Ecological Biochemistry

Principles of Environmental Toxicology
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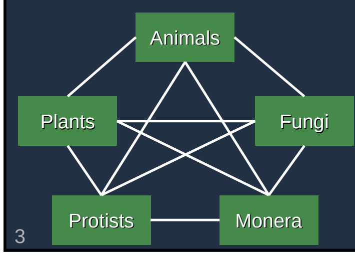
Learning Objectives

- Define ecological biochemistry.
- Explain biochemical adaptation and the roles of secondary compounds.
- Describe detoxification and the primary metabolic pathways in plants and animals.
- Explain the key processes and factors involved in biotransformation & biodegradation.
- Explain the concepts of sequestration, bioaccumulation, and biomagnification.
- Contrast different forms of ecological biochemical interaction.

Ecological Biochemistry

- Coupling of the observational science of ecology with the molecular science of biochemistry.
- Ecological interaction.



Ecological Biochemistry, 2

- Synthesis and transformation of chemicals in the environment, as the result of biochemical processes in an organism, to aid in species survival.
- Includes:
  1. Biochemical adaptation.
     - Biosynthesis.
  2. Detoxification.
     - Biodegradation, biomineralization.
  3. Bioaccumulation, biomagnification.
  4. Ecological biochemical interaction.

Biochemical Adaptation

- The metabolic flexibility of a living organism to fit into a changing environment, improving chances for survival and reproduction.
  - Evolution
    - Many generations.
  - Acclimatization.
    - Lifetime of an individual.
- Challenge: decipher the strategy of the natural world.
  - Example: THC

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Competitive, Symbiotic Interactions

- Plant ↔ Plant ↔ Animal…
  - Secondary plant compounds.
- Animal ↔ Animal…
  - Venoms, toxins.

Toxins and Survival Strategy

- Organisms often synthesize or use toxins in their survival strategy.
  - "The most conspicuous non-event in the history of the angiosperms is the failure of insects and other herbivores to attack plants on a wide-scale." (Feeny, 1975).
    - Plants dominate the landscape, hence plants must be "broadly repellent" to animals as food and "toxic" in the widest sense.
    - Overcoming the defense strategy of plants by insects and herbivores is a part of their survival strategy.

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Secondary Plant Compounds

- Hypothesis:
  developed in plants as survival mechanism.
  - Offensive and defensive biosynthesis.

Nitrogen Compounds

	Number of structures	Physiological activity
Alkaloids	6,500	Toxic, bitter
Amines	100	Repellent, hallucinogenic
Amino acids	400	Many toxic
Cyanogenic glycosides	30	Poisonous (as HCN)
Glucosinolates	75	Acrid, bitter

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Terpenoids

	Number of structures	Physiological activity
Monoterpenes	1,000	Pleasant smells
Sesquiterpene lactones	1,500	Some bitter & toxic
Diterpenoids	2,000	Some toxic
Liminoids	100	Bitter
Cucurbitacins	50	Bitter & toxic
Cardenolides	150	Toxic & bitter
Carotenoids	500	Color

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Phenolics

	Number of structures	Physiological activity
Simple phenols	200	Anti-microbial
Flavinoids	4,000	Often colored
Quinones	800	Colored, sometimes toxic

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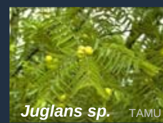
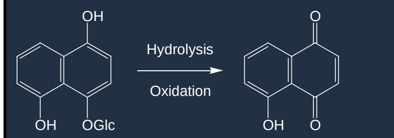
Example: The Walnut Tree

- Allelopathy: "biochemical interactions between all types of plant" (Molisch, 1937).
- Since the time of ancient Greece, the walnut tree *(Juglans nigra)* has been observed to kill nearby vegetation.
  - Moderately toxic to some insects, horses, dogs, humans.
- Produces a bound form of a toxin, which deposits in nearby soil through leaves, stems and roots.

Example: The Walnut Tree, 2

- Leaching causes hydrolysis and oxidation, releasing jugalone, a powerful herbicide.





Bound form of toxin

Jugalone (5-Hydroxynaphthoquinone)

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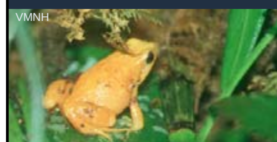
Harnessing Biosynthesis

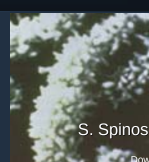
- Numerous secondary chemical compounds from nature have been used throughout the ages by humans in their own survival.
  - Medicines.
  - Hunting, warfare.
  - Pesticides.
  - Flavors.



***Phyllobates terribilis*, Poison dart frog**
Batrachotoxin – a steroidal alkaloid

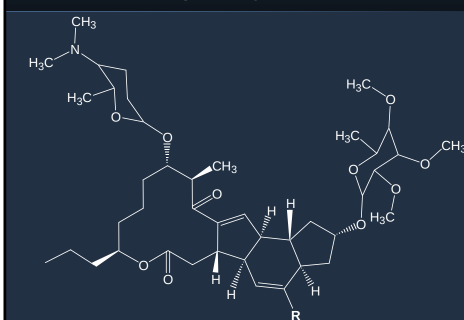
Insecticide Biosynthesis: Spinosad

- Spinosad is a mixture of spinosyn A and spinosyn D factors that are produced by the soil actinomycete, *Saccharopolyspora spinosa*.
- Causes rapid excitation of the insect nervous system.
  - Effects chewing worms.
- Off target: non-toxic to mod. toxic (e.g. fish LD_{50} 5 mg/L).
- Rapid biodegradation.



Spinosyn Macrolides

Dow



Spinosyn A: R = H
Spinosyn D: R = CH_3

Detoxification

- Adaptation of organisms to natural and “unnatural” chemicals which threaten their survival.
  - Both evolutionary and acclimatization processes.
- Includes the pathways for metabolism and biotransformation of toxicants.
  - Phase I & II; immune system.
  - Microbial biodegradation and biomineralization.
  - Phase change; sequestration.

Phase I & II

- In animals, primary PII pathways include glucuronide or sulfate formation.
- In plants, glucoside formation is the key detoxification reaction.
  - Carried out by glucosyltransferase enzyme and uridinediphosphateglucose as a co-factor.
  - More water soluble; pushed into the cell vacuole.
  - Highly phytotoxic phenols.
    - Glucosylation.
  - Systemic fungicidal agrichemicals.
    - Often “antimetabolite” glucoside forms with systemic activity.

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Detoxification of Herbicides

- Selective ability and rate of certain plants to detoxify herbicides is a primary weed management strategy.
- e.g. 2,4-dichlorophenoxyacetic acid (2,4-D).
  - Extremely active auxin (growth hormone).
  - Causes plant to grow too fast; upsets growth cycle.
  - Some crop plants degrade it and do so at a fast rate.

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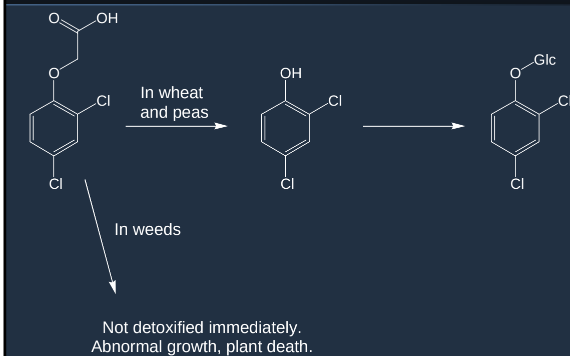
Plant Metabolism of 2,4-D



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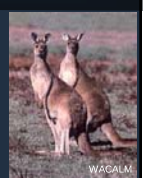
Fluorocitrate and Kangaroos



- Fluorocitrate found in legume pasture plants of Western Australia.
  - *Gastrolobium* and *Oxylobium.*
- Highly lethal (TD 1 mg/1080 kg).
  - Leaf concentrations can be 2.6 g/kg.
- The rat and gray kangaroo of Western Australia have evolved resistance.
  - *In vivo* defluorination w/ glutathione.
  - Other kangaroos from areas w/o these plants are not tolerant.

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Microbial Processes

- Microbial biotransformation/biodegradation: microbe induced change or breakdown of natural and synthetic chemical compounds.
  - In the natural world, some of these are higher trophic level xenobiotics or contaminants, some are not.
    - Scale is important (dose).
- Causes.
  - Detoxification.
  - Nutrition.
  - Respiration.

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Factors in Microbial Activity

- Evolutionary diversification.
- High specific surface area.
- High rate of population turnover.
- Survival of tolerant and acclimatized organisms.
- Heritable change in population characteristics.

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Microbial Processes

- Key processes are enzymatic or respiratory in the microbially induced changes of the chemical.
- Enzyme processes: catalytic reactions. Hurst
- Respiratory process: thermodynamic reactions.
  - Aerobic.
    - Use O_2 as terminal electron acceptor in respiration.
  - Anaerobic.
    - Use NO_3^{2-}, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2 as terminal electron acceptor.
  - Modulates biogeochemical redox rxns.
- Fungi: enzymatic.

Bacteria Processes

- Metabolism of a bacterium can detoxify a chemical using PI & PII type processes for its own survival, rendering it less- or non-toxic to itself and sometimes higher life forms.
- Biomineralization takes the process all the way to inorganic chemicals (e.g. CO_2, H_2O).
- Co-metabolism is the "gratuitous" oxidation of a chemical by an organism (no energy recovery).
  - Also: the pooled action of a community.

Bioremediation

- Intrinsic bioremediation.
  - Natural process, typically *in situ*.
- Biostimulation.
  - Addition of nutrients.
- Bioaugmentation.
  - Inoculation.
- Enzymatic processes can allow "harvesting" of the enzyme from culture for engineered treatments of chemical waste.

Phase Change

- Detoxification strategy of an organism sometimes uses a phase change to assist in reducing toxicity and increasing elimination.
- Example:
  - Volatilization of bioalkylated metals and metalloids by microbes and plants.
    - As, Se, Pb, Hg, Sb…

Sequestration, Bioaccumulation

- Often, an important process for management of some toxicants by an organism.
- Inorganic examples:
  - Mammalian incorporation of Pb into bone.
  - Plant incorporation of Se into seleno-amino acids.
  - Microbial reduction of selenate/selenite to Se^0 and incorporation of the solid into the cell.
- Can be exploited in hazardous waste clean-up.
  - Phytoremediation.

Sequestration, Bioaccumulation, 2

- Organic examples:
  - Accumulation of non-polar compounds in adipose tissue (e.g. DDT, dioxins).
- In both inorganic and organic cases, the organism can contain higher levels of a sequestered contaminant than the surrounding environment by factors as high as 10^2-10^3 (BCF).
- Food chain implications: a contaminant can "magnify" up a multi-trophic level food chain.
  - Low dose at lower levels; high dose and toxicity at upper levels.

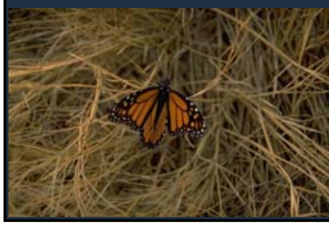
PCB Biomagnification

Lake Food Chain	Concentration (mg/kg)	Magnification
Phytoplankton	0.0025	1
Zooplankton	0.123	49
Smelt	1.04	416
Lake Trout	4.83	1930
Gull Eggs*	124	49,600

*Egg shell thinning, teratogenesis, immune dysfunction, death.

Ecological Biochemical Interaction

- Transfer of biosynthesized secondary compounds between different organisms.
  - Food web interaction.
  - Part of a survival strategy.



Milkweed-Monarch-Blue Jay

- Classic example of plant-animal co-evolution.
  - Insects using plants toxins against high predators.
  - Plants.
    - Milkweed (Aslepias currassavica); Nerium oleander.
  - Insects.
    - Monarch butterfly (Danaus plexippus); others.
  - Bird.
    - Blue Jay (Cyanocitta cristata bromia).

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Chain of Events

1. Milkweed produces bitter, toxic cardiac glycosides as a passive defense.
2. Feeding Monarch butterfly caterpillar adapts to these toxins and stores them.
3. Adult butterfly flies away with sequestered c. glycosides.
4. Blue Jay tries feeding on the butterfly, vomits.
5. Blue Jay avoids brightly colored Monarch butterflies.

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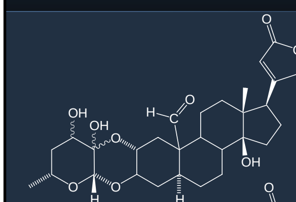
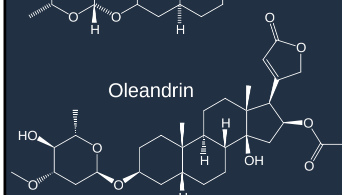
Cardiac Glycosides



Calotropin

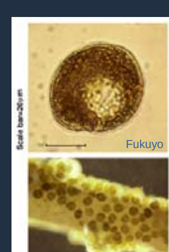


Oleandrin

Ciguatera Fish Poisoning

- The most commonly reported marine toxin disease in the world. NIEHS
  - Associated with consumption of contaminated reef fish such as barracuda, grouper, and snapper (vector fish).
- 50,000 people per year: debilitating neurologic symptoms, including profound weakness, temperature sensation changes, pain, and numbness in the extremities.

Ciguatoxin

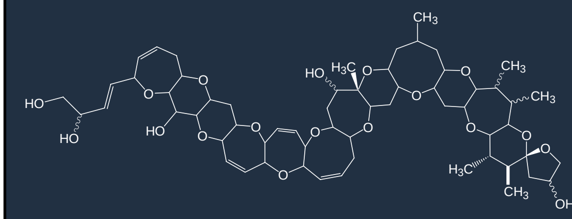


- The dinoflagellate, *Gambierdiscus toxicus* produces ciguatoxin throughout tropical regions of the world.

Ciguatoxin, 2

- The two most common toxins associated with Ciguatera are Ciguatoxin and Maitotoxin
  - Some of the most lethal natural substances known (mice 0.45 µg/kg ip).
- Ciguatoxin, a lipid soluble substance, opens voltage dependant sodium channels in cell membranes which induces membrane depolarization.
  - Lethality is usually seen with ingestion of the most toxic parts of fish.

Ciguatoxin, 3



Pyrrolizidine Alkaloids & Moths

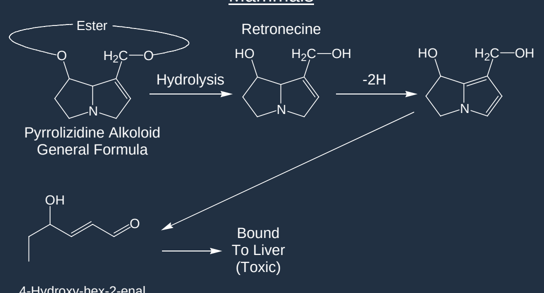
- Plants.
  - Groundsel *(Senecio vulgaris).*
  - Ragwort *(S. jacobaea).*
- Insects.
  - Tiger moth *(Arctia caja).*
  - Cinnabar moth *(Tyria jacobaeae).*

Senecio Alkaloids

- Highly toxic pyrrolizidine alkaloids are found in plant species of the genus *Senecio.*
- Poisonous to foraging wildlife and livestock.
  - Strong hepatotoxins.
- Also found in comfrey *(Symphonium officinale).*
  - Used in herbal medicines and tea (!).

Pyrrolizidine Alkaloid Metabolism

Mammals



Senecio-Moth Interaction

- Pyrrolizidine alkaloids from the plants are sequestered and stored by the moths.
- The moths can biotransform one alkaloid *in vivo*, into another.
  - Toxic alkaloids are even present in the insect eggs.
  - Bright colors and patterns also present.
- Moth will also feed on foxglove *(Digitalis purpuria),* a source of cardiac glycosides.

Pyrrolizidine Alkaloid Metabolism

Lepidoptera

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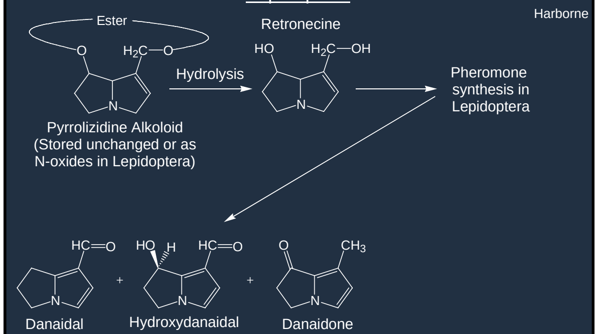

43

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Pyrrolizidine Alkaloid Metabolism

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- In mammals, PAs metabolize to a pyrrole.
  - Can bind to macromolecules (DNA).
  - Can breakdown to a more reactive metabolite.
- In lepidoptera, PAs can act as essential pheromone precursors.
  - Biotransformed into “love dust”.