Alkaloids Part 2: Chemical Ecology

Example 1. Pyrrolizidine Alkaloids (PAs) - Senecionine-N-oxides

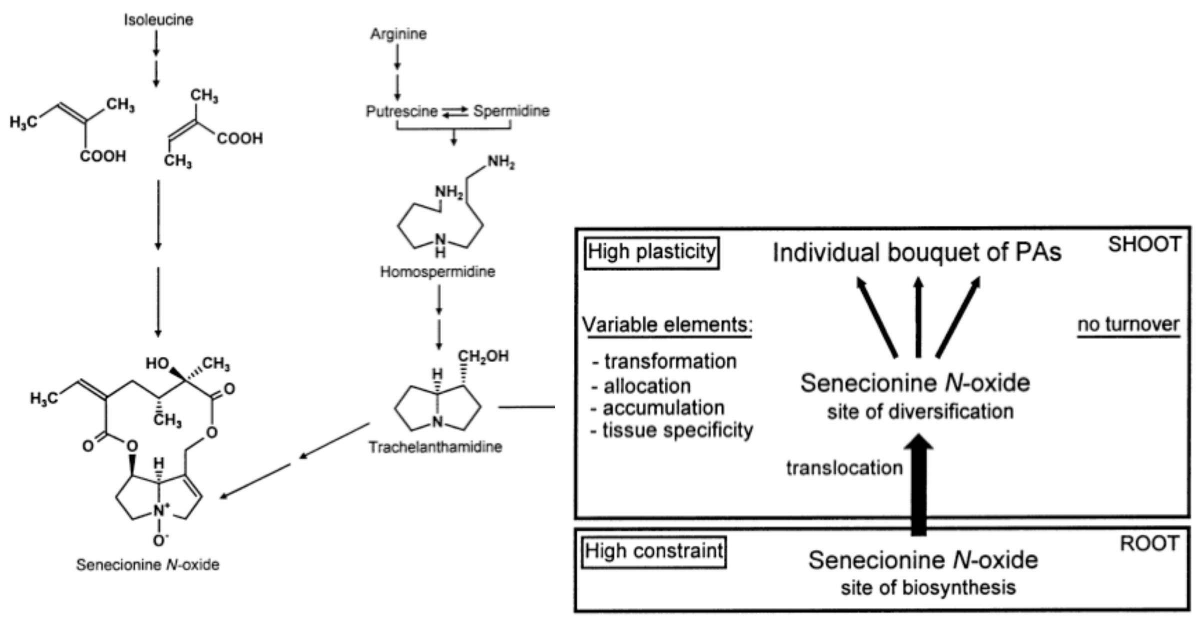
Background

- basic structural type, but many variations
- occurrence in plants in Compositae, Boragincaceae, Orchidaceae
- extremely toxic to both mammals and insects but complex interactions
- documented co-evolution with specialist insects, and sophisticated use of plant defenses by insects

Biosynthesis

Senecionine-N-oxide synthesized from two separate pathways/moieties

- **necine** moiety, from **arginine** via putrescine (*decarboxylate, condense, cyclize*)
- **dicarboxylic acid** moiety, from **isoleucine** => both form the **macrocyclic diester = senecionine**
- key enzyme: **homospermine synthase**. Evolved independently at least four time - different plant families - from deoxyhypusine synthase, and enzyme of primary metabolism (activates eukaryotic translation initiation factor eIF5A)
- five distinct structural types, that vary in macrocyclic diester bridge component



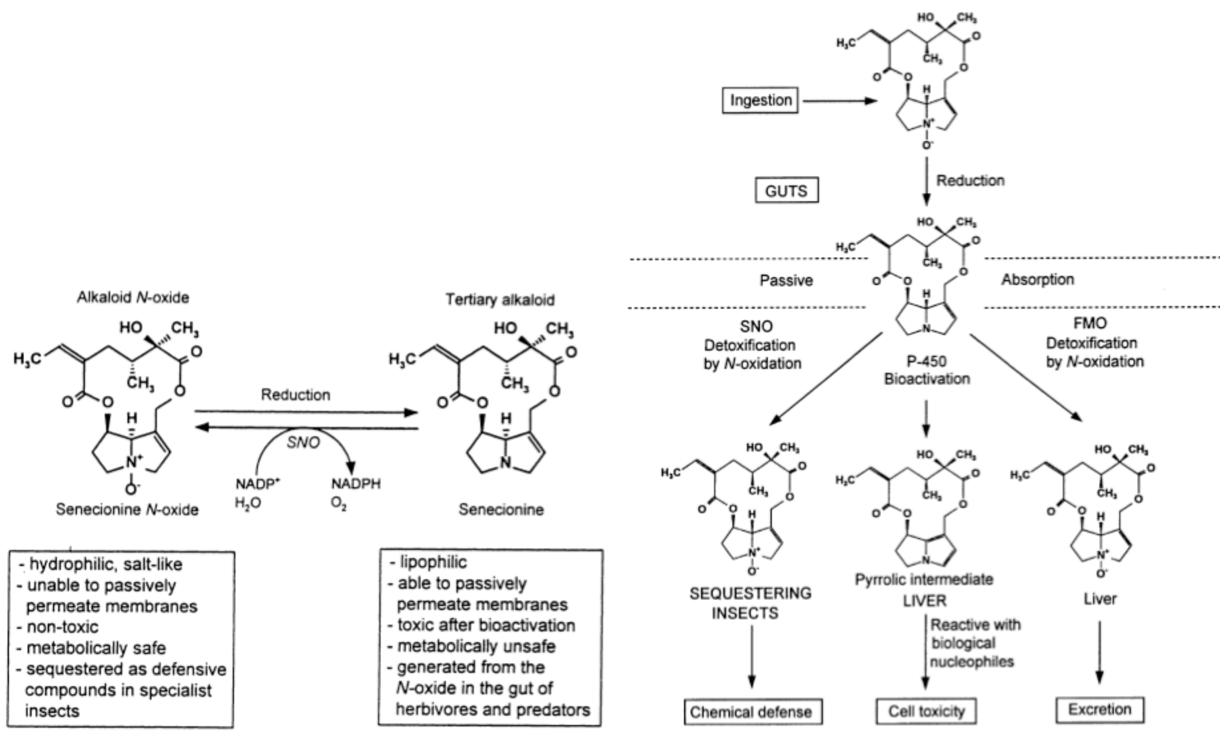
Pyrrolizidine alkaloid localization and transport

- root synthesis of senecionine, then transported to shoot
- final accumulation of alkaloids is in flowers and shoot apex
- further biochemical elaboration and structural diversification (decoration of macrocycle) occurs in shoot
- population differentiation (diverse mixtures of pyrrolizidine alkaloids in different plant populations) - > variable target for pests.

Pyrrolizidine Alkaloid Toxicity and its Avoidance

- PA-containing plants are generally avoided by herbivores (bitter taste, toxicity)
- N-**oxide** forms are stored in plant, these are the less toxic form (see figure)
- after ingestion: N-oxide are reduced to **tertiary amine** in reducing conditions in gut
- in mammals, a liver **cytochrome P450 oxidase** (general detoxification enzymes, xenobiotics) actually activates the toxin by converting to **pyrrolic** compounds (=alkylating agents that damage biomolecules, and cause liver damage)
- generalist insects are similarily susceptible to this
- however, specialist insects have a **specific** senecionine N-oxidase that keeps PA in N-oxide form and prevents the toxic effects

Interestingly, guinea pigs are immune to the pyrrolizidine alkaloids. It turns out they have non-specific oxidase which maintains the PA as the N-oxide form, and it can be excreted with out causing damage.



Additional specialist insect adaptations to the pyrrolizidine alkaloids

i) **tolerance** (see above - senecionine N-oxidase)

ii) uptake and **sequestration**:

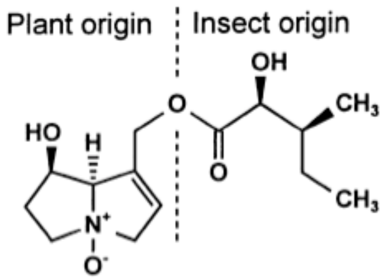
- cinnabar moths tolerate and accumulate PAs (keep in the N-oxide form)
- specialized storage and **secretion** by *Oreina* beetles (spray predators with PAs)

- in American arctiid moths, the PAs are sequestered, and *retained through metamorphosis* (larvae to adult moth), and ultimately incorporated into eggs and sperm. They protect the eggs and larvae.
- alkaloid-sequestering species are brightly colored to warn predators

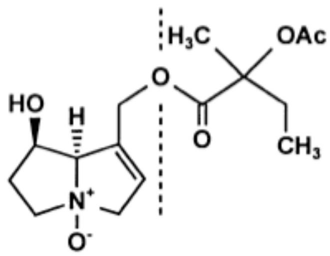
iii) derivatives used as **pheromones**

- in some sequestering moths, the PAs are converted to mating pheromones (hydroxydanaidal]
- hydroxydanaidal is placed on specialized organs (hair pencils) of male as a courtship pheromone (= PA content of male signaled via hydroxydanaidal)

iv) **pharmacophagy** (Danainae butterflies) - take up pyrrolizidine alkaloids from non-food source (stems or nectar). Adults secrete saliva and absor the chemicals via mouth -> obtain alkaloids for protection



Creatonotine *N*-oxide


Callimorphine *N*-oxide

Example 2. Nicotine alkaloids from *Nicotiana* (tobacco)

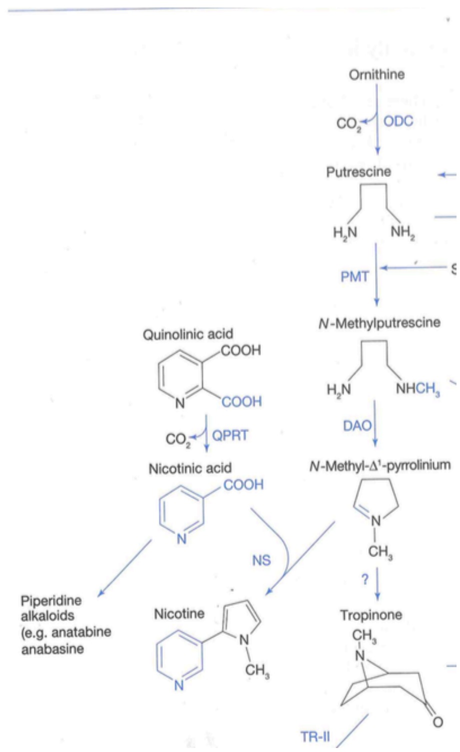
Background

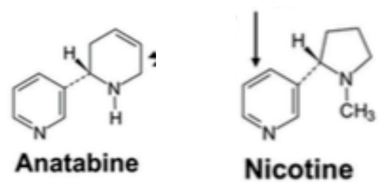
- structural family: nicotine, nornicotine, anabasine
- occurrence: Nicotiana *spp*. (flowers, seeds, young leaves)
- highly toxic to mammals and insects, binds and stimulates cholinergic synapses
- in humans at higher doses causes: "nausea, vomiting, diarrhea, mental confusion, convulsions, respiratory paralysis "
- highly addictive (interacts w. dopamine...)

Biosynthesis

Two ring structure with two components:
- nicotinic acid (as in NAD/NADH) (from aspartate)
- pyrrole (furan ring), synthesized from arginine (ornithine)

- note: decarboxylation first
- two key enzymes to remember: *putrescine methyltransferase (PMT), nicotine synthase*
  NB: low nicotine mutants of tobacco have low PMT
- synthesis occurs root and nicotine is transported to shoot in phloem





Nicotine synthesis is induced by wounding

- this is somewhat unusual for an alkaloid
- 'topping' of commercial tobacco plantations is known to increase nicotine synthesis
- putrescine methyltransferase is wound-induced in root. This supports the idea of a defensive function
- a good example of **systemic signaling**. It is now known to require methyl jasmonate)

Direct experimental evidence of nicotine for defense function against insect pests

(*Nicotiana attenuata* system, Ian Baldwin)

- RNAi-mediated suppression of *putrescine methyltransferase* to a reduction of nicotine levels for bioassays
- both constitutive and induced nicotine levels are affected.
- insects bioassay effects seen only after the
- lower nicotine leads to faster growth of insect pests (Steppuhn et al. data, PLoS 2: e217 (2004).

Interesting counteradaptation by specialist insects

i) *Manduca sexta* (tobacco hornworm) tolerates otherwise lethal doses of nicotine (metabolism / excretion)

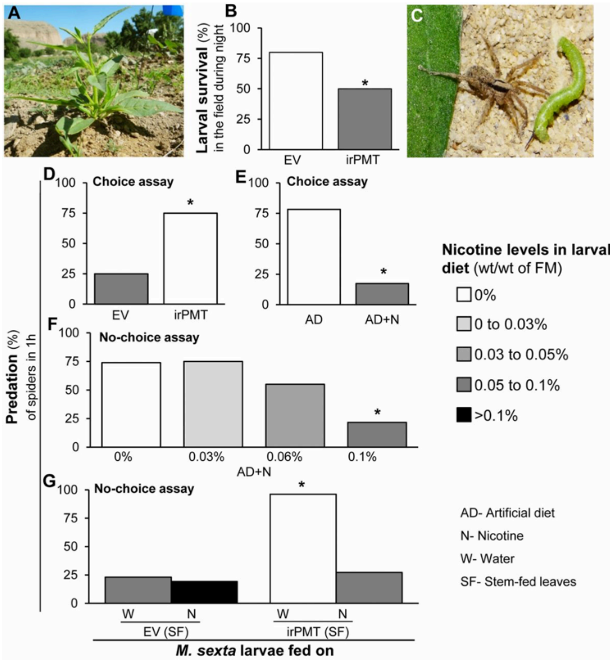
- larvae can at least partially suppresses *nicotine induction* during feeding
- on the other hand, nicotine in larvae helps defend against parasitoid wasp predators of *Manduca* (lower rates of emergence on high-nicotine *Manduca*.)

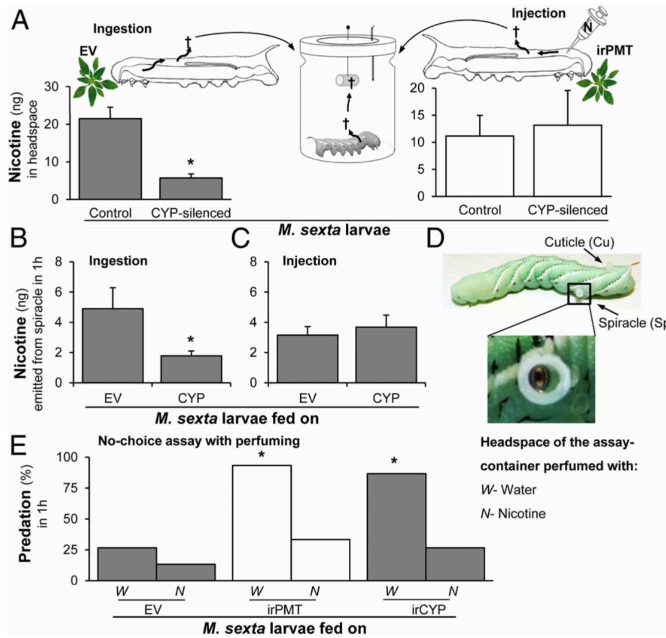
ii) Nicotine in nectar prevents overconsumption and promotes shorter feeding times

iii) Nicotine is used by tobacco hornworm to repel wolf spiders (Kumar et al., PNAS 111: 1245 (2014)

- larvae feeding on low nicotine N. attenuata (PMT-RNAi silenced) are preferred by nocturnal spiders
- observations and loss of nicotine suggest that **nicotine is emitted** from **spiracles** of larvae ("***defensive halitosis***")
- larvae feeding on plants expressing a CYPB46 RNAi gene show reduced nicotine emission (ie the larvae cannot emit nicotine). these are more vulnerable to predation.

Key control experiments - inject larvae with nicotine to show this is the key factor of manipulations)





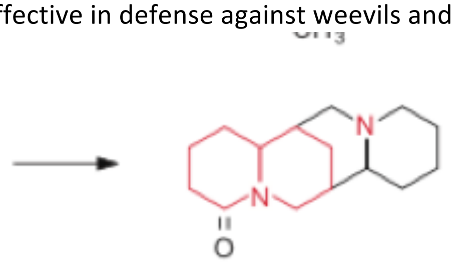
Example 3. Quinolizidine alkaloids (lupines - *Lupinus* sp.) [lupanine]

Lupines (legume family) are common native species, highly toxic due to alkaloids, only eaten by herbivores if starving.
- strong biological effects in mammals: "loss of coordination, convulsions, liver damage, .. crooked calf disease (teratogen)"
- cattle poisoning, especially in autumn, from high alkaloid seeds of lupines on range.
- wild hares learn to avoid high-alkaloid lupine (feeding deterrent)

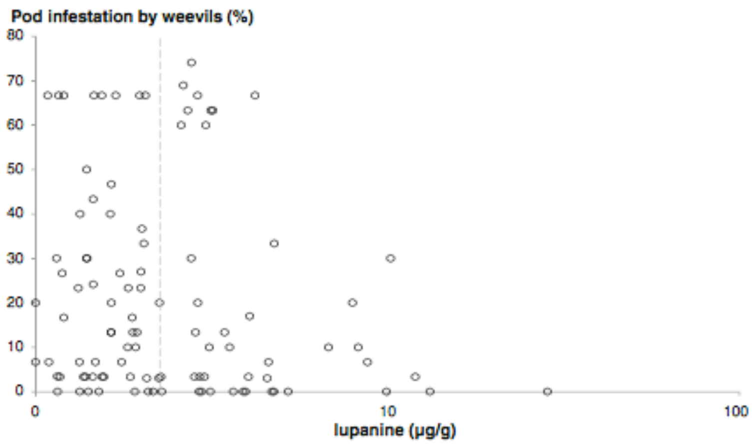
Other plant species containing quinolizidine alkaloid, but may not synthesize them:

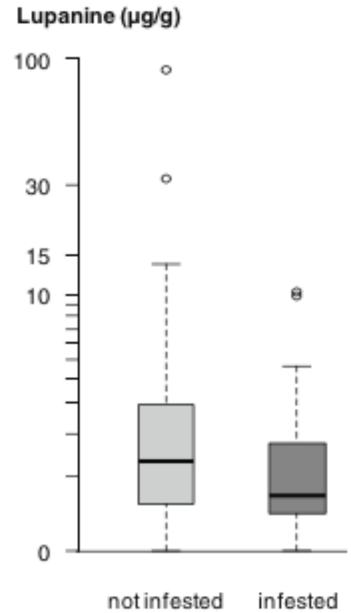
- Indian Paint Brush (*Castilleja* spp): does not synthesize, but is a root parasite of lupine roots - steals alkaloids!
- other common plants (*Laburnum anagyroides:* **cytisin***; Cytisus scoparius:* **spartein**)
- Gorse (like Scotch broom)
  Why are so many invasive species highly toxic?? Is it an invasive species advantage? [see *Naturwissenschaften (2012) 99:883*].
- lupanine appear to be effective in defense against weevils and rust (though data looks preliminary)

3 Lysine



Lupanine
(Chinolizidine)





4. Solanine and Potato Steroid Alkaloids (Human chemical ecology)

Biological effects:

- can lead to respiratory failure in humans (mammalian defense?)
- antifungal agent in potato and tomato, also toxic to Colorado potato beetle (anti-herbivory)

Implications for human diet:

- solanidin is found in edible potatoes at very levels, so it doesn't have discernable effects. However, higher levels can be found in other organs (leaves, flowers, fruit)
- synthesis in tubers is induced by greening (triggered by light) - store potatoes in the dark!
- wild potato species (Peru, Bolivia) contain high levels of these compounds. Presumably commercial varieties were selected for lower levels (at the risk of greater disease incidence or herbivory)

Geophagy (eating of soil or clay) = binding of alkaloids by special clays

- detoxification and removal of alkaloids.
- effectiveness can be shown in lab experiments (binding to clay)

Concluding thoughts on alkaloids

- there are many very toxic plant chemicals (ie alkaloids) in common plant species
- toxic chemicals are present in many food plants, and in nature
- plant toxins are effective against generalist insects or herbivores, but specialists have evolved counter-adaptations
- alkaloids are part of evolved chemical warfare systems between plants and insects (of different trophic levels)

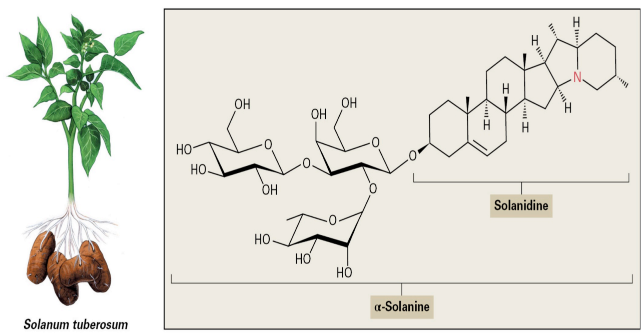


FIGURE 24.34 *Structure of the steroid alkaloid glycoside α-solanine from* Solanum tuberosum *(potato). The aglycone solanidine is derived from cholesterol.*

Biochemistry & Molecular Biology of Plants, Second Edition. Edited by Bob B. Buchanan, Wilhelm Gruissem, and Russell L. Jones.

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DETOXIFICATION FUNCTION OF GEOPHAGY AND DOMESTICATION OF THE POTATO

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Abstract—Detoxification as the adaptive function of geophagy is demonstrated from field and historical data associating clay consumption with the domestication of potentially toxic potatoes. In vitro analyses showed that the glycoalkaloid, tomatine, was effectively adsorbed by four classes of edible clays over a range of simulated gastrointestinal conditions. These results, in conjunction with reports of geophagy by nonhuman primates, suggest geophagy as a solution to the impasse chemical deterrents pose to the process of domestication and to chemical constraints on plant exploitation by non-fire-using hominids. The inorganic component of the chemical environment deserves increased attention from chemical ecologists.

Key Words—Geophagy, hominid, primate, detoxification, glycoalkaloids, tomatine, domestication, potatoes, clay–organic interactions.

Study Question

Describe the evidence (using specific examples and experiments) that demonstrates the importance of alkaloids in plant-insect interactions or their effectiveness in defense

[Think of different types of arguments you could use, in terms of different adaptations, that demonstrate the efffectiveness of alkaloids as protective agents]