is the adjusted retention time of the *smaller* alkane

$t'_r(N)$ is the adjusted retention time of the *larger* alkane

- **12:Ac, 14:Ald, and 14:OH have KI similar to the EAD-active peak**
- **(9*E*)-14:Ald (EAD-active) has a different KI from the pheromone**
- **KI of the EAD-active peak indicates two conjugated double bonds**

Identification of sex pheromone

Conclusions from experiments A and B

- **(9*E*)-14:Ald has a different KI and elicits lower EAG- and behavioural activity than the pheromone**

- **the pheromone may be tetradecadienal (TDDA) with double bonds located around C - 9**

=> to prepare all isomers: 7,9-, 8,10- and 9,11-TDDA and test responses on GC-EAD

Identification of sex pheromone

D) GC-EAD of synthetic isomers:

EAG of positional isomers TDDA:

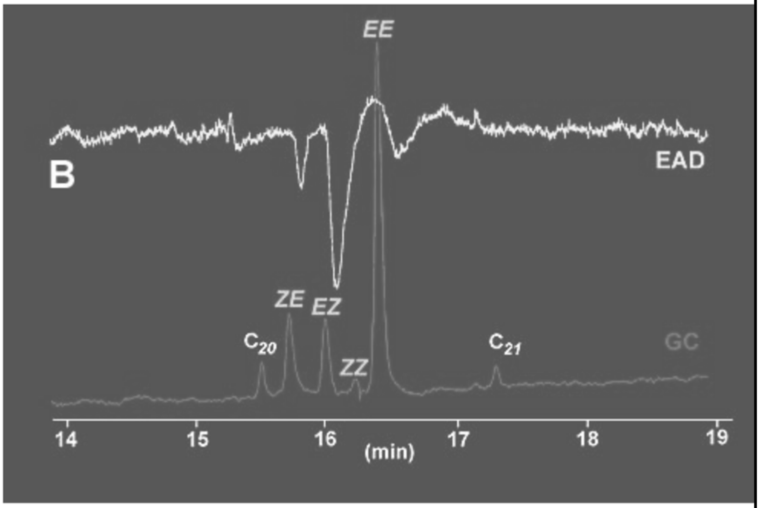
9,11- < 7,9- << 8,10

GC-EAD recordings:

(*E,Z*)-8,10-TDDA elicits highest response of all isomers

KI and EAD responses to 8*E,*10*Z*-TDDA are identical with natural extract

8,10-TDDA



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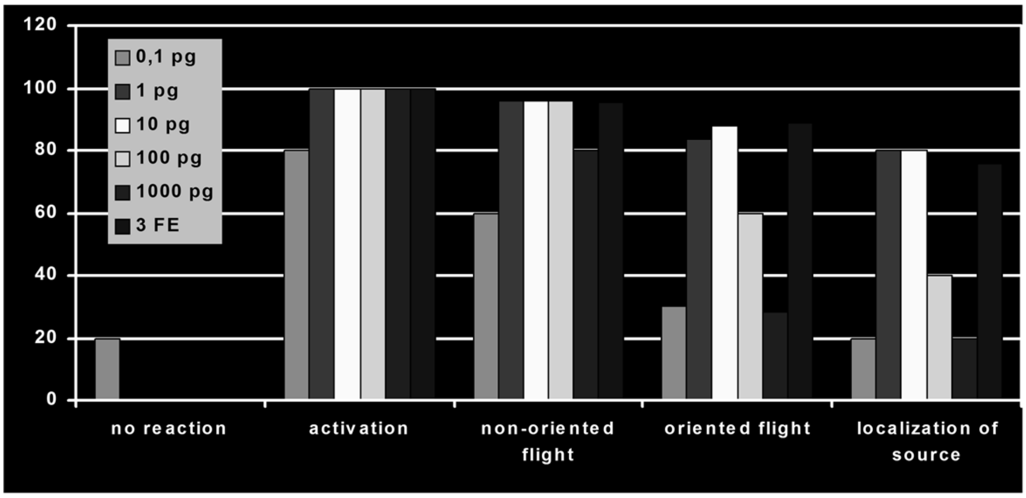
Svatoš A. et al.: Identification of a new lepidopteran sex pheromone in picogram quantities using an antennal biodetector: (8*E*,10*Z*)-Tetradeca-8,10-dienal from *Cameraria ohridella*. *Tetrahedron Lett*. **1999**, *40*, 7011-7014.

Bioassays - Synthetic pheromone in the wind tunnel, dose response

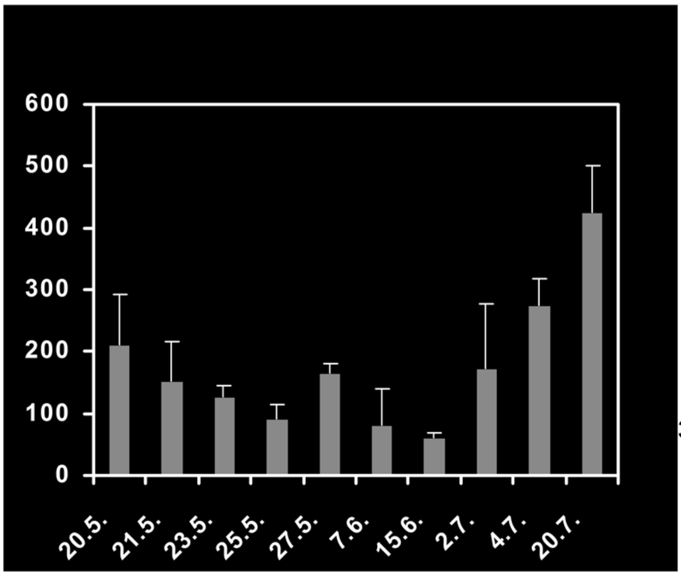
(n = 25 males, 3-4 days old, day time 9-11 AM, air flow 0,4 m/s)

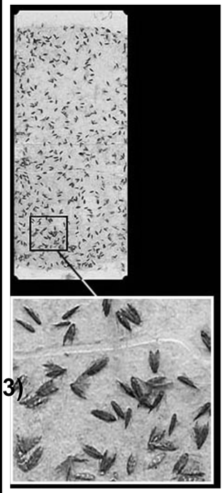


Svatoš A. et al.: Identification of a new lepidopteran sex pheromone in picogram quantities using an antennal biodetector: (8*E*,10*Z*)-Tetradeca-8,10-dienal from *Cameraria ohridella*. *Tetrahedron Lett*. **1999**, *40*, 7011-7014.

Field tests of the synthetic pheromone

Delta traps, checked daily

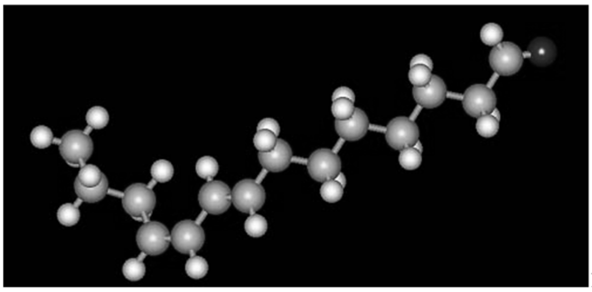




glue trap
(420 males)

Conclusions:

- using combinatorial approach (EAG mapping and Kováts retention index, (8*E,*10*Z*)-tetradeca-8,10-dienal was determined as sex pheromone of horse chestnut leafminer and synthesised in the laboratory
- chromatographic, EAG, and behavioural properties of synthetic and natural pheromone are identical
- synthetic substance is a specific attractant for males in traps



Social insects

The most complex living systems

Dominate terrestrial ecosystems – pollinators, predators, decomposers

Ants	≈ 10^{17} individuals	≈	6×10^{10} kg
Humans	≈ 7×10^{9} individuals	≈	7×10^{10} kg
Termites	≈ 2×10^{18} individuals	≈	1×10^{12} kg

Hölldobler & Wilson 2009: The Superorganism: The Beauty, Elegance & Strangeness of Insect Societies. *W.W. Norton & Co.*

Bignell 2003: Termites: 3000 variations on a single theme. *Inaugural lecture as Professor, Queen Mary University of London.*

Social insects have been existing for 100 millions years, humans only 100 thousands years

(Eu)social insects

- overlapping generations
- division of labour, reproduction asymmetry
- cooperative care of the brood
- existence of sterile casts
- (cast polymorphism)

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Definition of Eusociality

Many animals live together as a group, but they are not necessarily social (e.g. a school of fish) because there is a very precise definition when it comes ot sociality. True sociality (eusociality) is defined by three features: 1). There is cooperative brood-care so it is not each one caring for their own offspring, 2). There is an overlapping of generations so that the group (the colony) will sustain for a while, allowing offspring assist parents during their life, and 3). That there is a reproductive division of labor, i.e. not every individual reproduces equally in the group, in most cases of insects, this means there is one or a few reproductive(s) ("queen", or "king"), and workers are more or less sterile.

Insect societies are based on self-sacrificing, altruism

- part of the society gives up their own reproduction and help others to take care of the brood, colony defence, search for and collecting of food (foraging)
- traits of insect society: coherence/solidarity, diligence, non-selfish submission of an individual to the interest of the society

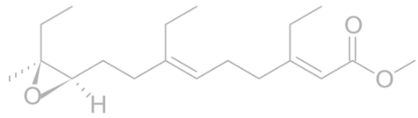
superorganismus

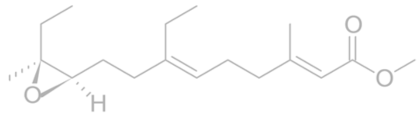
Social insects - bees, ants, termites Life in a colony is chemically controlled

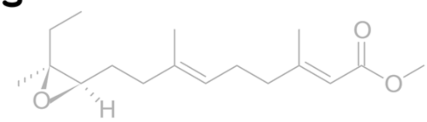
- morphologically distinct casts have different position and function in a colony
- casts: queen (royal pair in termites), workers, drones, soldiers, brood (larvae, nymphs, cocoons)
- combinations of smell and taste signals and receptors used for orientation and communication
- different glands produce secretions of different compositions and functions
- cuticular hydrocarbons – recognition signal

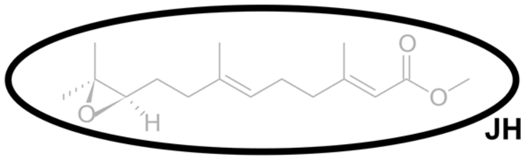
Regulation of the colony development

- nutrition factors
- juvenile hormone
- behaviour
- communication signals


JH 0


JH I


JH II


JH III

Social insects & chemistry

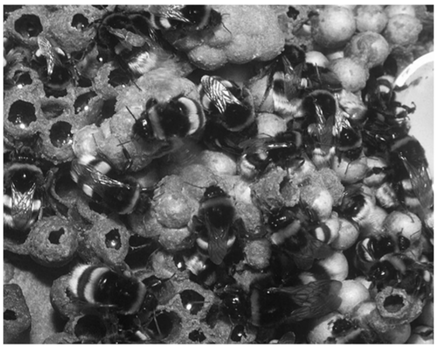
Chemical signals - deeply involved in all aspects of social life
chemical communication – pheromones
from recognition to division of labour – releaser pheromones
caste regulation & queen dominance – primer pheromones
chemical defense – defensive chemicals



Šobotník et al.: Chemical warfare in termites. *J. Insect Physiol.* **2010**, *56,* 1012-1021.

Bumblebees

queen

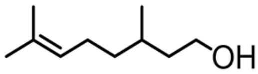


laboratory colony

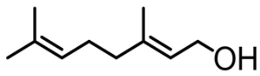
Bombus terrestris

male

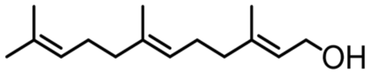
Marking pheromones of bumblebees



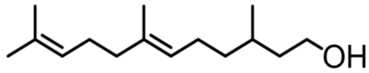
citronellol



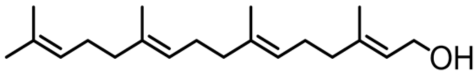
geraniol



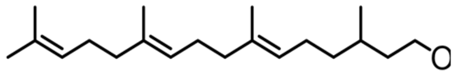
farnesol



dihydrofarnesol



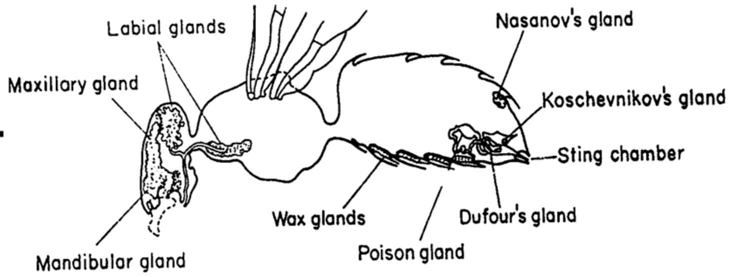
geranylgeraniol



geranylcitronellol

aliphatic alcohols, aldehydes, esters of fatty acids

Bees



queen's pheromone:
(*E*)-9-oxodec-2-enoic acid

- hinders development of more queens
- hinders development of ovaries in workers
- keeps workers in “working mood”
- is attractive for drones

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Slessor K. N. et al.: Semiochemical basis of the retinue response to queen honey bees. *Nature* **1988,** *332,* 354-356.

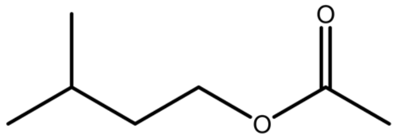
Swarming in bees

lack of queen's pheromone in a colony

workers who register lack of pheromone start to feed a new queen (royal jelly)

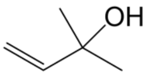
- after emergence, the young queen flies on her "wedding" flight, she mates and returns to the maternal hive
- the old queen leaves her hive followed by a group of her faithful workers - swarming

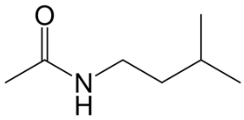
Alarm pheromone - bees

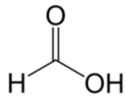


- Some components of the bee's venom elicit alarm behaviour in other bees.
- The active compound is isoamyl acetate - "banana odour" - that causes excitement of bees and elicits their aggressive behaviour.

Alarm pheromone of other Hymenoptera





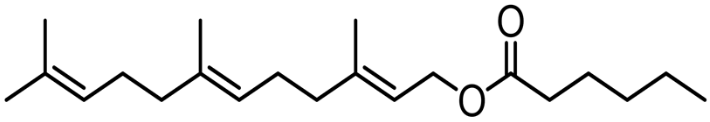


hornets wasps ants (*Formica*)

Ants genus *Formica* (formic acid), hornets (2-metyl-3-buten-2-ol), honey bee (amyl acetate), wasps*Vespula maculifrons* and *V*. *squamosa* (N-3-metylbutylacetamide).

Insect “cuckoos"

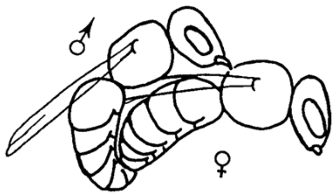
- nest parasites – egglaying in a host nest
- bumblebee (*Bombus*) *vs.* cuckoo bumblebee (*Psithyrus*) – strong queen, fights to get into a host nest
- solitary bees of the genus *Andrena* – odour marking of a nest (Dufour’s gland)
- **farnesyl hexanoate** in the secretion

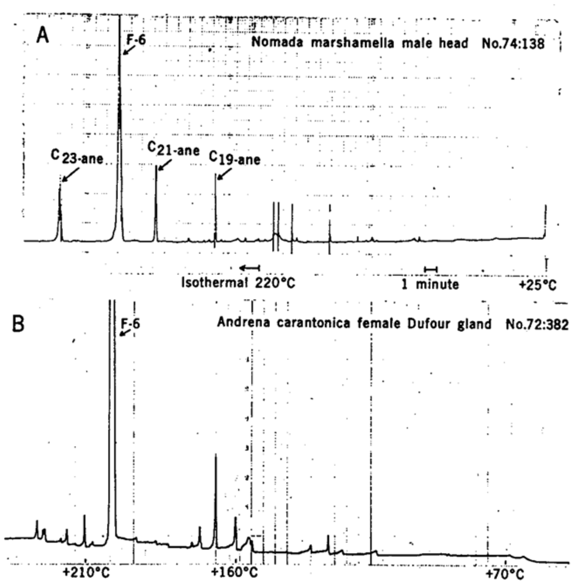


Tengö J. & Bergström G.: Cleptoparasitism and odor mimetism in bees: Do *Nomada* males imitate the odor of *Andrena* females? *Science* **1977**, *196*, 1117.

Parasitic bee of the genus *Nomada*

- identical compound in male's mandibular gland – transferred to the female during mating
- enters the host nest easily ("home odour")





Tengö J. & Bergström G.: Cleptoparasitism and odor mimetism in bees: Do *Nomada* males imitate the odor of *Andrena* females? *Science* **1977**, *196*, 1117.

Cast polymorphism in ants

Camponotus sansabeanus

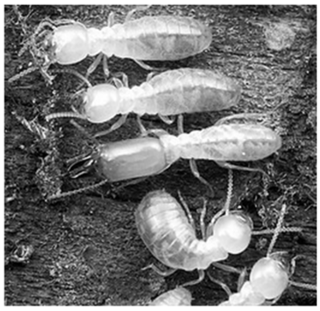
Myrmecocystus mexicanus

Hymenoptera – allometry; termites – formation of special structures

Cast polymorphism in ants

Pheidole californica

Pheidole rhea



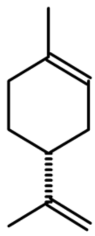
Reticulitermes

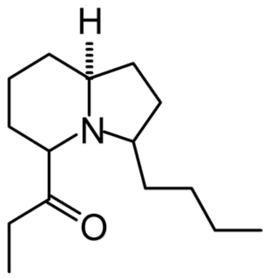
Hymenoptera – allometry; termites – formation of special structures

Ants

- ◆ trail pheromone – marking of a trail to food source (foraging)
- ◆ calling for help with found food (recruitment)
- ◆ alarm pheromone – calling for help in case of danger
- ◆ defence of some species - poison gland

Exaple: *Myrmicaria eumenoides* - poison gland – mixture of limonene and 2 isomeric indolizine alkaloids



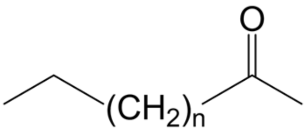


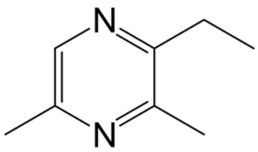
toxicity: limonene << alkaloids << mixture

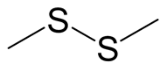
Kaib M., Dittebrand H.: The poison gland of the ant *Myrmicaria eumenoides* and its role in recruitment communication. *Chemoecology* **1990**, *1*, 3-11.

Francke W., Schröder F., Walter F., Sinnwell V., Bauman, H., Kaib M.: New alkaloids from ants - identification and synthesis of (3R,5S,9R)-3-butyl-5-(1-oxopropyl) indolizidine and (3R,5R,9R)-3-butyl-5-(1-oxopropyl)indolizidine, constituents of the poison gland secretion in *Myrmicaria eumenoides* (Hymenoptera, Formicidae). *Liebig's Ann. Chem*. **1995,** *995*, 965-977.

Alarm pheromone of other ant species







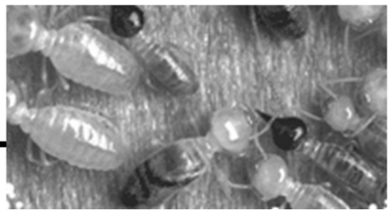
other ant species

Necromones (ants and bees)

- identification of a dead body of a conspecific individual
- products of decomposition of animal material (oleic acid and other fatty acids)
- ants – objects marked with necromone are removed from the colony

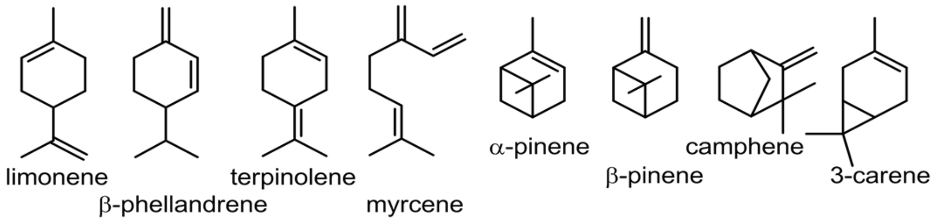
Test – a living worker marked with a necromone is pulled out from the colony as long as she smells of fatty acids.

Necromones, consisting of oleic and linoleic acids, help animals identify the presence of dead conspecifics (crustaceans and hexapods)



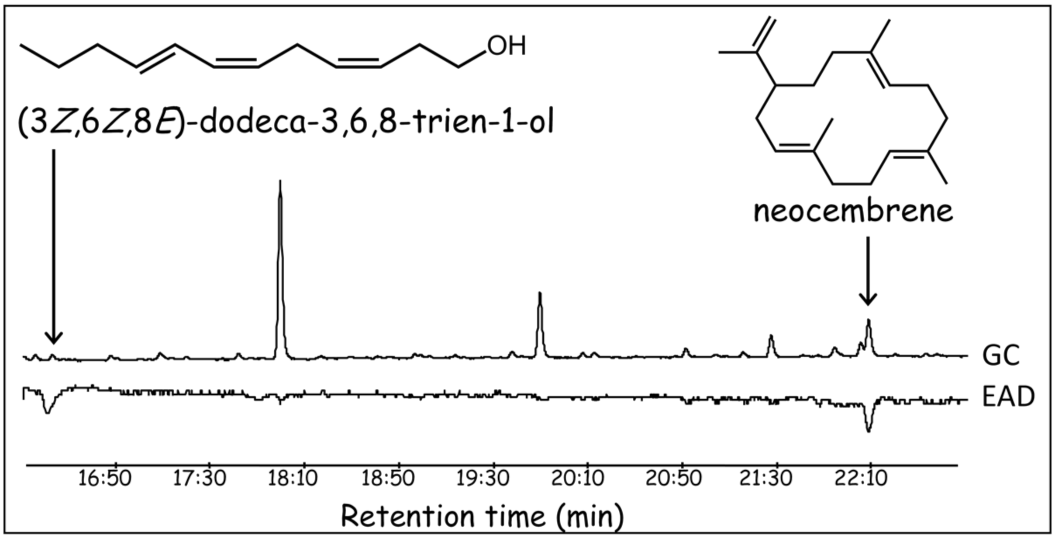
Termites

- ◆ queen's pheromone not known
- ◆ some signals similar to ants
- ◆ cast of soldiers produces effective defence compounds, some species have a fully chemical strategy (Nasutitermitinae)
- ◆ monoterpenic hydrocarbons function as alarm pheromone



Chemodiversity in termites

Trail-following pheromone in *Prorhinotermes simplex*



Sillam-Dussès et al. 2009: *J. Insect Physiol.* **55**: 751-757.

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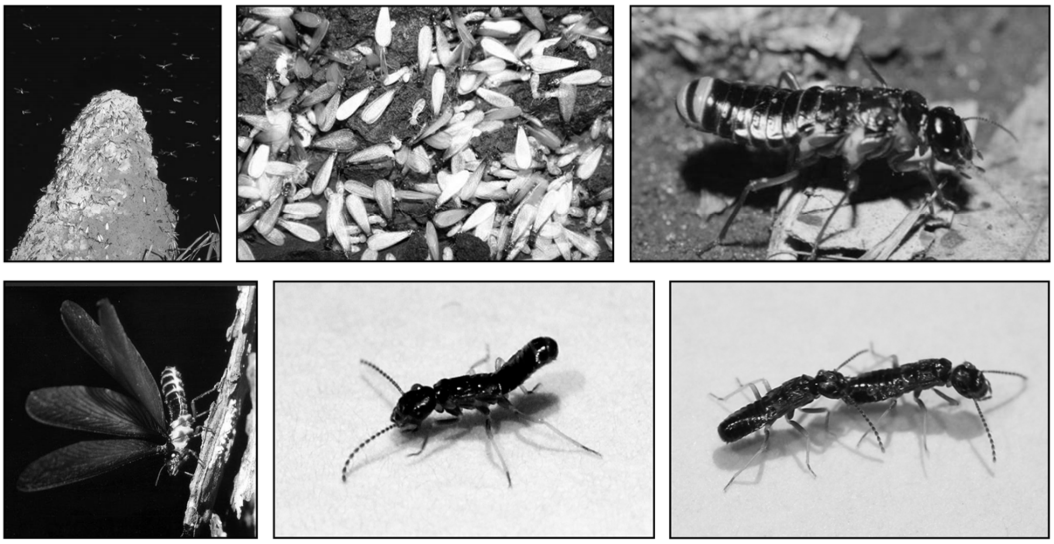
náš modelový druh P. simplex

vysvětlit, že stopovací feromon je používán k nalezení cesty k potravě

vysvětlit krátce EAG a spojejí GC-EAD

Chemodiversity in termites

Sexual communication in *Prorhinotermes simplex*



Hanus et al. : *Insectes Soc.* **2009**, *56,* 111-118.

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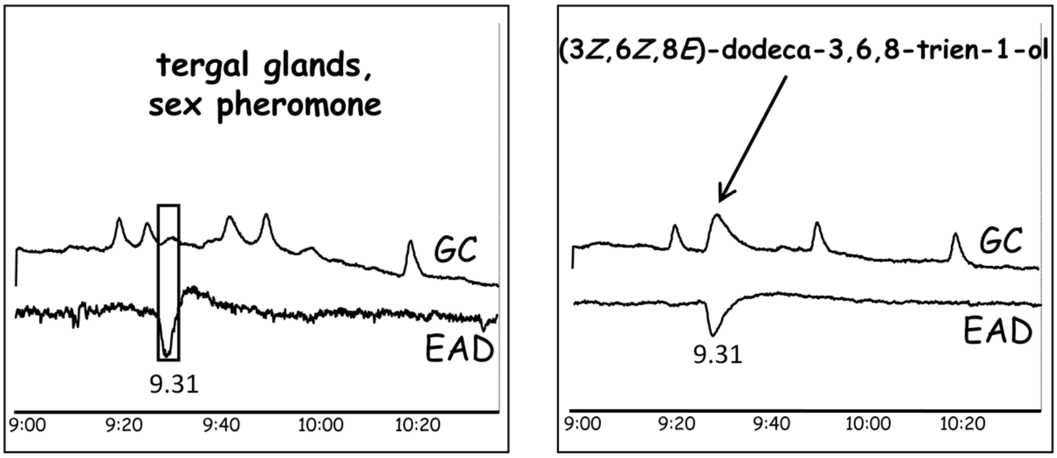
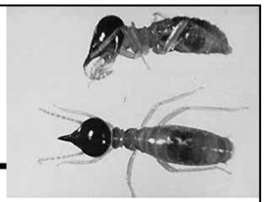
Rojení: vylétání okřídlených královen a králů, pak ztráta křídel, královna volá a nakonec dělají tandem.

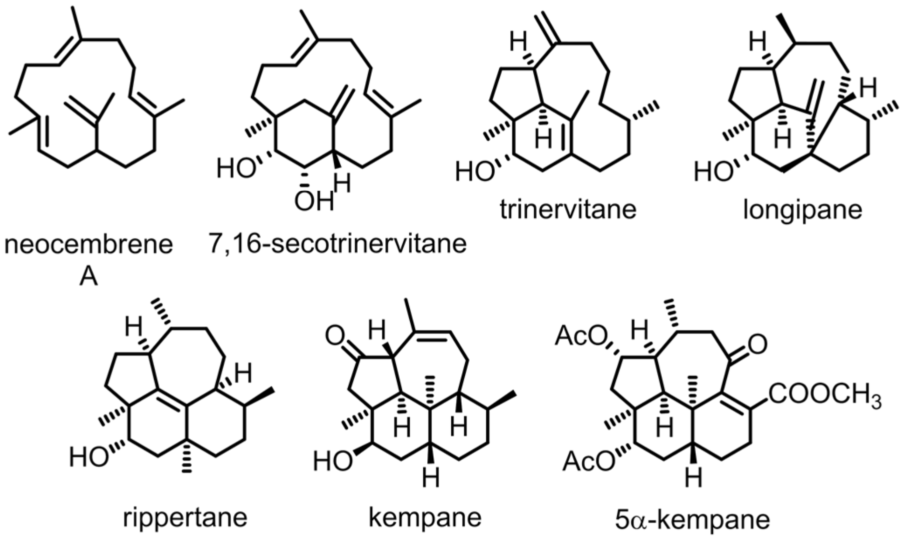
Chemodiversity *vs* parsimony in termites



Hanus et al. : *Insectes Soc.* **2009**, *56,* 111-118.

Defence compounds of termites

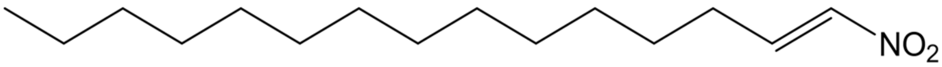




Šobotník J, et al.: Chemical warfare in termites. *J. Insect Physiol.* **2010,** *56,* 1012-1021.

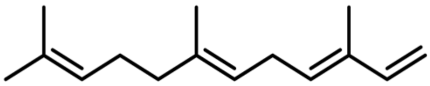
Defence compounds of termites

Prorhinotermes simplex soldiers



(*E*)-1-nitro-pentadec-1-ene

soldiers' cuticle can detoxify the compound

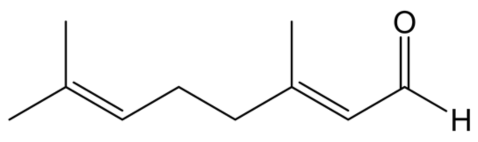


α-farnesene

component causing alarm behaviour

Misuse of alarm pheromone

- Cleptoparasitic bee *Lestrimelitta melao* misuses **citral** (alarm pheromone) for attacks of related stingless bees (*Trigona, Mellipona*).
- Citral is alarm pheromone of both, the parasite and its host.



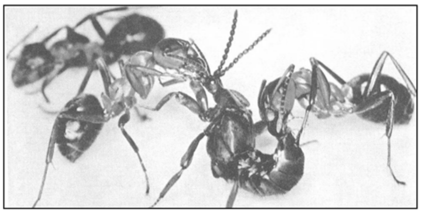
citral

Misuse of chemical signals
Chemical mimicry and camouflage

- **Imitate the code of a host**: surface identification pheromones (cuticular hydrocarbons)
- **Myrmecophily** (termitophily): species living in ants' or termites' nests due to chemical adaptations
- **Symphily** – high adaptation, guests produce attractive or calming substances, common in ants
- **Commensalism** – partial adaptation, feed on organic waste of a colony, common in ants; benefit for guests, neutral for host

  Synechtry – parasites feeding on host's brood or stored food; low adaptation, but strong defence means (Staphylinidae, caterpillars of lycaenid butterflies)

Myrmecophilic beetle *Atemeles pubicollis* (Staphylinidae) offers a secretion of abdominal gland and accepts feeding by ants



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In ecology, commensalism is a class of relationship between two organisms where one organism benefits but the other is neutral (there is no harm or benefit). There are three other types of association: mutualism (where both organisms benefit), competition (where both organisms are harmed), and parasitism (one organism benefits and the other one is harmed).

Atemeles pubicollis, a staphylinid beetle, lives as a guest in colonies of ants.

Ant-insect interactions

Ants tend a wide variety of insect species, most notably lycaenid butterfly caterpillars and hemipterans.[5] Forty-one percent of all ant genera include species that associate with insects.[17] In all ant-insect associations the ants provide some service in exchange for nutrients in the form of honeydew, a sugary fluid excreted by many phytophagous insects.[5] Interactions between honeydew-producing insects and ants is often called trophobiosis, a term which merges notions of trophic relationships with symbioses between ants and insects. This term has been criticized, however, on the basis that myrmecophilous interactions are often more complex than simple trophic interactions, and the use of symbiosis is inappropriate for describing interactions among free-living organisms.[5]

Hemiptera

Some of the most well-studied myrmecophilous interactions involve ants and hemipterans (earlier grouped in the order Homoptera which included the Auchenorrhyncha and Sternorrhyncha), especially aphids. There are around 4000 described species of aphids, and they are the most abundant myrmecophilous organisms in the northern temperate zones.[3][5] Aphids feed on the phloem sap of plants, and as they feed they excrete honeydew droplets from their anus. The tending ants ingest these honeydew droplets then return to their nest to regurgitate the fluid for their nestmates (see trophallaxis).[1] Between 90-95% of the dry weight of aphid honeydew is various sugars, while the remaining matter includes vitamins, minerals, and amino acids.[3] Aphid honeydew can provide an abundant food source for ants (aphids in the genus can secrete more honeydew droplets per hour than their body weight) and for some ants aphids may be their only source of food. In these circumstances, ants may supplement their honeydew intake by preying on the aphids once the aphid populations have reached certain densities. In this way ants can gain extra protein and ensure efficient resource extraction by maintaining honeydew flow rates that do not exceed the ants' collection capabilities.[3] Even with some predation by ants, aphid colonies can reach larger densities with tending ants than colonies than without. Ants have been observed to tend large "herds" of aphids, protecting them from predators and parasitoids. Aphid species that are associated with ants often have reduced structural and behavioral defense mechanisms, and are less able to defend themselves from attack than aphid species that are not associated with ants.[3]

Ants engage in associations with other honeydew-producing hemipterans such as scale insects (Coccidae), mealybugs (Pseudococcidae), and treehoppers (Membracidae), and most of these interaction are facultative and opportunistic with some cases of obligate associations, such as hemipterans that are inquiline, meaning they can only survive inside ant nests.[5] In addition to protection, ants may provide other services in exchange for hemipteran honeydew. Some ants bring hemipteran larvae into the ant nests and rear them along with their own ant brood.[3] Additionally, ants may actively aid in hemipteran dispersal: queen ants have been observed transporting aphids during their dispersive flights to establish a new colony, and worker ants will often carry aphids to a new nesting site if the previous ant nest has been disturbed. Ants may also carry hemipterans to different parts of a plant or to different plants in order to ensure a fresh food source and/or adequate protection for the herd.

Lycaenid butterflies

Myrmecophily among lycaenid caterpillars differs from the associations of hemipterans because caterpillars feed on plant tissues, not phloem sap, and therefore do not continually excrete honeydew. Caterpillars of lycaenid butterflies have therefore evolved specialized organs that secrete chemicals to feed and appease ants.[3] Because caterpillars do not automatically pass honeydew, they must be stimulated to secrete droplets and do so in response to ant antennation, which is the drumming or stroking of the caterpillar's body by the ants' antennae.[2] Some caterpillars possess specialized receptors that allow them to distinguish between ant antennation and contact from predators and parasites, and others produce acoustic signals that agitate ants, making them more active and likely better defenders of the larvae.[18][19] As with homopteran myrmecophiles, ants protect Lycaenid larvae from predatory insects (including other ants) and parasitoid wasps, which lay their eggs in the bodies of many species of Lepidoptera larvae. The enemy-free space that ants can provide for lycaenids is significant: one study conducted by Pierce and colleagues in Colorado experimentally demonstrated that survival rates of *G. lygdymus* larvae declined 85-90% when ant partners were excluded.[20] These interactions do not come without an energetic cost to the butterfly, however, and it has been shown that ant-tended individuals reach smaller adult sizes than non-tended individuals due to the costs of appeasing ants during the larval stage.[21] Interactions with ants are not limited to the butterfly's larval stage, and in fact ants can be important partners for butterflies at all stages of their life cycle.[2] For example, adult females of many lycaenid butterflies preferentially oviposit on plants where ant partners are present, possibly by using ants' own chemical cues in order to locate sites where juvenile butterflies will likely be tended by ants.[19] Finally, while ant attendance has been most widely documented in Lycaenid butterflies (and to some extent riodinid butterflies), many other lepidopteran species are known to associate with ants, including many moths.[19]

Multiple levels of myrmecophily

Many trophobiotic ants can simultaneously maintain associations with multiple species.[17] Ants that interact with myrmecophilous insects and myrmecophytes are highly associated: species that are adapted to interact with one of these myrmecophiles may switch among them depending on resource availability and quality. Of the ant genera that include species that associate with ant plants, 94% also include species that associate with trophobionts. In contrast, ants that are adapted to cultivate fungus (leaf cutter ants, tribe Attini) do not possess the morphological or behavioral adaptations to switch to trophobiotic partners.[17] Many ant mutualists can exploit these multi-species interactions to maximize the benefits of myrmecophily. For example, some plants will host aphids instead of investing in EFN's, which may be more energetically costly depending on local food availability.[5] The presence of multiple interactors can strongly influence the outcomes of myrmecophily, often in unexpected ways.[22]

Significance of myrmecophily in ecology

Mutualisms are geographically ubiquitous, found in all organismic kingdoms, and play a major role in all ecosystems.[22][23] Combined with the fact that ants are one of the most dominant lifeforms on earth,[12] it is clear that myrmecophily plays a significant role in the evolution and ecology of diverse organisms, and in the community structure of many terrestrial ecosystems.

Evolution of positive interactions

Questions of how and why species coevolve are of great interest and significance. In many myrmecophilous organisms it is clear that ant associations have been influential in the ecological success, diversity, and persistence of species. Analyses of phylogenetic information for myrmecophilous organisms as well as ant lineages have demonstrated that myrmecophily has arisen independently in most groups multiple times. Because there have been multiple gains (and perhaps losses) of myrmecophilous adaptations, the evolutionary sequence of events in most lineages is unknown.[21] Exactly how these associations evolve also remains unclear.

In studying the coevolution of myrmecophilous organisms, many researchers have addressed the relative costs and benefits of mutualistic interactions, which can vary drastically according to local species composition and abundance, variation in nutrient requirements and availability, host plant quality, presence of alternative food sources, abundance and composition of predator and parasitoid species, and abiotic conditions.[17] Because of the large amounts of variation in some of these factors, the mechanisms that support the stable persistence of myrmecophily are still unknown.[22] In many cases, variation in external factors can result in interactions that shift along a continuum of mutualism, commensalism, and even parasitism. In almost all mutualisms, the relative costs and benefits of interactions are asymmetrical; that is, one partner experiences greater benefits and/or fewer costs than the other partner. This asymmetry leads to “cheating,” in which one partner evolves strategies to receive benefits without providing services in return. As with many other mutualisms, cheating has evolved in interactions between ants and their partners. For example, some lycaenid larvae are taken into ant nests where they predate on ant brood and offer no services to the ants.[3] Other lycaenids may parasitize ant-plant relationships by feeding on plants that are tended by ants, apparently immune to ant attack because of their own appeasing secretions. Hemipterophagous lycaenids engage in a similar form of parasitism in ant-hemipteran associations.[13] In light of the variability in outcomes of mutualistic interactions, and also the evolution of cheating in many systems, much remains to be learned about the mechanisms that maintain mutualism as an evolutionarily stable interaction.[23]

Species coexistence

In addition to leading to coevolution, mutualisms also play an important role in structuring communities.[22] One of the most obvious ways in which myrmecophily influences community structure is by allowing for the coexistence of species which might otherwise be antagonists or competitors. For many myrmecophiles, engaging in ant associations is first and foremost a method of avoiding predation by ants. For example, the caterpillars of lycaenid butterflies are an ideal source of food for ants: they are slow-moving, soft-bodied, and highly nutritious, yet they have evolved complex structures to not only appease ant aggression but to elicit protective services from the ants.[2]

In order to explain why ants cooperate with other species as opposed to predating on them, two related hypotheses have been proposed: cooperation either provides ants with resources that are otherwise difficult to find, or it ensures the long-term availability of those resources.[5]

Community structure

At both small and large spatiotemporal scales, mutualistic interactions influence patterns of species richness, distribution, and abundance.[24] Myrmecophilous interactions play an important role in determining community structure by influencing inter- and intraspecific competition; regulating population densities of arthropods, fungi, and plants; determining arthropod species assemblages; and influencing trophic dynamics.[5] Recent work in tropical forests has shown that ant mutualisms may play key roles in structuring food webs, as ants can control entire communities of arthropods in forest canopies.[13] Myrmecophily has also been key in the ecological success of ants. Ant biomass and abundance in many ecosystems exceeds that of their potential prey, suggesting a strong role of myrmecophily in supporting larger populations of ants than would otherwise be possible.[13] Furthermore, by providing associational refugia and habitat amelioration for many species, ants are considered dominant ecosystem engineers.[3][24]

Myrmecophily as a model system

Myrmecophilous interactions provide an important model system for exploring ecological and evolutionary questions regarding coevolution, plant defense theory, food web structure, species coexistence, and evolutionarily stable strategies. Because many myrmecophilous relationships are easily manipulable and tractable, they allow for testing and experimentation that may not be possible in other interactions. Therefore they provide ideal model systems in which to explore the magnitude, dynamics, and frequency of mutualism in nature.[13]

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Misuse of chemical signals

Chemical weapons of slavers

- Imitation of **surface pheromones** of hosts (*de novo,* acquirement of the host scent)
- Secretion of **calming** and **adoption** compounds (chemical nature not known)
- Use of **chemical fight propaganda** („Huráááá…“ smell) imitating alarm pheromone of host, stimulate dispersion and disorganisation (sesquiterpenes and hydrocarbons)
  source: Dufour's gland (10% of body weight)
- Use of **trail pheromone** on slaver raids.
- Success of a parasite is determined by **reading of the host communication code:** specific signals controlling the society

Misuse of chemical signals
Social parasitism and slavers

- **Social parasite** – **related and sympatric** species, in all Hymenoptera (ants 270, bees 3500 species). Example: bumblebee – cuckoo bumblebee.
- **Xenobiosis** – lowest level, neighbouring or shared nests, each colony has own queen and separated brood (cleptobiosis)
- **Temporary parasitism** – during start of a colony (*F. pratensis*). Young female kills the original queen and takes over the nest
- **Permanent parasitism** (2 forms):
  - **Slavery** (*dulose*) female kills the queen, the offspring run for slaver raids to steal workers .
  - **Inquilinism** – queen doesn't have her own workers.

Classification of pheromones

Primer pheromones

- cause long-term physiological changes změny (influence the function of neuroendocrine organs)
- subconscious responses in humans

Releaser pheromones

- elicit immediate changes in behaviour
- conscious responses in humans

Pheromones known in mammals

Territorial	Marking of a territory (deers, beasts of prey, rodents)
Sexual	Indicates sex or estrus (e.g. estrous females in dogs, monkeys)
Maternal	Recognition of own mother (olfactory imprinting)
Social	Determines social position in a pack
Reproduction	Stimulates or inhibits reproduction cycles of females

Do any of them exist in humans?

Humans‘ body odour

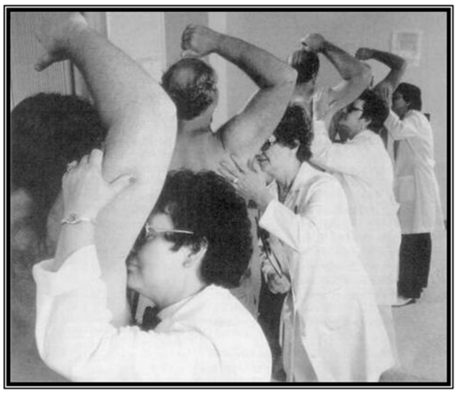
- role in social interactions
- perception on both, conscious and subconscious levels
- sweat glands, apocrine cells + skin bacteria
- genetically determined „odour signature“
- centre of interest - axillary region
- odour → information on sex, reproduction phase, age, health, nutrition, smoking, hygienic habits...

Research on human odours

- methods of odour experiments not unified
- restrictions during experiments
- *(no smoking, drugs, alcohol, aromatic food, cosmetics, exhausting physical activity, sex, sharing bed with a partner)*
- sample collection – T-shirts, cotton pads
- different time intervals for exposition

Odour studies

- fresh samples X stored frozen samples
- evaluators-volunteers – age 18–35, healthy, no hormonal contraception
- hedonic evaluation, subjective scale
- habituation → regeneration of odour receptors



1. sexual differences

- characteristic odours
- ability to distinguish odours ♀ and ♂
- male odour – musk type, intense
- female odour – sweet, less intense
- variability in female odour - menstruation cycle

2. self-recognition

- identification of own T-shirt
- self-evaluation – pleasant odour in ♀, unpleasant in ♂

3. odour of sexual partner

- 30 % people recognise partner's odour
- positive response

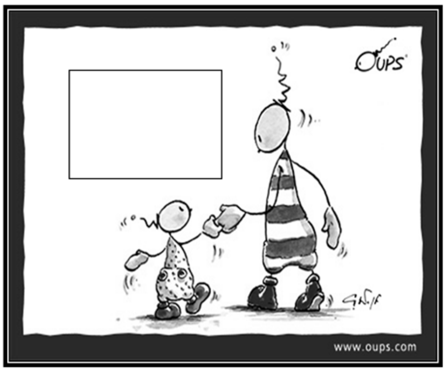
4. relatives

- individual odours similar in families

- close relatives have a similar, but still distinguishable odours
- odours of relatives is evaluated as less pleasant
- aversion of odours - father/daughter, brother/sister

5. mother and child

- first contact important for building relation
- time spent together the first day after delivery
  → mother recognises her baby's odour

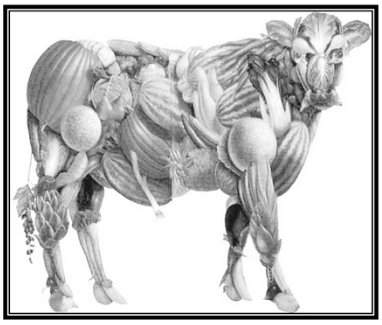


Mother's odour:

1) amniotic fluid
2) areola and papillae
3) axillary odour

- Genetic predisposition of individual odour → relatively stable during life
- Partial changes – **environmental factors**

1. **reproduction state**
   - menstruation cycle and ovulation
   - age and odour
2. **emotional state**
   - distinct odours of good-tempered or scared people



3. **nutrition**
   - garlic, onion, chilli, pepper, vinegar, fermented milk products, aromatic cheese, fish, alcohol
4. **infections and diseases**
   - diabetes, skin issues, parodontosis

Influence of meat consumption on attractiveness of human body odour

- 17 ♂ - 14 days of „meat“ diet
  and 14 days of „meatless“ diet
- last 24h collection of samples of axilliary odours
- 30 ♀ without hormonal contraception
- evaluation of pleasantness, attractiveness, masculinity, and intensity of fresh samples

Havlíček J., Lenochová P.: Chem. Senses **2006**, *31*, 747-752.

Results and conclusions

→ odours of men on meatless diet evaluated as more attractive, more pleasant, and weaker

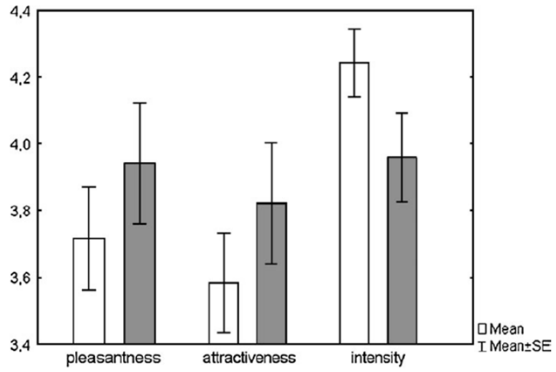


Figure 2 Mean ratings (±SE) of axillary odor pleasantness, attractiveness, and intensity when body odor donors were on meat diet (white bars) and when on nonmeat diet (gray bars). Differences are significant at $P = 0.01$ (repeated measures ANOVA).

Vomeronasal organ (VNO)

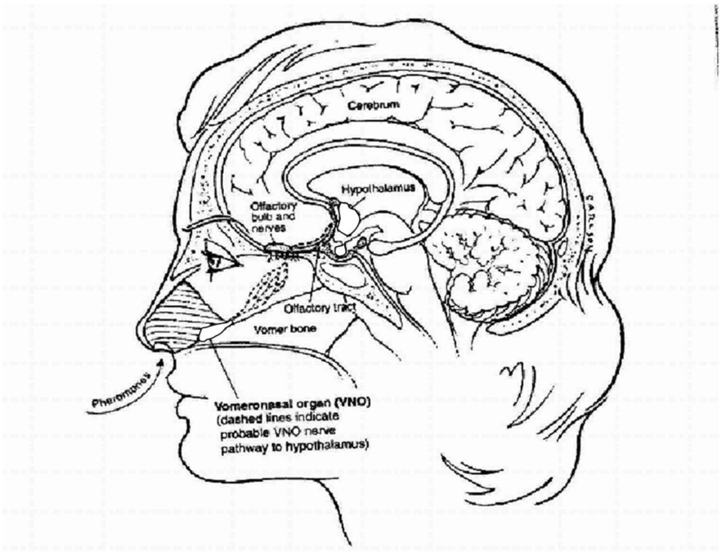
- 1703 first found and described in humans (Ruysh)
- 1811 biologist Ludwig L. Jacobson describes it in animals, but not in humans. „Jacobson's organ", term used in zoology since
- 1938 Elizabeth Crosby in textbook of anatomy describes VNO as non-functional relict existing in fetus only
- 1991 L. J. Stensaas and D. T. Morgan describe VNO ultrastructure in humans and confirm its presence in all patients
- 1996 D. L. Berliner prove steroid receptors in VNO

olfactory receptor ⟶ olfactory nerve ⟶ olfactory bulb

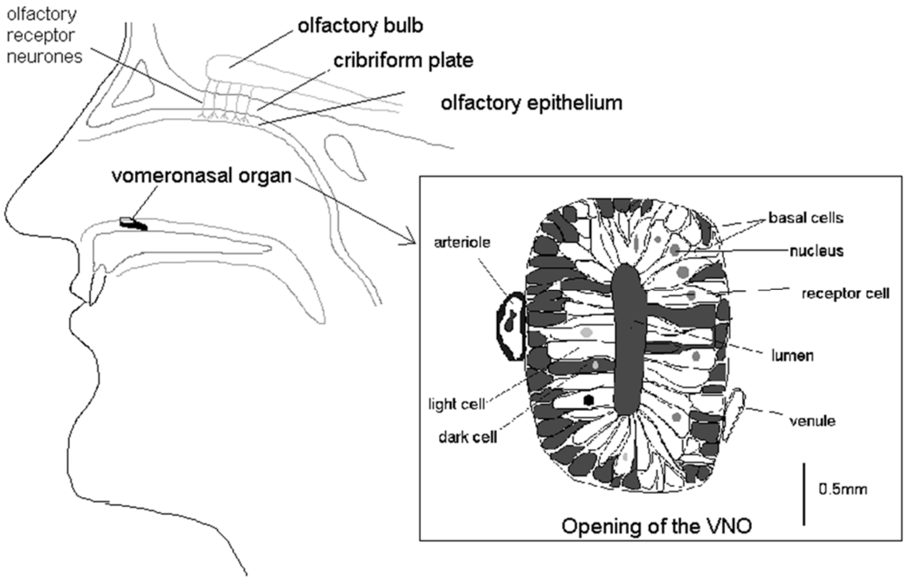
VNO receptor ⟶ VNO nerve ⟶ AOB* ⟶ hypothalamus

VNO receptor	VNO nerve	hypothalamus
found in humans, function proved	not yet found in humans	Controls emotions, sexual appetite, water metabolism, production of hormones, appetite, body temperature

* accessory olfactory bulb



This drawing shows the position of the vomeronasal organ (VNO), which detects pheromones, in the human nose. Pheromone molecules enter the nostrils and contact the sensory receptor cells in the VNO, which in turn send nerve impulses to the hypothalamus, a key control center in the brain.



VNO - functionality

Compounds occurring naturally on the human skin were found to cause a local depolarisation when applied directly to the VNO (Monti-Bloch and Grosser, 1991). The nature of these compounds was not disclosed. This depolarisation had the characteristics of a receptor potential. Furthermore these compounds did not cause a response from the olfactory epithelium and, olfactory stimulants (e.g. cineole) had no effect on the VNO. Using the same compounds sexual dimorphism was demonstrated in their effect on electrodermal activity (Monti-Bloch et al, 1994). These compounds were subsequently revealed to be 16-androstenes and estrenes (Berliner, 1993; 1994). The androstenes have been previously isolated from human sweat (secreted by the axillary apocrine glands) (Gower et al., 1985).

Another "vomeropherin", pregna-4,20-diene-3,6-dione (PDD), caused evoked potentials in the VNO and also changed gonadotropin pulsatility in males, resulting in a reduced level of luteinizing hormone (Berliner et al, 1996) and testosterone (Monti-Bloch et al, 1998). In addition, PDD decreased respiratory frequency, increased cardiac frequency and caused event-related changes of electrodermal activity in EEG pattern (Berliner et al, 1996).

Odour perception

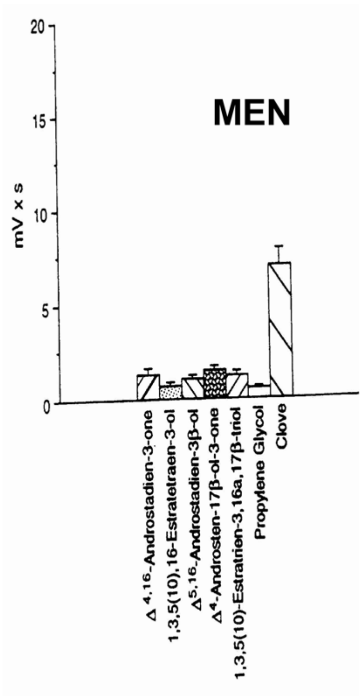
- G-proteins -- regulate cAMP
- upregulation of cAMP influences membrane ion channels and generate action potentials
- cca 1000 genes (mediators of odorants) were cloned
- every olfactory neuron expresses one receptor protein
- odour discrimination – which neuron was activated

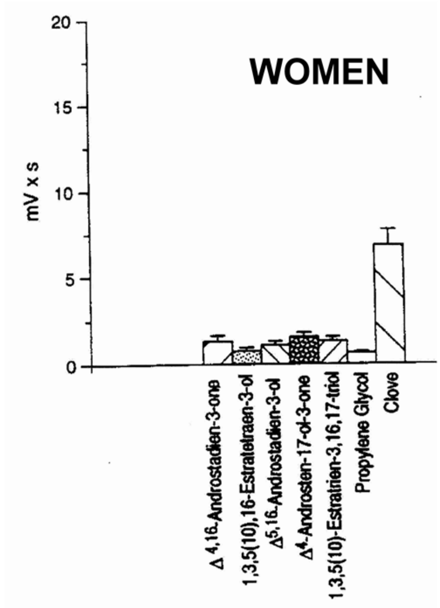
Nobel prize 2004 for research of the sense of smell!

VNO perception

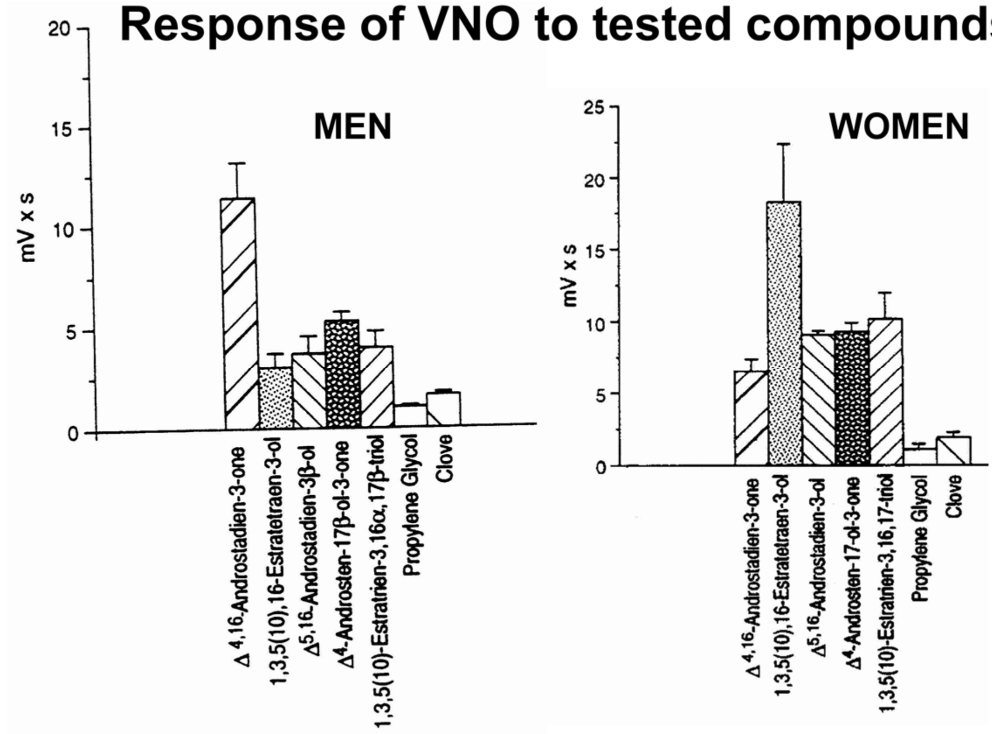
- G-proteins are not expressed
- analogs of olfactory receptors not found
- BUT: two families of VNO receptors
  are suggested

Response of olfactory receptors to tested compounds





Response of VNO to tested compounds



Reproduction pheromones of mice

Lee-Boot effect	Estrus of females kept together without a male is inhibited or changed
Whitten effect	Estrus of females kept together is synchronised by male's urine
Bruce effect	Urine of a male from different group restrains nidation of embyo in fertilised females
Vandenbergh effect	Start of puberty in females is speeded up by urine of matured males

Ablation of VNO removes all 4 effects

“Dormitory effect” (McClintock effect)

- women living together in dormitories or working together synchronise their period (female students, prisoners, factory workers...)
- published in 1971 in *Nature*
- study of 135 volunteers from a female dormitory
- synchronisation of periods depends on duration and intensity of living together
- some women have stronger influence than others (dominance)

Is this caused by chemical signals?

"Dormitory effect"
(McClintock effect)

- sweat extract of a dominant woman was presented to a group of volunteer women; solvent (ethanol) was applied to a control group

- in 4 months, the tested group synchronised their periods, while the control group kept their own rhythms

Synchronisation is caused by a signal/pheromone in human sweat.

How is the signal perceived and how does it work?

“Dormitory effect” (McClintock effect)

- experiments continue with laboratory rats
- rats exhibit the same effect when kept together in a cage
- Hypothesis – existence of 2 signals, one speeds up ovulation and the other postpones it (extends the estrus period)

Do women share the same model?

"Dormitory effect" (McClintock effect)

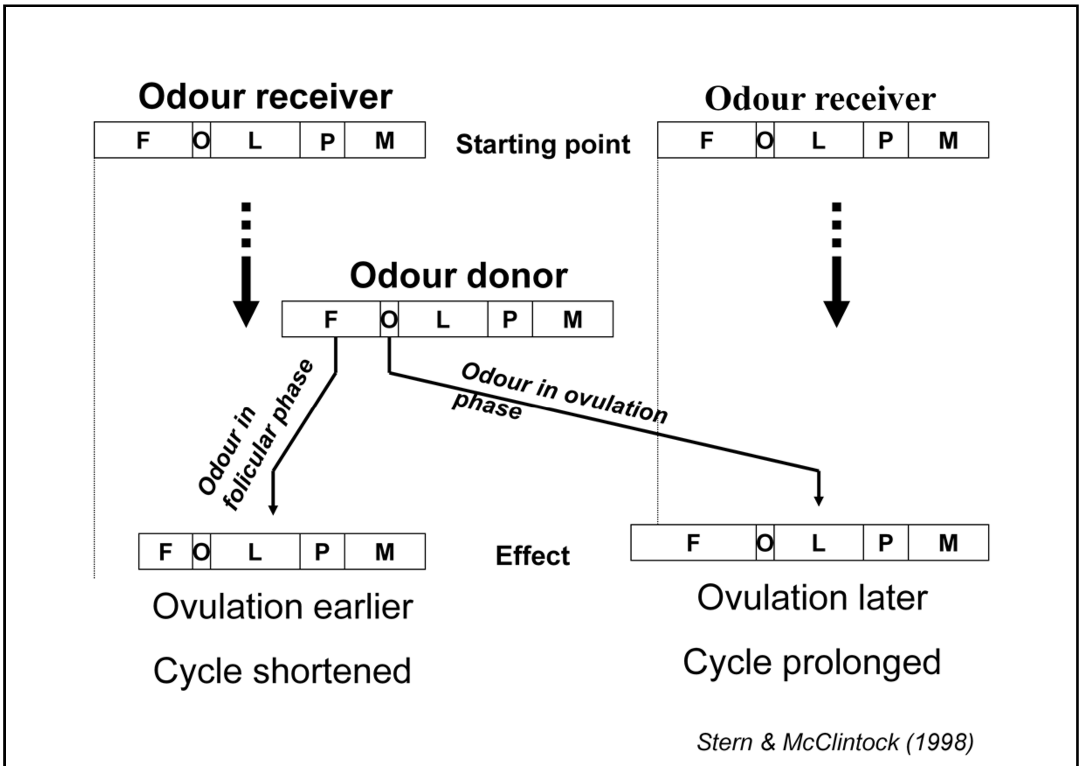
- experiment with 29 female volunteers

- sweat samples taken from 9 women in different phases of the cycle
- others were exposed to the samples

- sweat extracts verified the validity of the hypothesis in women, too:
- sweat of women in folicular phase shortens the folicular phase of receivers, but extends the ovulation when applied later

The sweat extract influenced the biological clock of volunteers.

First proof that volatiles influence the biological rhythm of another individual by means of neurohormonal mechanism.


Odour receiver
F
O
L
P
M
Starting point
Odour receiver
F
O
L
P
M
Odour donor
F
O
L
P
M
Odour in folicular phase
Odour in ovulation phase
F
O
L
P
M
Effect
F
O
L
P
M
Ovulation earlier
Cycle shortened
Ovulation later
Cycle prolonged
Stern & McClintock (1998)

"Dormitory effect"
(McClintock effect)

- baboon is the closest monkey that shows the same effect
- hypothesis - advantage for females to choose a partner
- female monkeys are receptive only in the phase of estrus
- if all females have synchronised cycles, they would mate not only with the dominant male, but some would mate with others (genetical variability)

Females can make a choice to some extent.

Lower risk of genetic load in case of α-male had a genetically conditioned disease.

Vomeropherines
(story of Prof. Berliner)

Apocrine cells may contain human pheromones.

- in fifties, David L. Berliner studied human skin and its products extracted from bandages

- one fraction had unusual effect on people: relaxation, increased self-confidence

- in sixties he stops the academic research and makes business with human pheromones

Story of Prof. Berliner continues

- in 1989 he founded Erox Corporation that organised a symposium in Paris in 1991; isolation and effects of vomeropherins were presented, but no chemical structures

- in 1993, Erox releases 2 perfumes (*Realm Man* and *Realm Woman*) with pheromonal effect (not aphrodisiacs)

In 1994, Berliner patents synthesis, structure, and application of 5 vomeropherines

Berliner became a media star

The Wall Street Journal, Vogue

Source of pheromone in humans

- secretions of skin glands (sweat, apocrine, fat), their components belong to "individual signature"

- glands: often hairy spots on skin, axillary region, areola and papillae, upper lip

- bacteria decompose the secretion components individually

- genetic, hormonal, metabolic, nutritive, psychical factors

- secretions contain steroid compounds – *vomeropherines*, synthesis of many analogs

Fragrance industry misuses research in this area

Pheromone = success, self-confidence, sexual abilities.

Is it based on scientific results?

Male sex pheromones

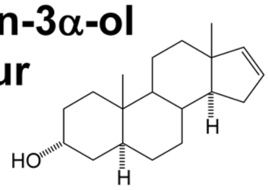
Fertility of female rats increases in the presence of male rats. Is it also valid for humans?

- **Winnifred Cutler: frequent contact with men (not necessarily sex) has a positive effect on women fertility**
- **studied the influence of men sweat on the women hormonal system and thus on the period**
- **tested group: women with irregular cycles and those having irregular sex with men**
- **the men sweat reduced the irregularity of cycles in most women**

Effect of male pheromone – even without regular sex – may contribute to women fertility.

Good news for women whose husbands often travel.

Vomeropherine 5α-16-androsten-3α-ol influences social behaviour



Female volunteers were exposed to androstenol for 17 h.

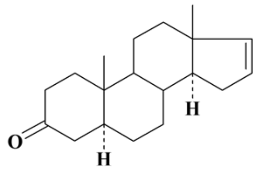
Next day, these women

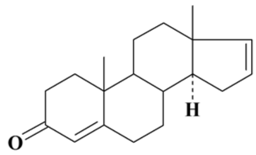
- communicated easier with men
- felt relaxed in contacts with men

Cowley & Brooksbank, *J. Steroid Biochem. 39*, 647 (1991).

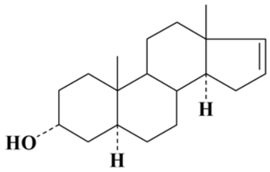
Vomeropherines and pheromones

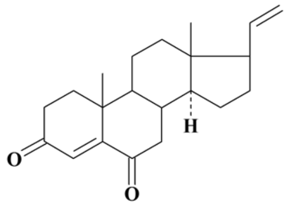
pheromone of boars





vomeroferin





Berliner D.L., Monti-Bloch L.: *US 6331534* (2001). Steroids as neurochemical stimulators of the VNO to alleviate pain. (*Chem. Abstr.* **136**, 37829; 2002).

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Androstadienone

Androstadienone is the current best candidate we have for a human pheromone. Is is a component of human (in particular male) secretions. Does androstadienone meet Karlson & Luscher (1959) "pheromone" criteria? In human studies in which androstadienone had access to the olfactory mucosa both physiological and psychological effects have been reported (Jacob and McClintock, 2000; Jacob, Hayreh and McClintock, 2001; Jacob, Garcia, Hayreh and McClintock, 2002 Bensafi et al., 2003; Lundstrom et al., 2003; Bensafi et al 2004a, 2004b; Cornwell et al., 2004). While none of these changes can be regarded as the behavioural changes required for a compound to qualify as a pheromone, Savic et al. (2001) demonstrated that androstadienone activated the hypothalamus in a gender-specific manner (it activated the hypothalamus in women but not men). Compared with other odorous substances, androstadienone activated the anterior part of the inferior lateral prefrontal cortex (PFC) and the superior temporal cortex (STP) in addition to olfactory areas (Gulyas et al., 2004). The PFC and STP have been implicated in aspects of attention, visual perception and recognition and social cognition.

Vomeropherines (perspectives)

Advantages: not applied through stomach or blood system, but through olfactosystem and hypothalamus influence the neuroendokrine system in nano- to picogramm quantities (no side-effects expected).

- **depressions, anxieties, premenstruation tension and other psychic issues**
- **pregna-4,20-dien-3,6-dion (PDD) decreases heart beat, breathing, enlarges capillaries**
- **obesity (control of appetite) or men impotence (control of libido)**
- **contraceptive (control of ovulation)**
- **hormone-dependent forms of cancer in men and women**

Will vomeropherines become therapeutics of the future?

Natural products from plants: Models for synthetic biocides

from Latin **-cida** (= to kill)
biocide kills all living organisms
pesticide kills pest organisms
(from human's point of view)

Natural products from plants: Models for synthetic biocides

insecticide	kills insects (insectum)
herbicide	kills plants (herba)
fungicide	kills fungi (fungus)
bactericide	kills bacteria (bacterium)

Pesticides

- Under general term “pesticides” sometimes belong also compounds that are not toxic, but remove the pest organisms by other mechanisms (repellents, chemosterilants, growth regulators, defoliants).

Requirements for pesticides

- to kill the target organism
- selectivity
- lowest effective dose
- harmless for beneficial organisms
- harmless for the environment
- non-toxic for humans
- (fast) degradation to harmless compounds

Insecticides – classification according to:

- target pests (aphicide for aphids...)
- target instar (ovicide, larvicide...)
- application (contact, systemic – transported in plant to target tissues eaten by herbivores...)
- origin (natural, synthetic)
- chemical structure (inorganic, organic)
- mechanism of effect

Mechanism of effect

- **Effect on:**
- nervous system - organophosphates, carbamates, pyrethroids (most common nowadays)
- growth and development - juvenoids, ecdysteroids, inhibitors of chitin synthesis (derivatives of benzoyl-urea)
- metabolism and energy production
- circulation system - some rodenticides (anticoagulants)

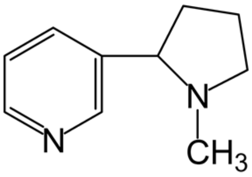
Alternative methods of pest management

Bioracional pesticides

- combination pheromones + insecticides (attract and kill)
- biological method (parasites, pathogenic fungi, *Bacillus thuringiensis, entomopathogenic* nematodes)
- development manipulation (analogs of hormones)
- physical methods

30 % loss of agricultural production world-wide due to pests and fungi

Insecticides – natural models

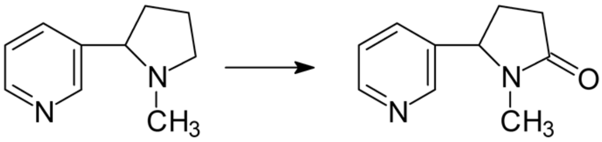


nicotine

- *Nicotiniana ssp.* (tobacco)
- toxic for vertebrates incl. humans (LD_{50} 55 mg/kg in rats)

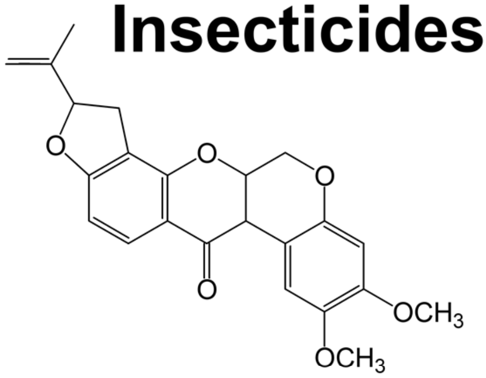
But:

Manduca sexta is a pest on tobacco. Detoxication principle:



nikotine used since 1700

Insecticides – natural models

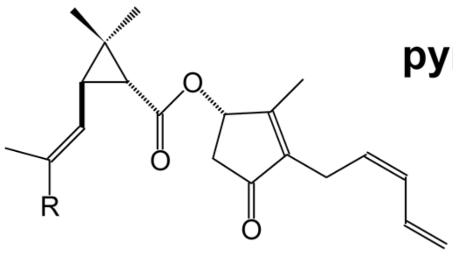


rotenone

- from roots of tropical plants *Derris* (Papilionaceae, Africa)
- used first as arrow poison
- specific effect (toxic for fish, less toxic for mammals)
- traditional „derris powder“ (dried and ground root)
- decomposes quickly (oxidation) when exposed to air and UV light

rotenon LD50 25-75 mg/kg

Insecticides – natural models

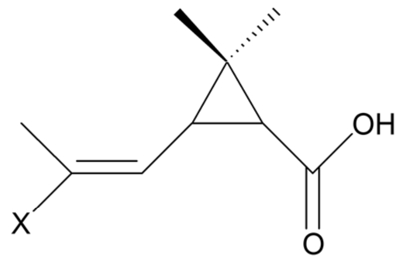


pyrethrins

- from flowers of tropical daisies (*Chrysanthemum*)
- „Dalmatian insect powder“, later extract used in form of spray in households (Kenja - 10 000 t/year)
- low toxicity for mammals (400 mg/kg)
- structural analogs - **pyrethroids**

Pyrethrins since 1820

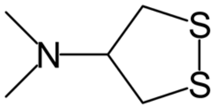
Natural pyrethrins



$X = CH_3$ chrysantemic acid

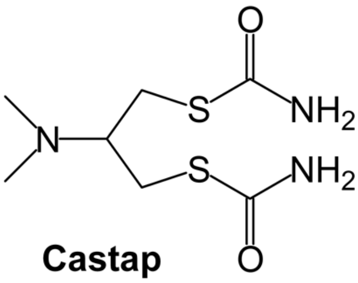
$X = COOCH_3$ pyrethric acid

Insecticides – natural models

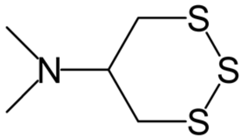


nereistoxin
(*Nereis ssp*. – marine worm)

Synthetic analogs



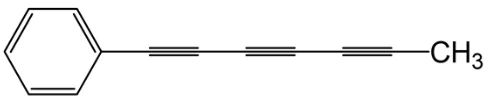
Castap



company Sandoz

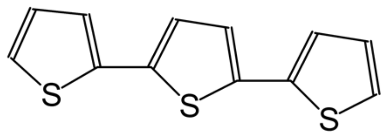
Insecticides – natural models

- **photosensitive**
- polyacetylenyes and polythienyls, furocoumarins
- effective after irradiation by UV light (also sunlight)
- inhibit mitosis



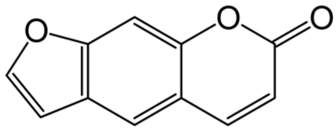
Compositae, effect comparable with DDT

Insecticides – natural models



α-terthienyl

- African marigold (*Tagetes*); alkylated synthetic analogs are more effective



psoralen

- furocoumarins (bergamot oil, hogweed)

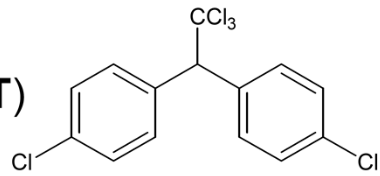
α-terthienyl is allelopathic compound of Tagetes genus

Synthetic insecticides

Structural types:

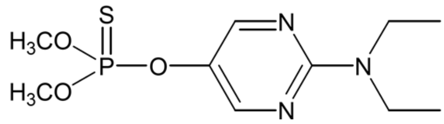
inorganic - contain arsenic, fluorine, barium, sulphur, selenium etc.
mostly stomach poisons
non-specific (toxic to mammals, too)

organic - main types:
chlorinated hydrocarbons (**DDT**)



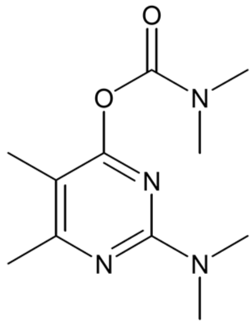
Organic insecticides

organophosphates – esters of phosphoric acid and its sulphuric analogs (**Actellic**); inhibition of cholinesterase (nervous system)



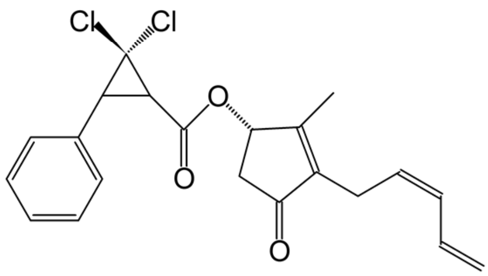
carbamates – derivatives of methylcarbamic acids (**Pirimor**); the same mechanism as organophosphates

nitrocompounds (**dinitrophenols**)



Organic insecticides

Pyrethroids – structurally similar esters; synthetic analogs (**Biolit**); high activity (5-50 g per 1 ha, i.e. 200x less than older types of insecticides)



- **fenethrin** (Farkaš, Šorm - IOCB)
- synergic effects of some compounds (lignanes)

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First **pyrethroids** were unstable in light and oxygen

Synthetic since 1947

30% insecticides used in the world are pyrethroids

Relatively expensive factory production (complicated structures), but low doses, higher stability, lower ecotoxicity (good degradability)

Traditional pyrethroids contain cyclopropane ring (Ambush)

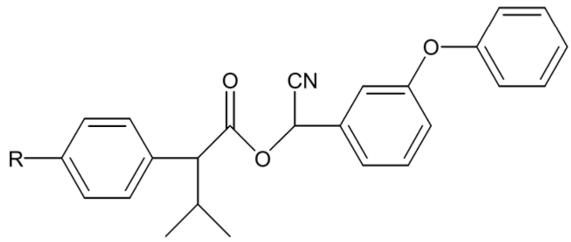
Later pyrethroids – no cyclopropane ring in the molecule

toxicity 100 – 450 mg/kg

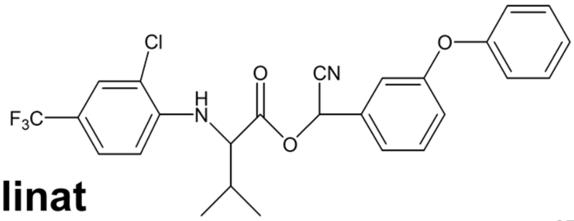
Synthetic pyrethroids

structure of both ester moieties changed

Non-traditional pyrethroids



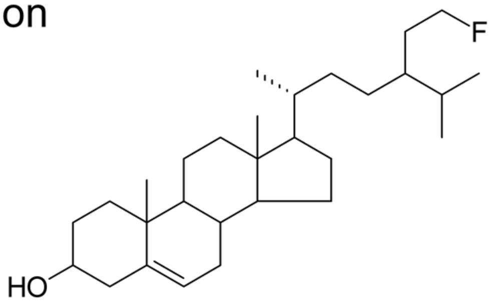
Fenvalerat
R = Cl or $OCHF_2$



Fluvalinat

Insecticides based on fluoroacetic acid

fluoroacetic acid - FCH_2COOH – it enters Krebs' cycle (citric acid cycle) as substrate, but the cycle is inhibited by fluorocitric acid formed, inhibition of respiration



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

Chemosterilants

- inhibit mitosis
- in adult insect instars, they inhibit development and maturation of gonads
- some of them were discovered in search for cancerostatics

Chemosterilants – types of compounds

- Alkylation reagents, aziridines, 5-fluorouracil, analogs of folic acid, triazines, derivatives of urea.
- Chemosterilants are effective per orally, but the practical use is limited (mutagenic effect on mammals).

Chemosterilants – possible use

- sterilisation of males and their release to wild (used for tse-tse flies, sterilisation of males using gamma-irradiation)

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Proportion of sterile males must be 10:1 to reach decrease in population density

Repellents

- repellents are perceived by insect sense of smell (volatile compounds)
- insects are not killed, they run away from the odour source

Requirements for commercial repellents

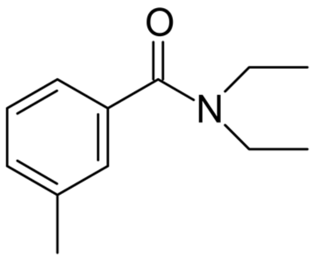
- to repel insects
- to stay on the skin longer time
- to be effective longer time
- not to damage clothes
- not to smell unpleasantly

Known natural (plant) repellents

- myrcene, limonene, capsaicin, valeric, isovaleric, and butyric acids, coumarin
- extract of black pepper, sweet basil leaves, tansy flowers (*Tanacetum vulgare*)

Repellents of stinging insects

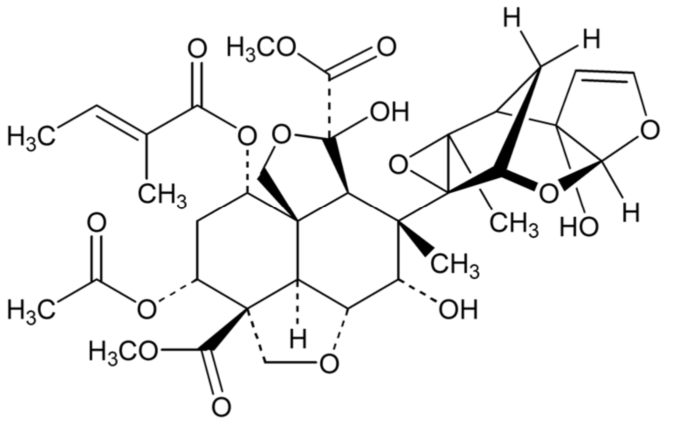
- ***N,N*-diethyl-3-toluamide**
- the most common synthetic repellent of mosquitoes (Off)
- repels ticks, up to 10 h effect



Johnson (Off)

Feeding deterrents (antifeedants)

Azadirachta indica („neem tree“)



azadirachtin

Azadirachtin

- most potent antifeedant known
- registered for use in organic growing, orchards
- application - 1% solution in organic plant oil, diluted to 0,3% water emulsion, spraying dose 1000 l/ha (apple trees, root vegetables)

Insect Growth Regulators

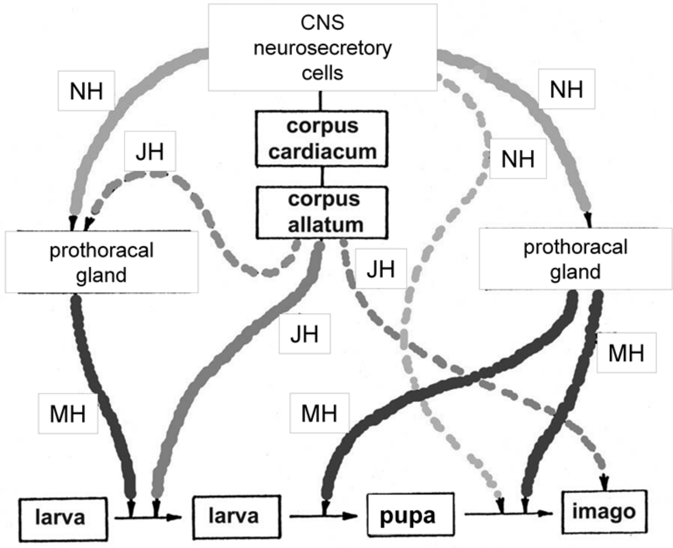
- **selective effect on:**

- embryo growth and development
- larva growth and development
- metamorphosis to adults
- development and function of reproduction organs

Insect hormones

- neurohormones
- juvenile hormones (JH)
- moulting hormones (ecdysones)

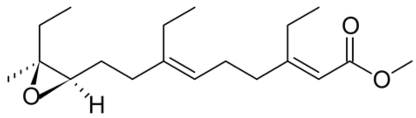
Application of IGR in biologically unsuitable dose of phase leads to the disruption of development and emergence of individuals unable to survive or to reproduce.



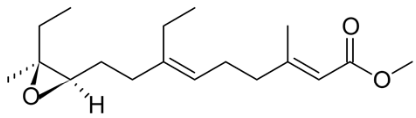
General scheme of hormonal regulation in insects

NH neurohormones
JH juvenile hormones
MH moulting hormones

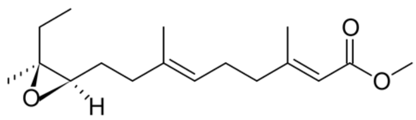
Structures of juvenile hormones



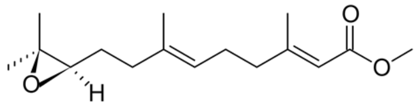
JH 0



JH I

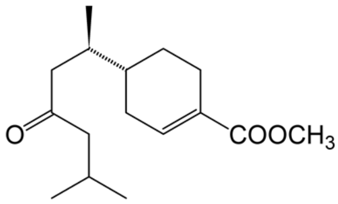


JH II

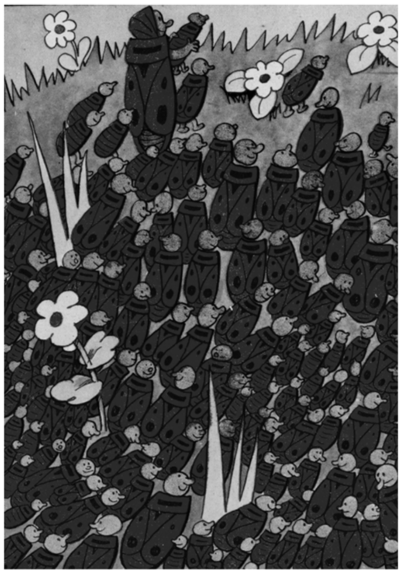


JH III

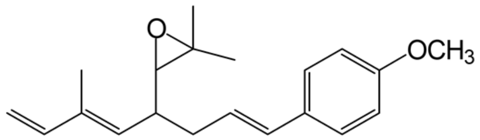
Juvenoids from plants



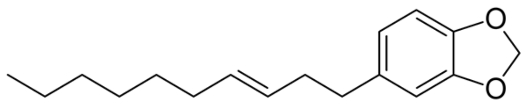
juvabione
„paper factor“
Sláma 1965
Canadian fir
(*Abies balsamea*)
effect on bugs



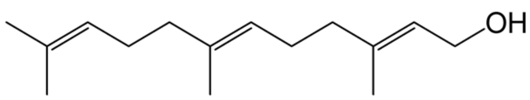
Other natural juvenoids



juvocimen 2
sweet basil (*Ocimum basilicum*)
more active on bugs that JH I
(by several orders of magnitude)

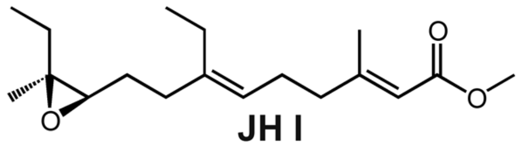


juvadecene
from pepper-related plant
(*Macropiper*)

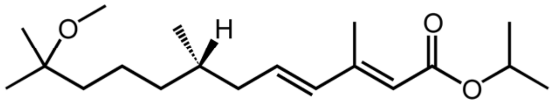


farnesol
from faeces of mealworm
(*Tenebrio*)

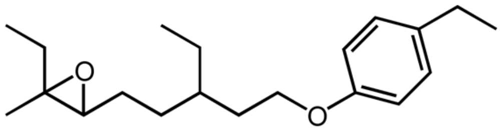
Synthetic juvenoids



JH I

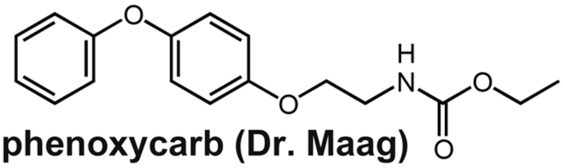


(S)-methoprene (Zoecon)

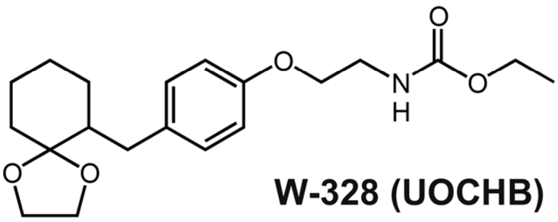


epophenonane (Hoffmann - La Roche)

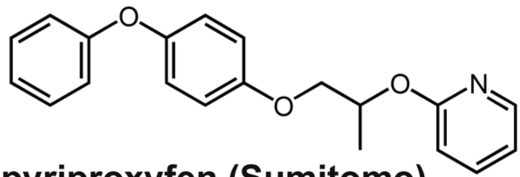
Synthetic juvenoids



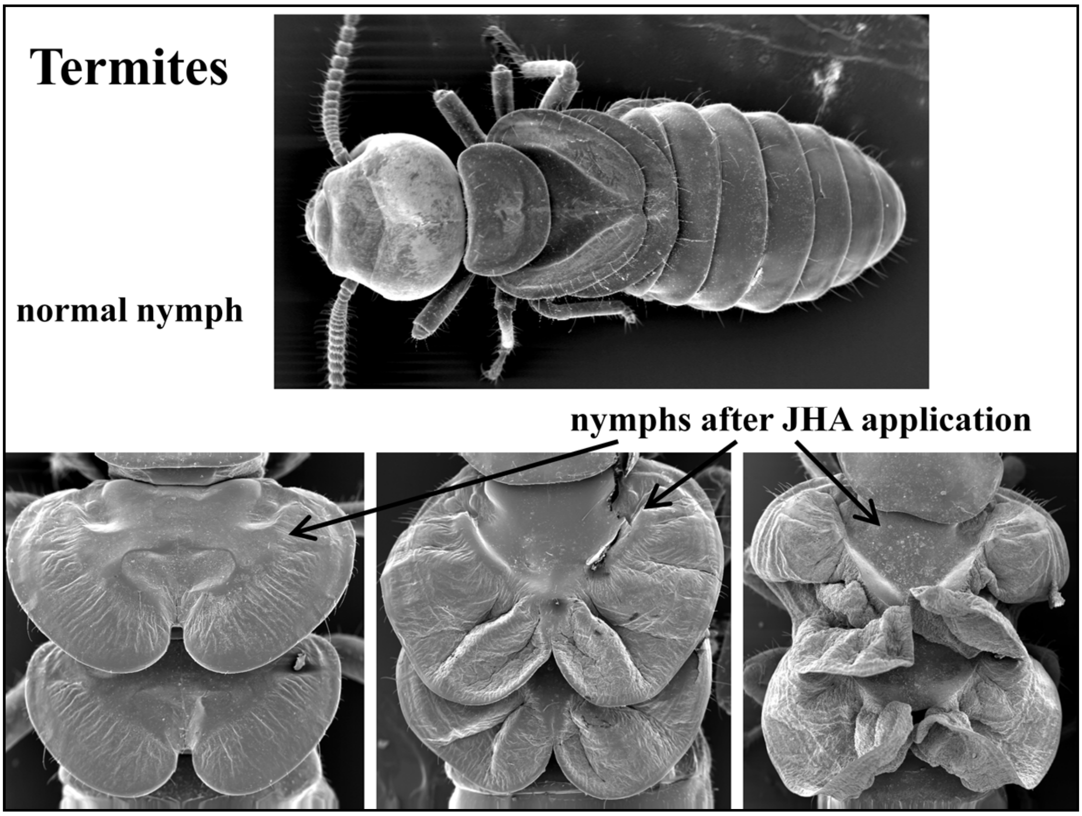
phenoxycarb (Dr. Maag)



W-328 (UOCHB)



pyriproxyfen (Sumitomo)


Termites
normal nymph
nymphs after JHA application

Juvenoids

- Influence cast determination (up to 90 % of soldiers in a colony)

worker

Prorhinotermes simplex

soldier

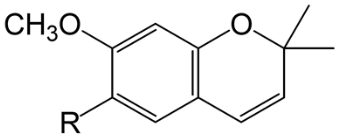
Juvenoids

- specific effect on insects
  (doses μg–ng per individual)
- non-toxic to mammals
- longer-time effect
- not very stable (suitable formulation needed)
- used as 3rd generation insecticides
- use in production of silk (extension of larval phase and thus the weight of cocoons, increase of silk production by up to 50 %)

Juvenogenes

- precursors of JH or juvenoids (prohormones)
- higher stability
- better solubility in water
- possible systemic application (watering, transport in plant, degradation to juvenoid in the target tissues)

Anti-juvenoids

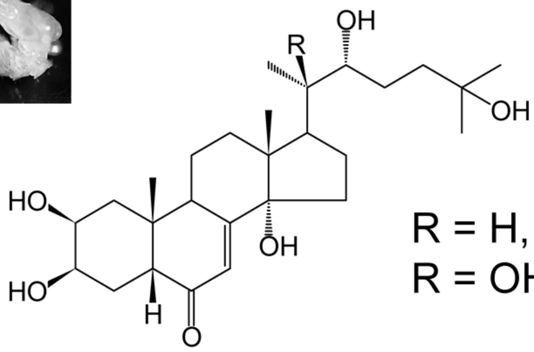


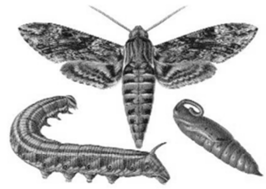
R = H nebo OCH_3
precocenes (chromene derivatives) from Compositae (*Ageratum houstoniatum*)

- inhibition of *corpus allatum* and thus the JH biosynthesis
- consequence – early metamorphosis of underdeveloped larvae to deformed or sterile adults (adultoid)
- antijuvenoids influence the insect development in all larval instars except for the last one (JH is not produced in the last instar)
- 4th generation insecticides

Ecdysteroids and phytoecdysteroids

moulting hormones, ecdysones





R = H, ecdysone
R = OH, ecdysterone

25 mg ecdysone obtained from 500 kg cocoons of silk moth (1954)

Ecdysteroids and phytoecdysteroids

- Phytoecdysteroids – structurally related compounds with the same effect – present in different plants in high content (fern *Polypodium vulgare* (osladič) or yew *Taxus baccata*); probable function – defence from herbivores.
- Ecdysteroids are used in silk production for manipulation of the moth development.

Moulting – all life stages (larva-larva, larva-pupa, pupa-adult)

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