The Alkaloids

Alkaloids in the skin glands of poison frogs:

A chemical defense against predation

What are Alkaloids?

- Low molecular weight Nitrogenous compounds
- 20% of plant species have been found to contain them
- Mainly involved in plant defense against herbivores and pathogens
- Utilization by human

3000 Year History of Alkaloids use by Humans

- In most human history, alkaloids from plant extracts have been used as ingredients in potions (liquid medicine) and poisons

- Ancient people used plant extracts containing alkaloids for treating a large number of ailments including: snakebite, fever and insanity

3000 Year History of Alkaloids use by Humans

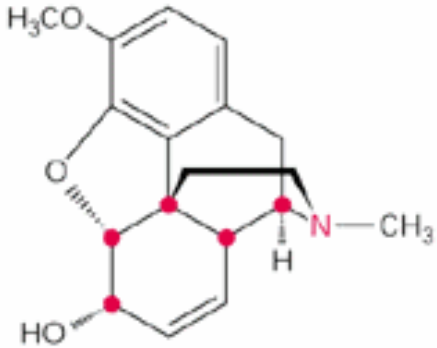
In the middle east- the latex of Opium Poppy (Papaver) was already used at 1200 B.C.

Alkaloids of Opium Poppy (Papaver)

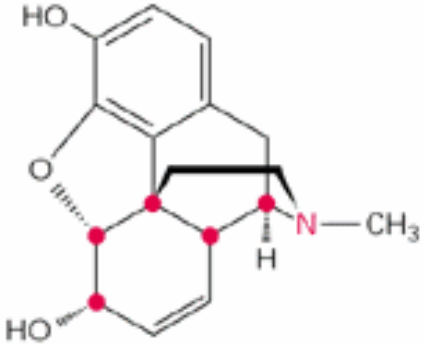
- Capsule releasing latex when wounded
- Latex contains the alkaloids morphine and related alkaloids such as codeine

Alkaloids of Opium Poppy (Papaver)

Papaver somniferum



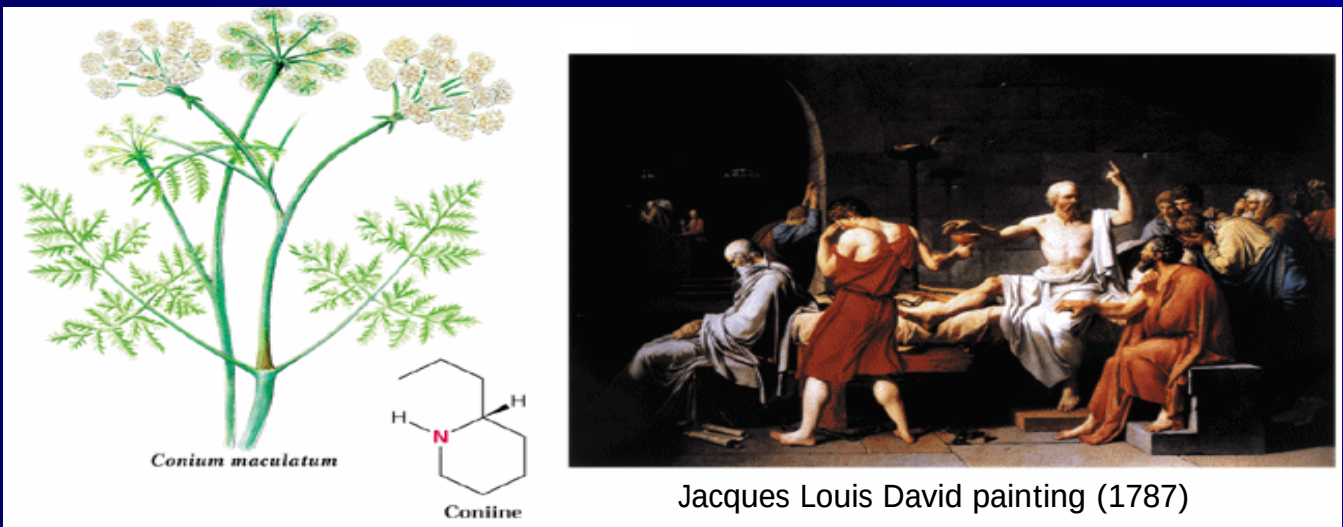
Codeine



Morphine

Bufo marinus frog accumulates high amount of morphine in its skin

Later the use of Alkaloids Spread to the West

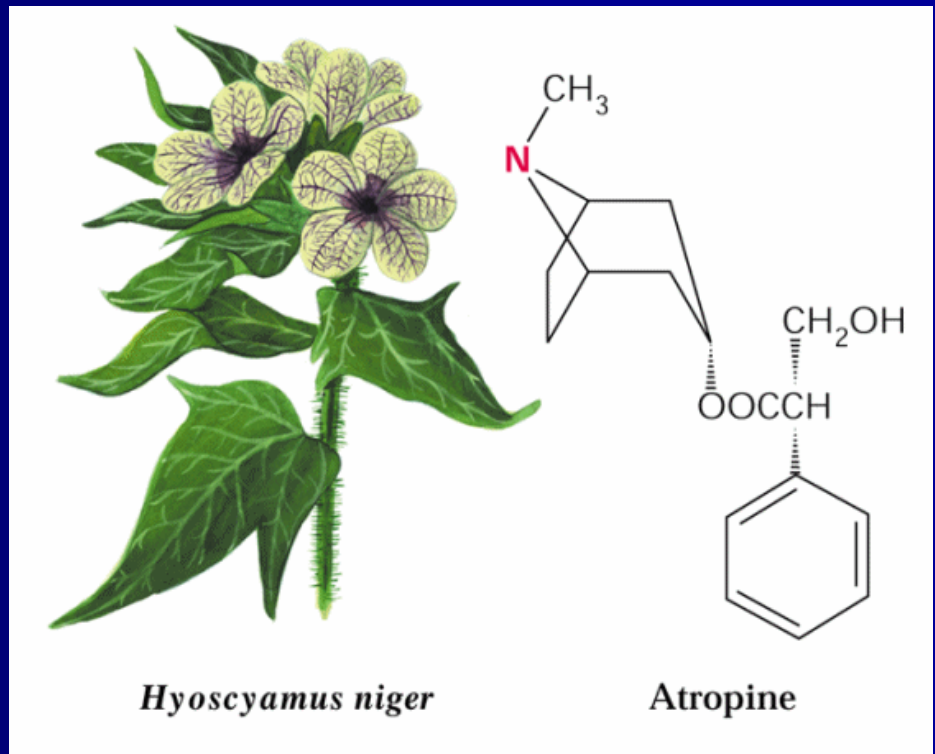


Jacques Louis David painting (1787)

- The piperidine alkaloid coniine (the first alkaloid to be synthesized)
- Coniine is extremely toxic, causing paralysis of motor nerve endings
- "The death of Socrates"- the philosopher Socrates drank and extract of coniine-containing hemlock (339 B.C.)

In Egypt-

Queen Cleopatra used extracts of henbane (Hyoscymus) to expand her pupils and appear more attractive to her male political competitors

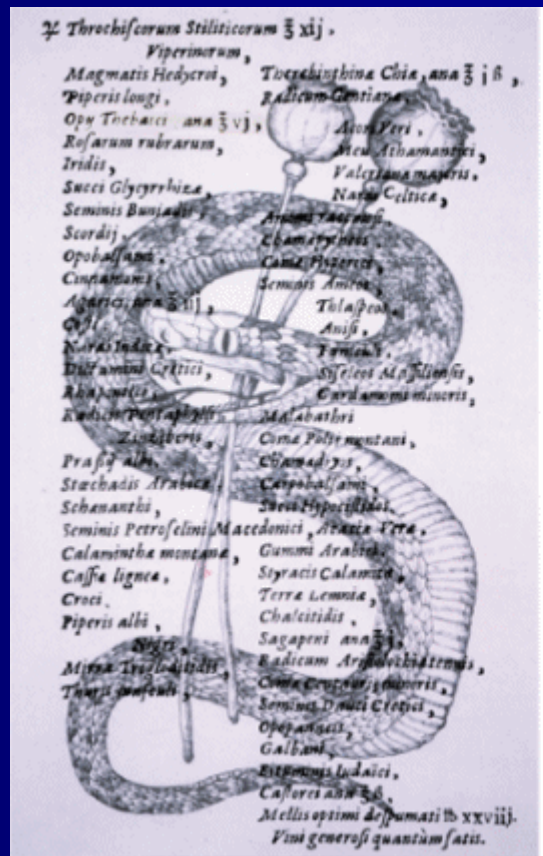


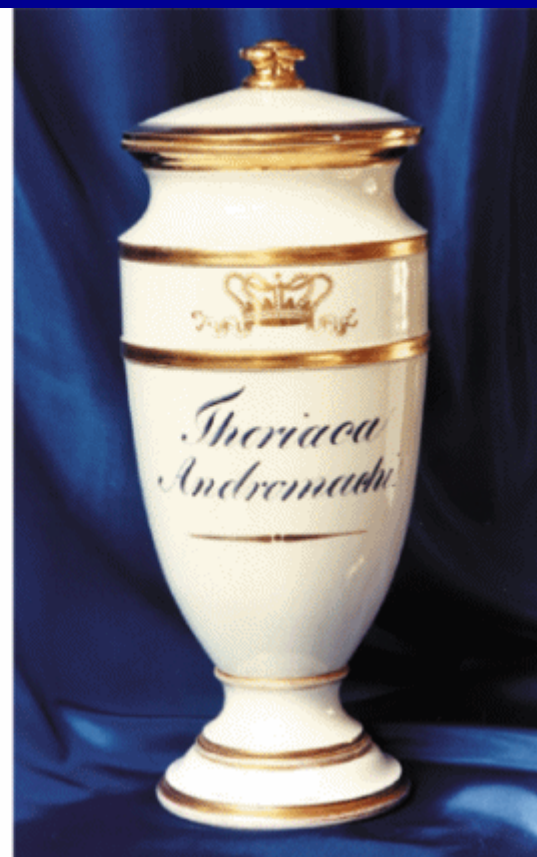
Hyoscyamus niger Atropine

Alkaloids of Opium Poppy (Papaver)

- Theriak, a mixture of opium, dried snake meat and wine
-
- One of the oldest and long lived medications in the history of mankind

- Against spiders, scorpions and snakes





Alkaloids of Opium Poppy (Papaver)

• Morphine named for Morpheus, the god of dreams in the Greek mythology

• Friedrich Serturner isolated Morphine at 1806 and this gave rise to the study of alkaloids

• In 1819, Carl Meissner (Halle) gave the name alkaloids after the plant *al-qali* from which soda was isolated (sodium carbonate called alkali in arabic)

• Alkaloids first defined as pharmacologically active nitrogen containing basic compounds of plant origin (nowadays expanded)

Alkaloids are not Unique to Plants

- Alkaloid bearing species have been found in nearly all classes of organisms: frogs, ants, butterflies, bacteria, sponges, fungi, spiders, beetles and mammals

- Not always synthesized de novo in the organisms but rather taken up

- Some animals, such as frogs produce toxic alkaloids in the skin or secretory glands

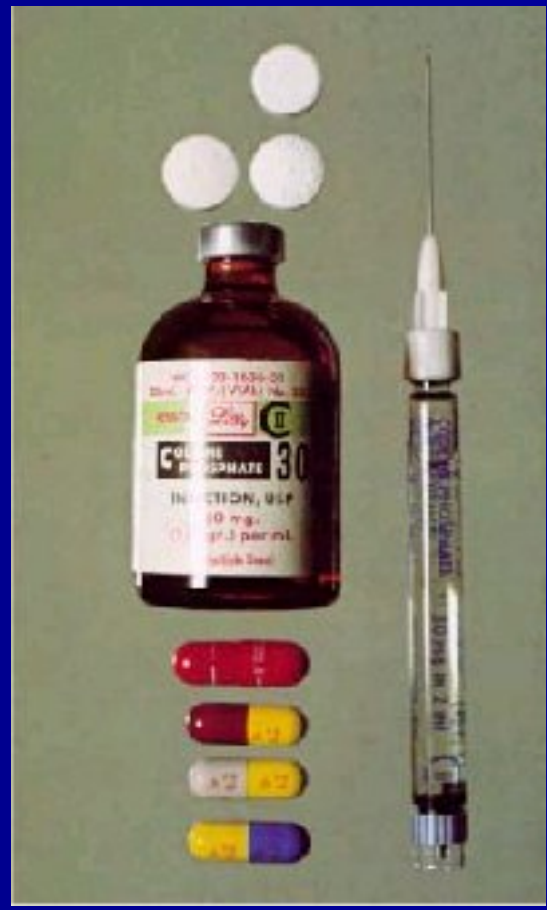
- Insects, use plant alkaloids as a source of attractants, pheromones and defense substances

Alkaloids in Modern Medicine

Atropine-
antidote to nerve gas poisoning

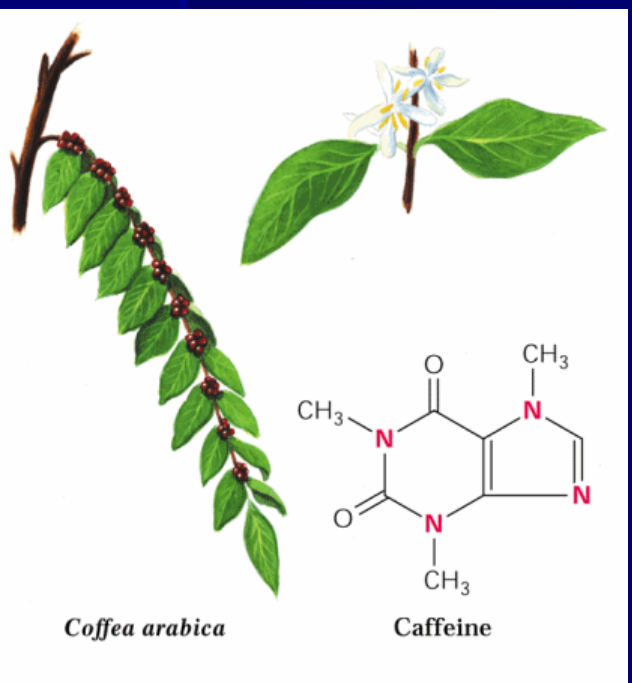
Alkaloids in Modern Medicine

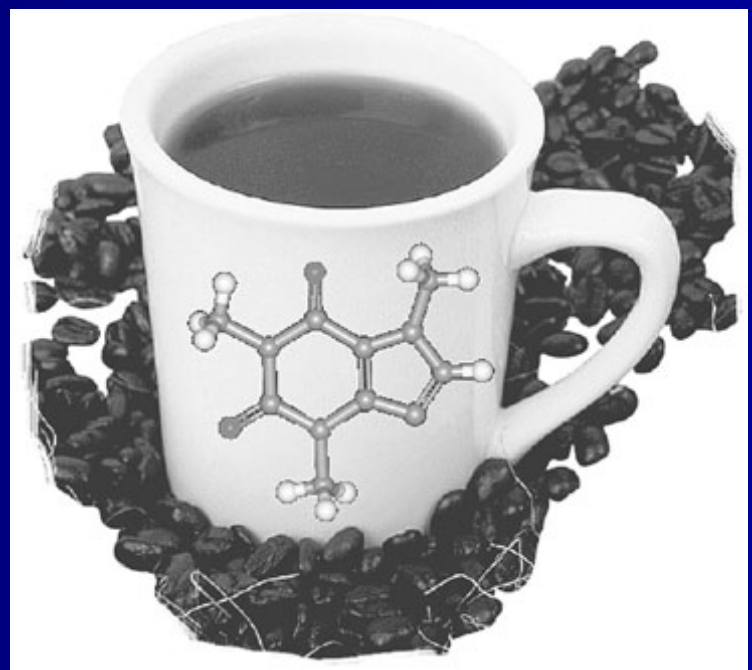
Codeine, Morphine, - Analgesic (painkiller)

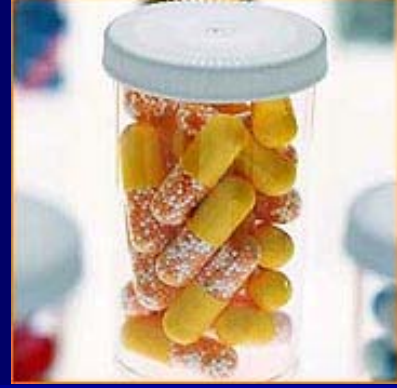


Alkaloids in Modern Medicine

Caffeine- central nerve system stimulant





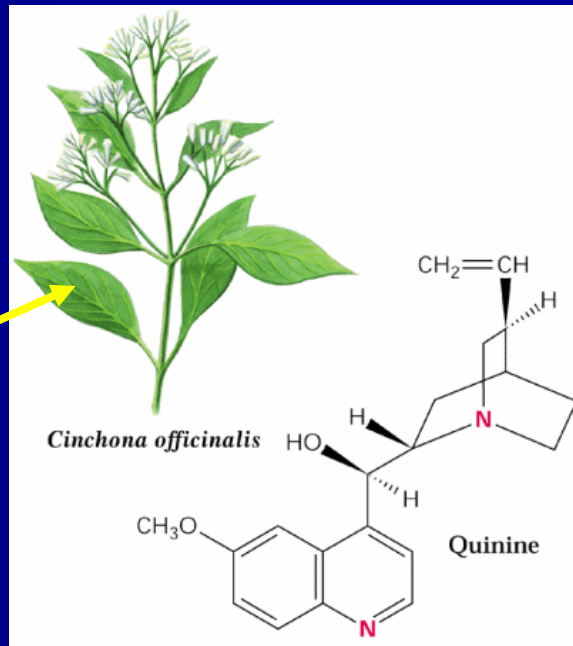


Alkaloids in Modern Medicine

Quinine- antimalarial, facilitated exploration of the tropics

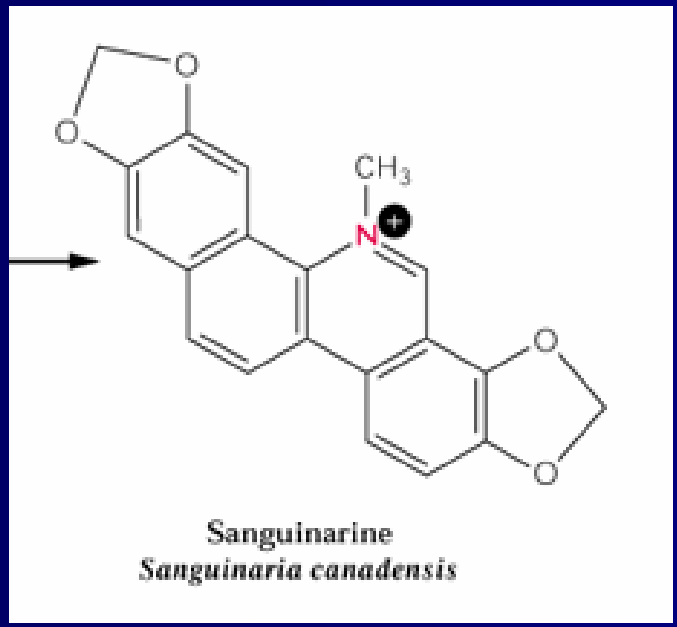
- A monoterpenoid indole alkaloid

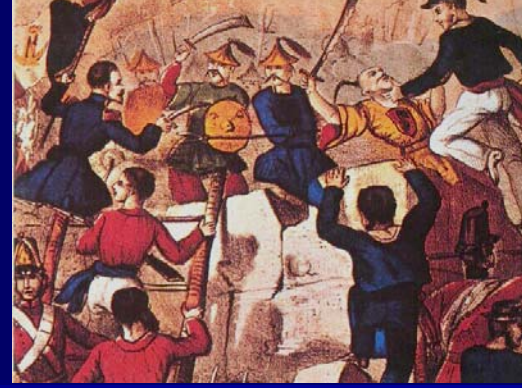
- Prepared from the bark of



Alkaloids in Modern Medicine

Sanguinarine- Antibacterial showing antiplaque activity, used in toothpastes and oral rinses





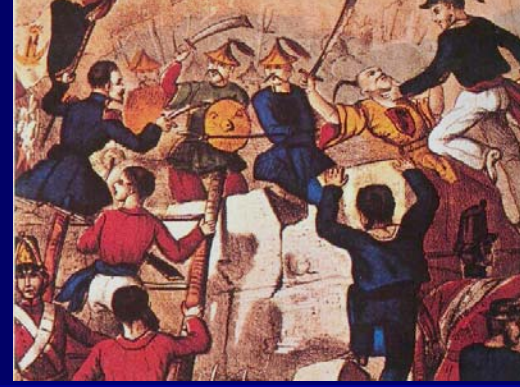
Alkaloids and Geopolitics

- The Opium Wars (Anglo-Chinese wars) between China and Britain (1839-1859)
- Due to a trade deficit Britain had to start using silver for the trade with India (in exchange for silk, porcelain and tea)

- The Brits started smuggling Opium from British India into China to reduce the amount of silver they exchange

- China lost in both wars

Alkaloids and Geopolitics



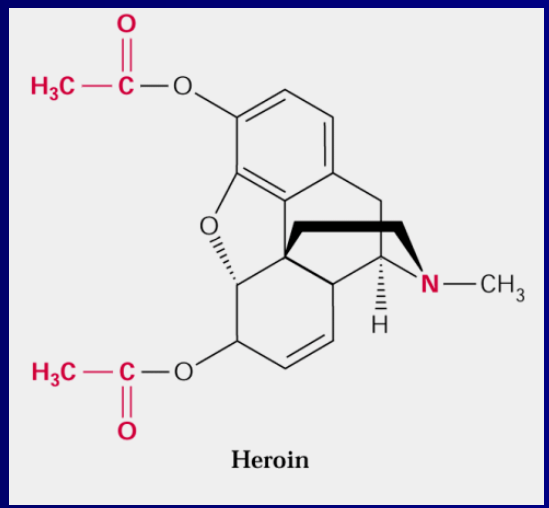
- China forced to tolerate the opium trade and sign unequal treaties opening several ports to foreign trade and giving Hong Kong to Britain

- Several countries followed Britain and forced unequal terms of trade onto China. This foreign influence led to the downfall of the Qing dynesty (1911)

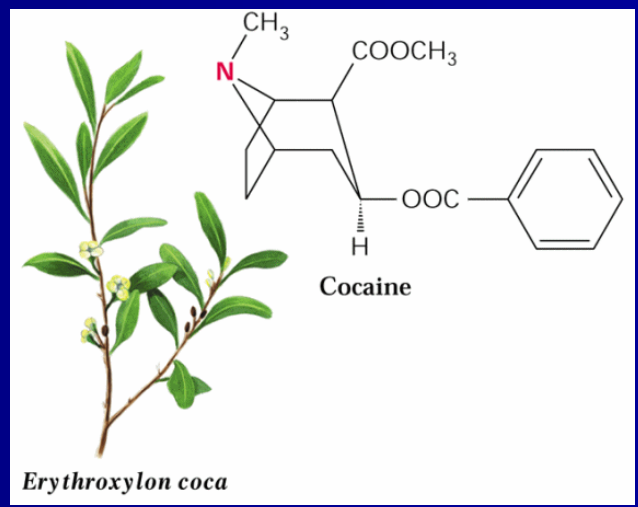
Alkaloids and Geopolitics

- Efforts underway to eradicate production of the semisynthetic compound HEROIN (derived by acetylation of Morphine)

- Also eradication of Cocaine, a natural alkaloid from the coca plant



Heroin



Cocaine

Erythroxylon coca

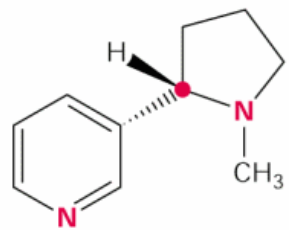
The Role of Alkaloids in Plants?

- Since the discovery of Morphine 12,000 alkaloids isolated
- Alkaloids as other secondary metabolites are produced in a unique pattern
- Large investment in nitrogen- it is clear that they have an eco-chemical role

Alkaloids and Plants Chemical Defense

- Wide range of physiological effects on animals
- Antibiotic activity
- Toxic to insects
- Feeding deterrents
- Example- NICOTINE from Tobacco, one of the first insecticides and is most effective
- Herbivory induces Nicotine formation in Tobacco

Nicotiana tabacum



Nicotine

Alkaloids and Plants Chemical Defense

- Caffeine also an effective insect toxin

- Found in leaves and beans of Cocoa, Coffee, cola, mate', and tea

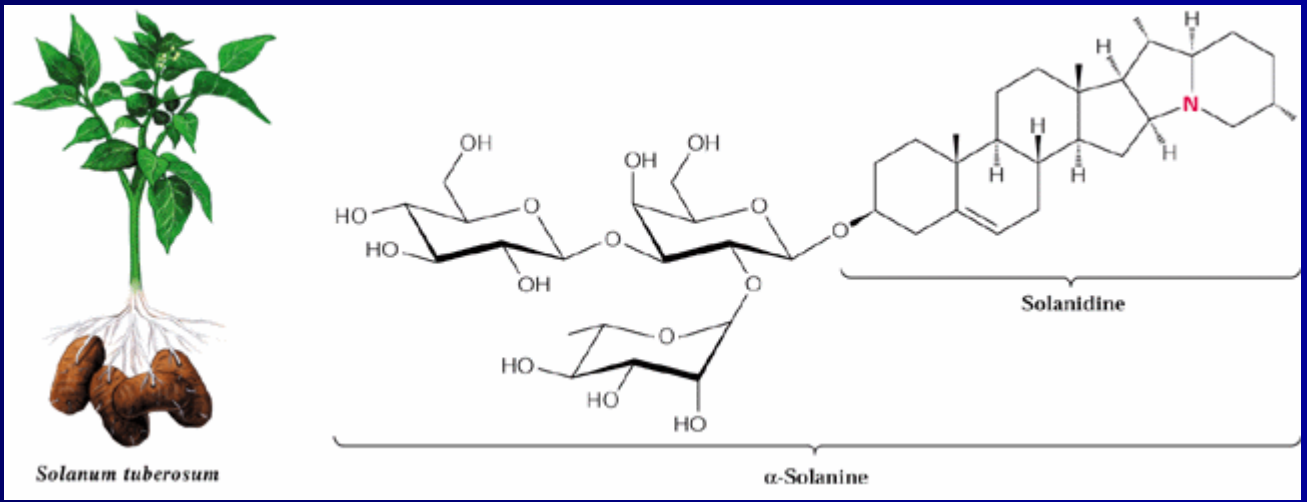
- Caffeine will kill larvae of the Tobacco horn-worm (Manduca sexta) within 24 hours in dietary concentration present, below those found in fresh coffee beans or tea leaves

Inhibits the phosphodiesterase thathydrolyzes c- AMP

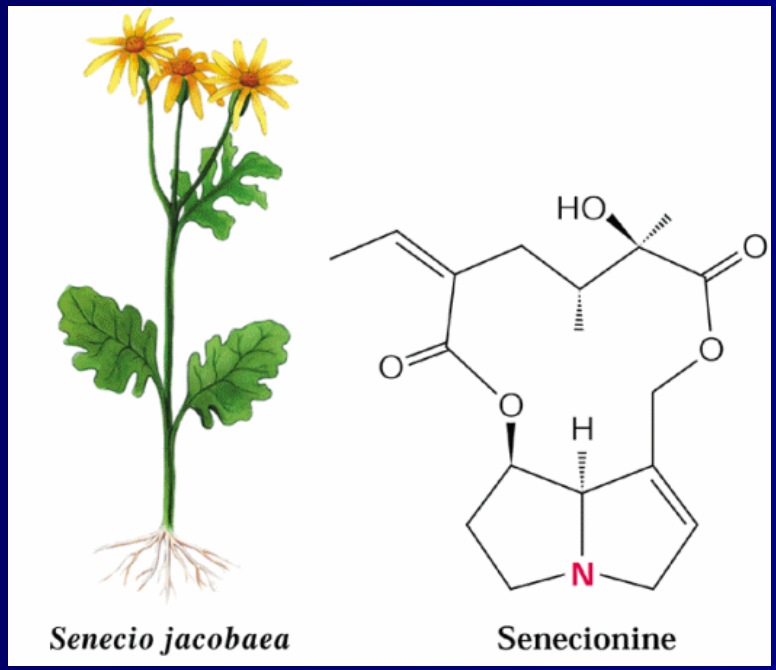
Alkaloids and Plants Chemical Defense

- Alpha-solanine, a steroid alklaoid is a cholinesterase inhibitor found in potato tuber

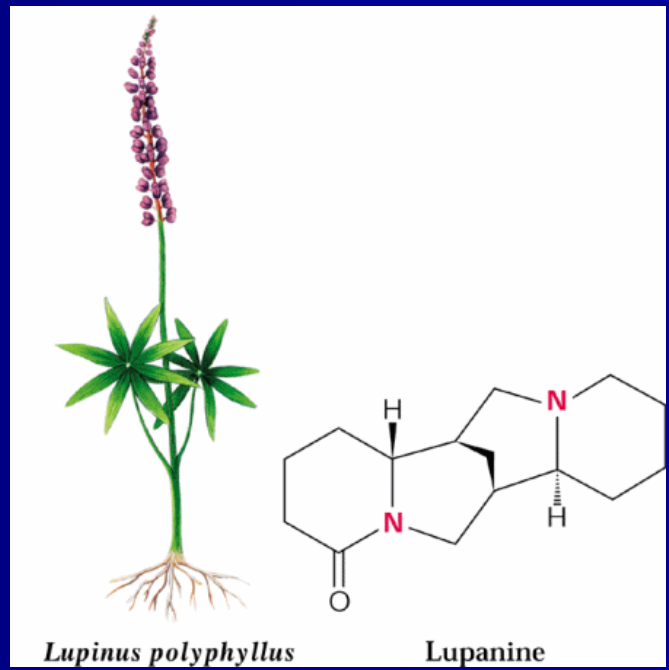
- Teratogenicity/embryotoxicity of sprouting potatoes



Alkaloids and Plants Chemical Defense

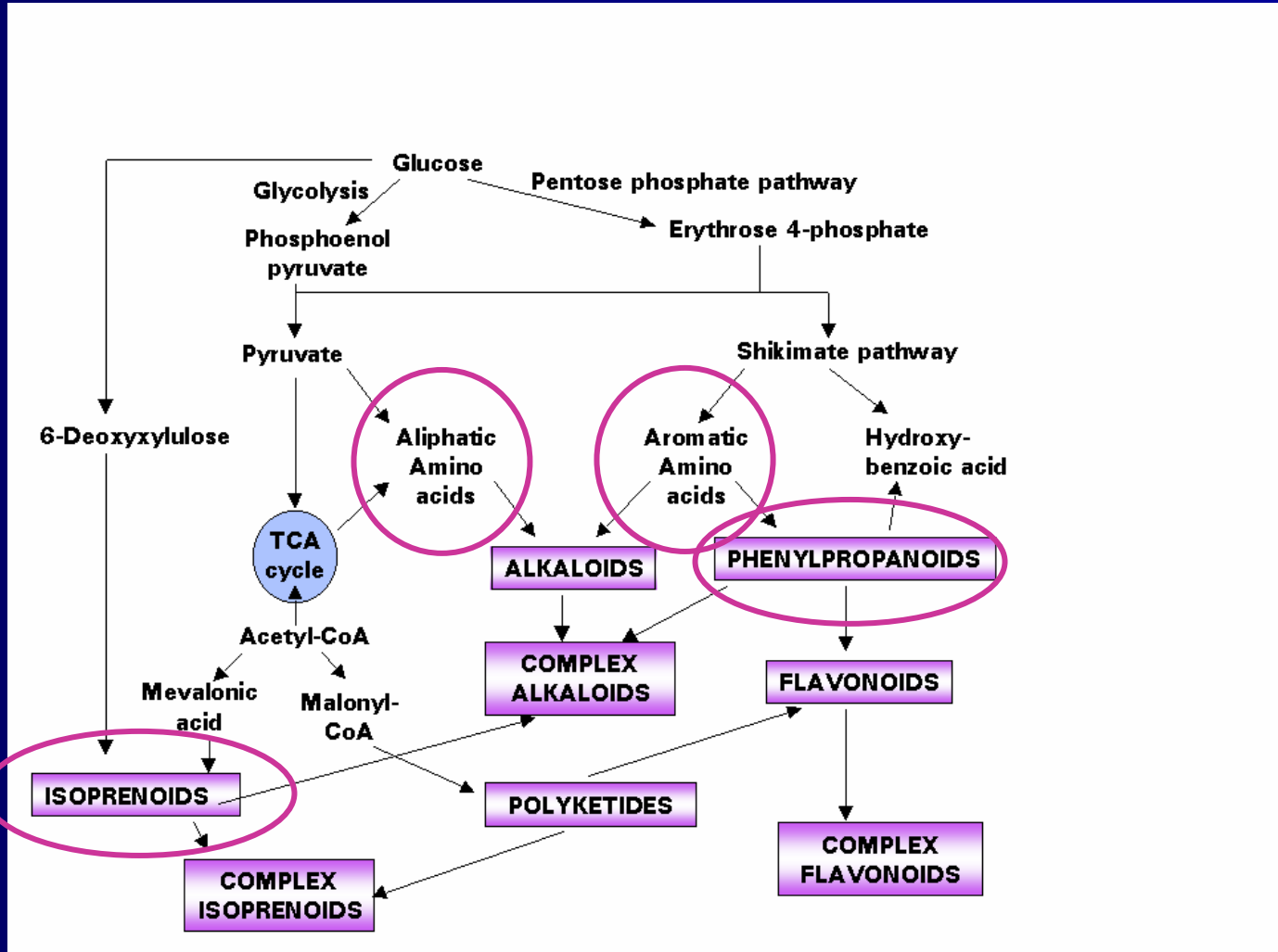


Pyrrolizidine alkaloid- toxic to mammals (family asteraceae)



Quinolizidine alkaloid (occur in the Lupinus genus)- Lupanine is a bitter compound, feeding deterrent

Alkaloid Biosynthesis



Alkaloid Biosynthesis

- Alkaloids in most cases are formed from L- amino acids
- Tryptophan, tyrosine, phenylalanine, lysine and arginine as precursors
- Produced alone from the above precursors or in combination with other chemicals such as terpenoid moieties
- One or two transformations can covert the above amino acid precursors to very specific secondary metabolites

Alkaloid Classes

- Terpenoid Indole Alkaloids
- Benzylisoquinoline Alkaloids
- Tropane Alkaloids
- Purine Alkaloids
- Pyrrolizidine alkaloids
- Other alkaloids: Quinolizine, Steroidal glycoalkaloids,

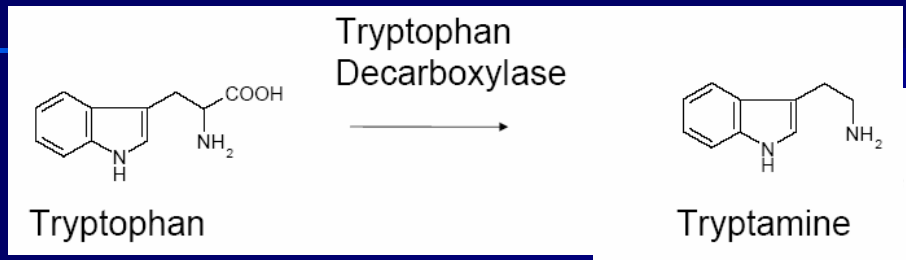
Terpenoid Indole Alkaloids

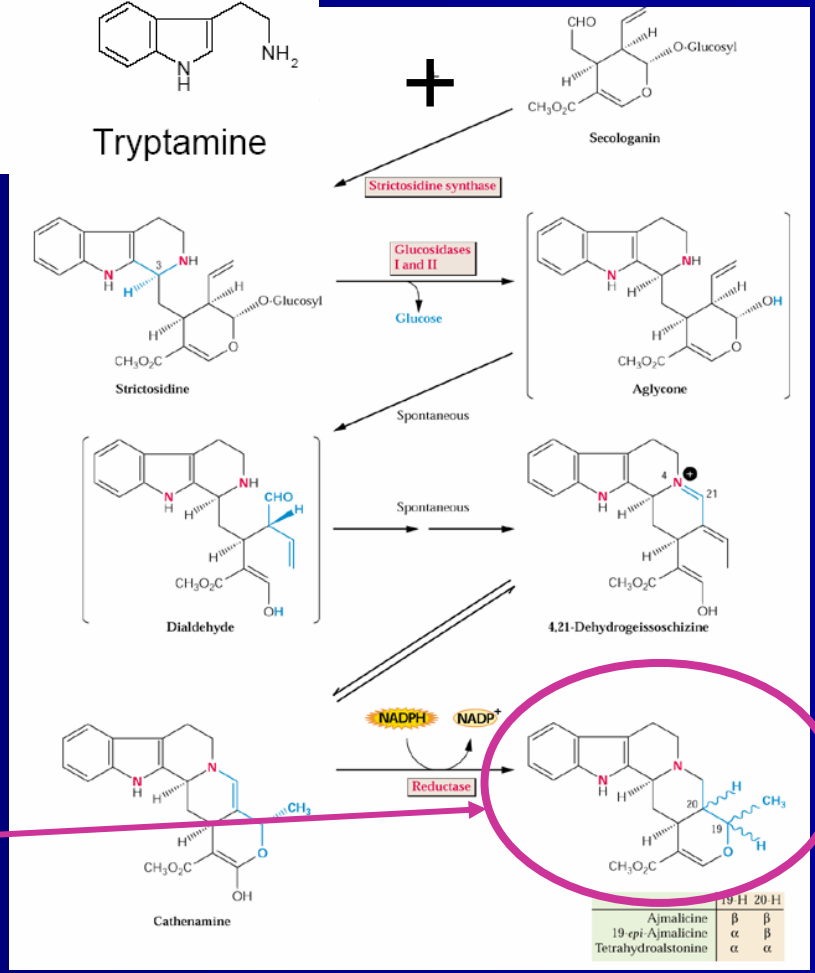
- Large group of about 3,000 compounds

- Indole moiety provided by Tryptamine (derived from Tryptophan) and a Terpenoid component

- Moneterpenenoid indole alkaloid:
the iridoid glycoside secologanin (derived from the monoterpene Geraniol) and Tryptamine

Monoterpenoid Indole Alkaloids (1800 known)

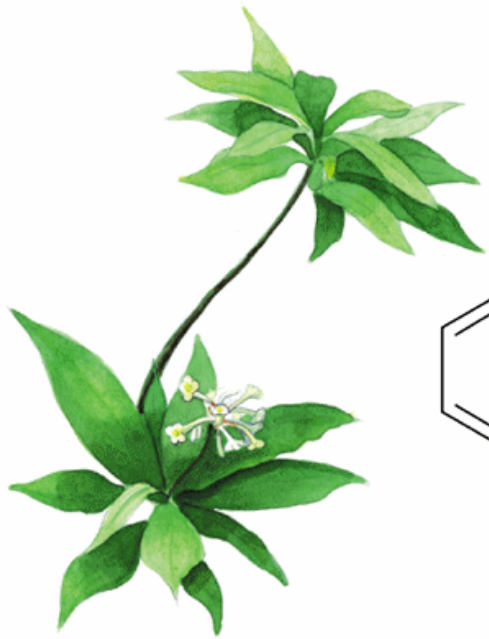




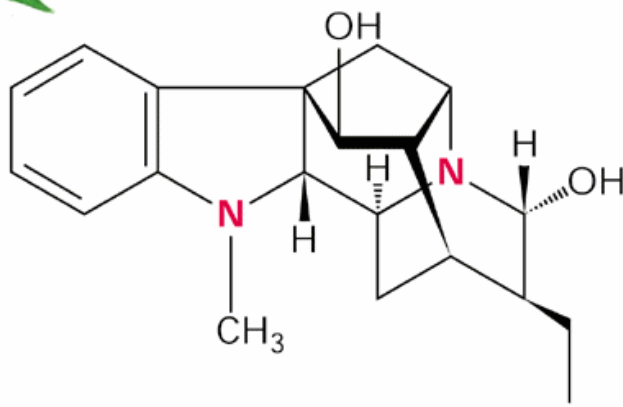
- Ajmaline: the first alkaloid for which biosynthesis was clarified at the enzyme levels

Monoterpenoid Indole Alkaloids

- Antiarrythmic (suppresses heart rythnus) that functions by inhibiting glucose uptake by heart tissue mitochondria

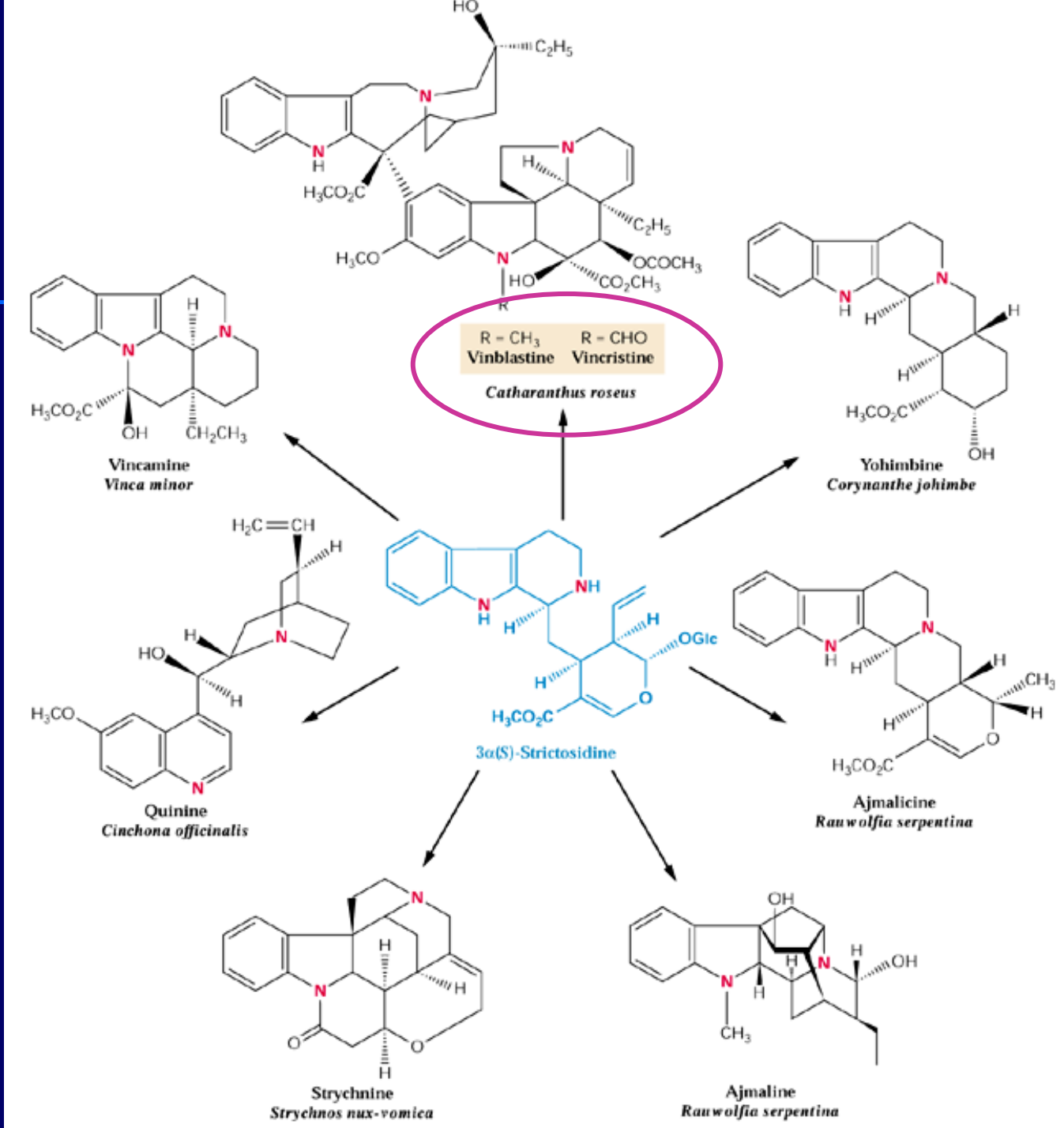


Rauwolfia serpentina



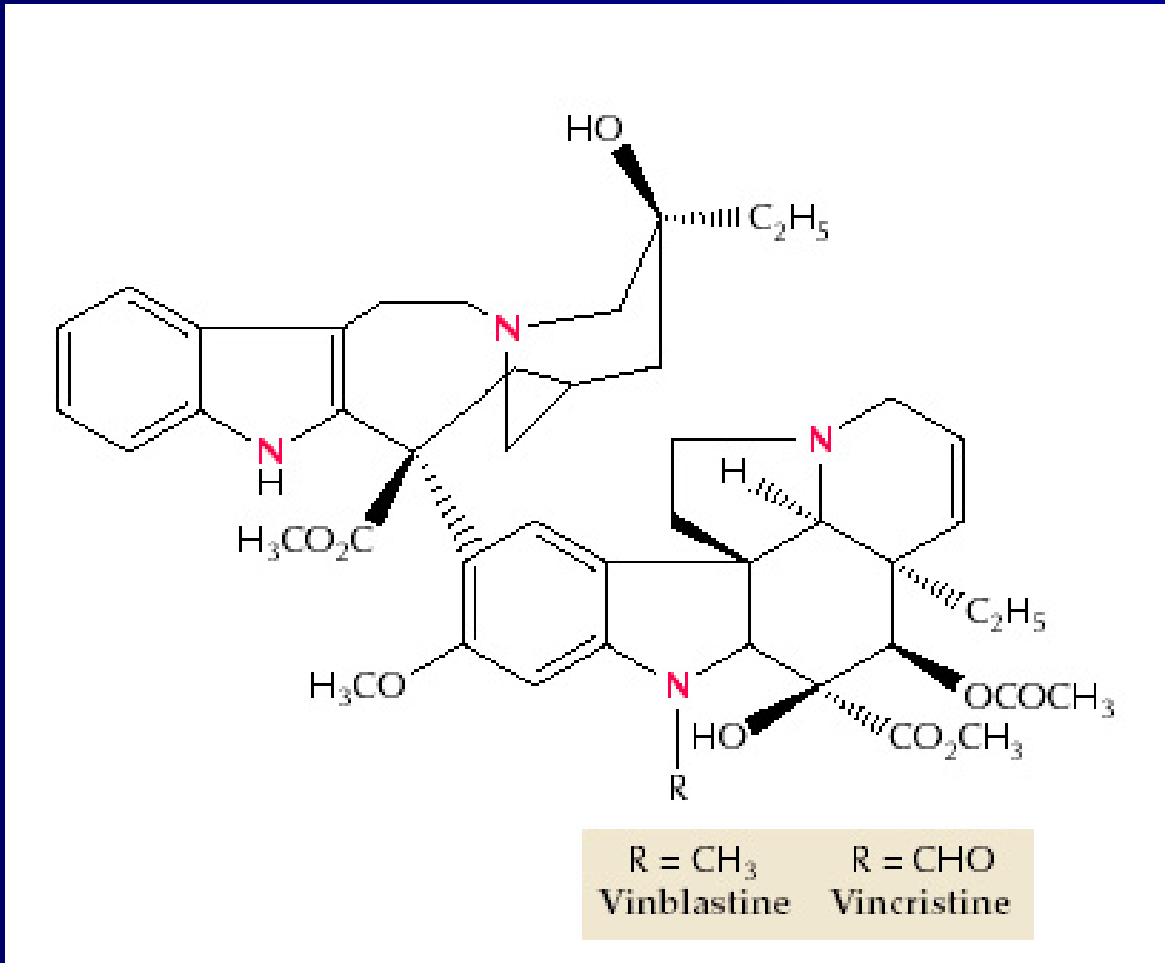
Ajmaline

Monoterpenoid Indole Alkaloids



Madagascar periwinkle (Catharanthus roseus/ vinka) Terpenoid Indole Alkaloids

Catharanthus Vinblastine and Vincristine



Catharanthus Vinblastine and Vincristine

- Both compounds commonly used for cancer therapy
- Bind microtubules and inhibit hydrolysis of GTP and thus arresting cell division at metaphase
- Bind Tubulin at different domains compared to Colchicines
- Also inhibit protein, nucleic acids and lipid biosynthesis

Catharanthus Vinblastine and Vincristine

- Reduce protein kinase C that modulates cell growth and differentiation
- Vinblastine is a component of chemotherapy for metastatic testicular cancer, Hodgkins desease and other lymphomas
- Vincristine is the prefferd treatment for acute leukemia in children
- Both drugs expensive, catharanthus the only source (low levels)

Catharanthus Terpenoid Indole Alkaloids

Example of metabolism in multiple type of tissues coupled to metabolism in different subcellular compartments

Subcelluar location of metabolism

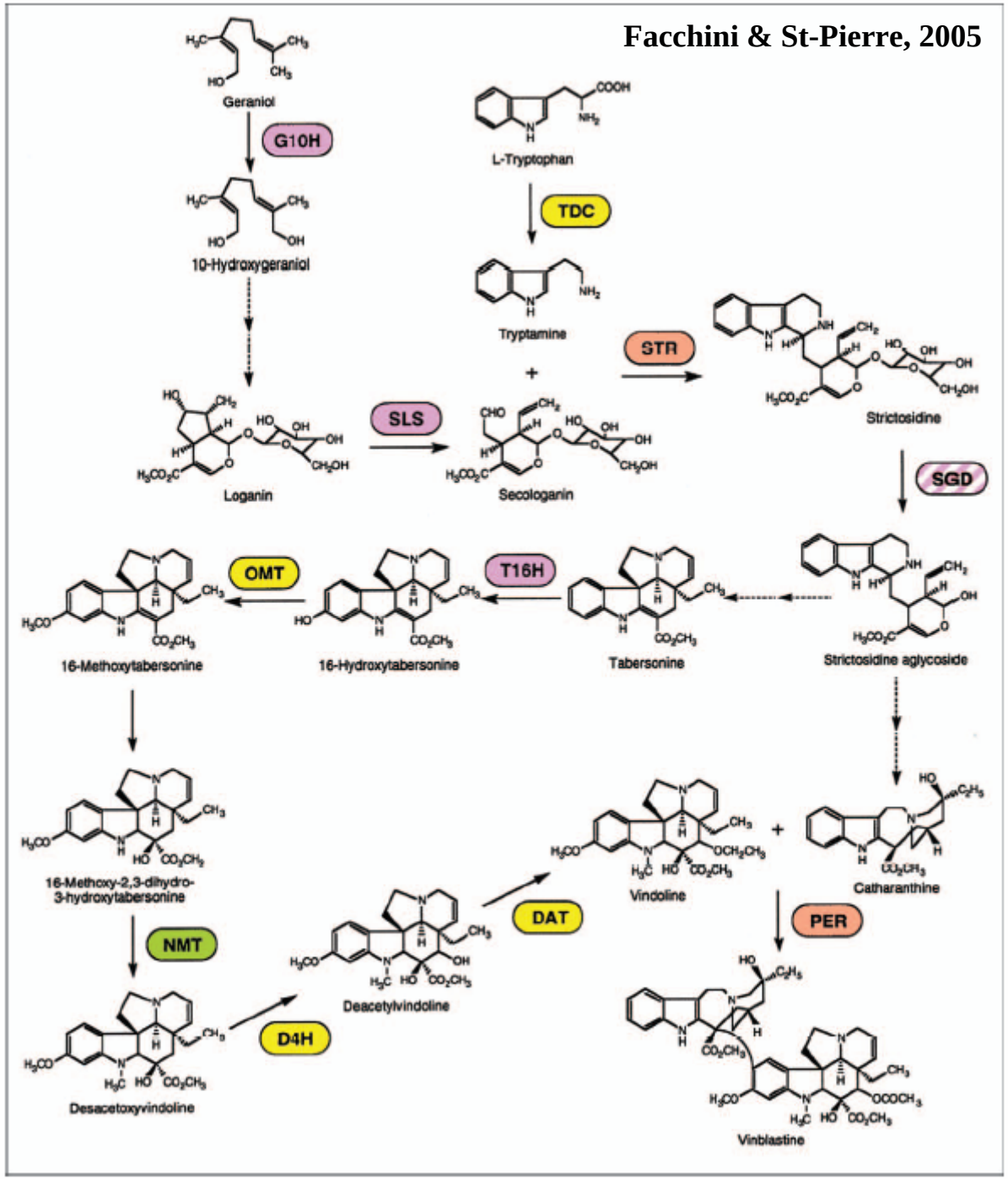
cytosol

vacuole

ER membrane

outer ER

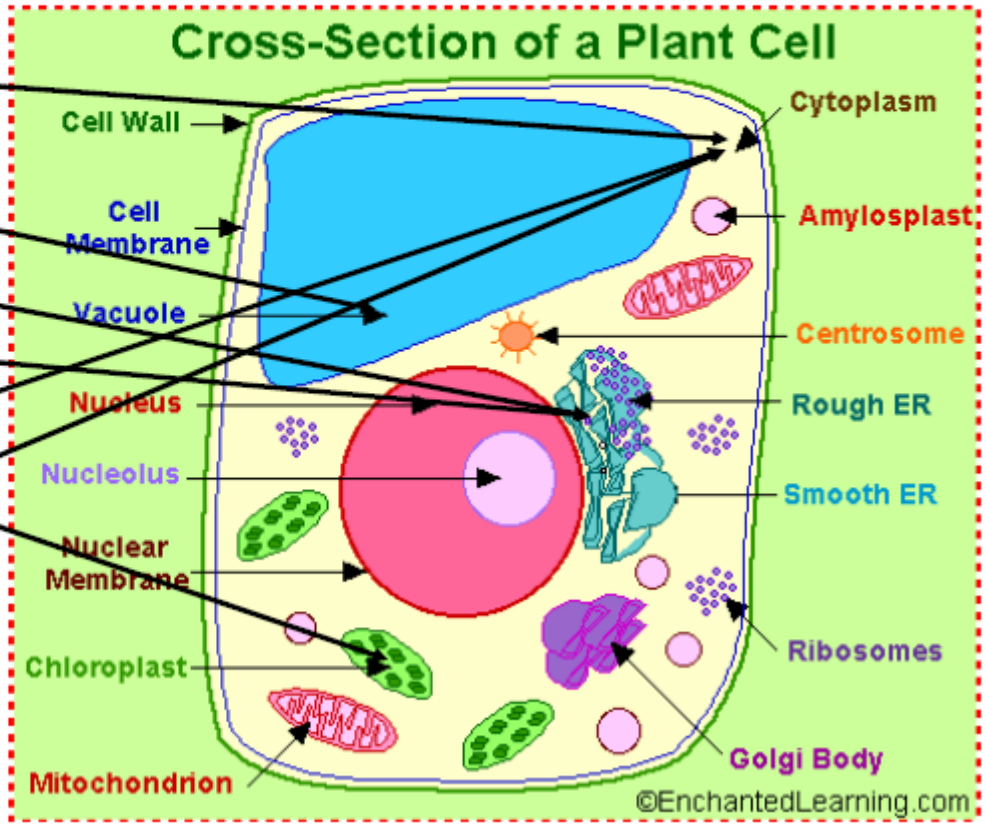
chloroplast



Catharanthus Terpenoid Indole Alkaloids

Subcellular Complexity

- TDC
- STR
- SGD
- T16H
- NMT
- D4H
- DAT



Metabolism of Monoterpenoid Indole Alkaloids in Three Different Catharanthus Cell types

- Extensive subcellular trafficking of pathway intermediates

- Geraniol 10-hydroxylase: internal phloem parenchyma of aerial organs

- Tryptophan decarboxylase (TDC), Secologanin synthase (SLS) and Strictosidine synthase (STR) to epidermis of aerial organs and the apical meristems of roots

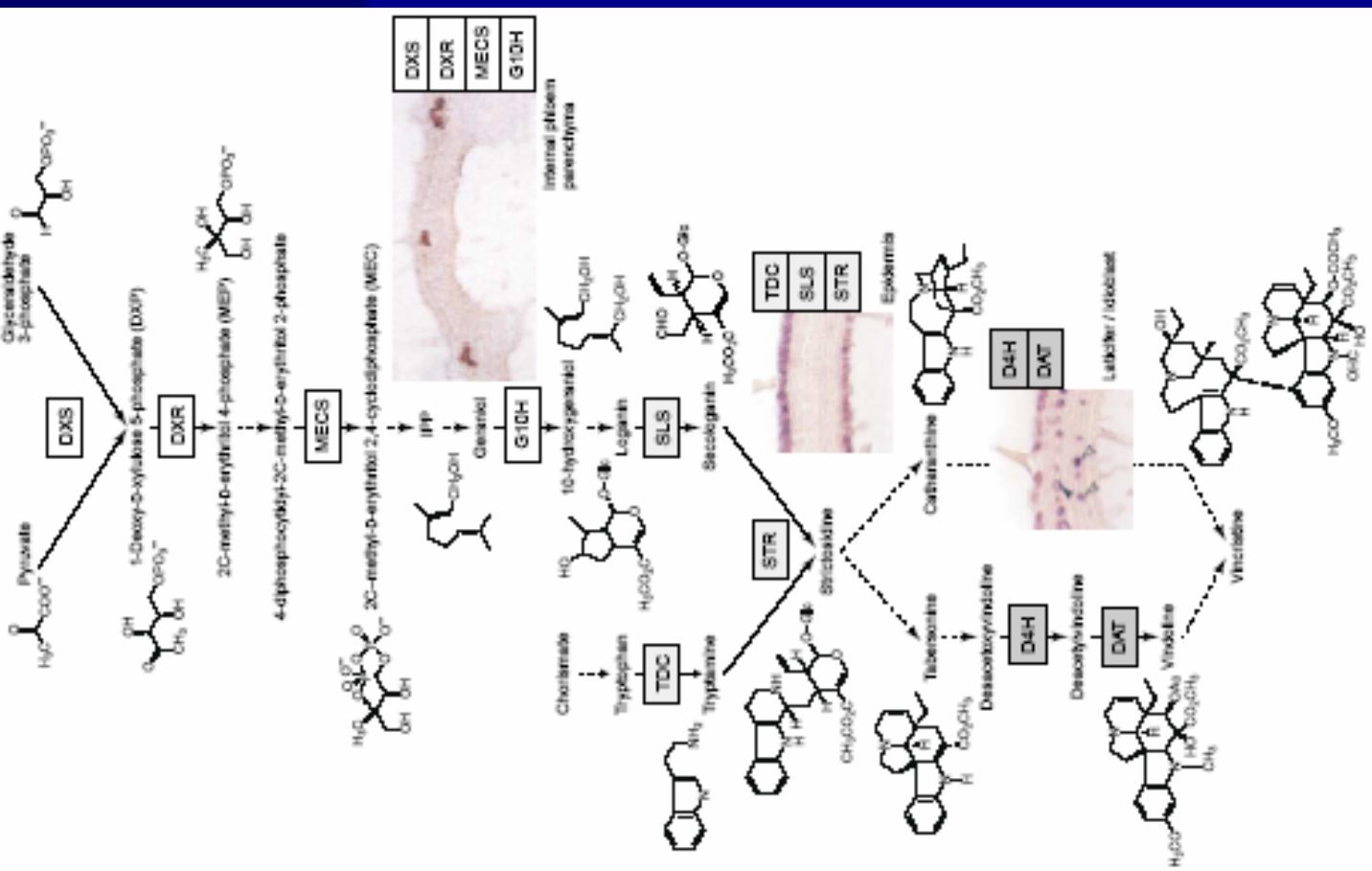
Metabolism of Monoterpenoid Indole Alkaloids in Three Different Catharanthus Cell types

- Deacetylvindolineacetyltransferase (DAT) and desacetoxyvindoline 4-hydroxylase (D4H) to the laticifers and idioblasts of leaves and stems

- Vindoline pathway intermediates must be translocated between cell types

Metabolism of Monoterpenoid Indole Alkaloids in Three Different Catharanthus Cell types

Kutchan, 2005



Regulation of Monoterpenoid Indole Alkaloids in Catharanthus

ORCA3, a Jasmonate-Responsive Transcriptional Regulator of Plant Primary and Secondary Metabolism

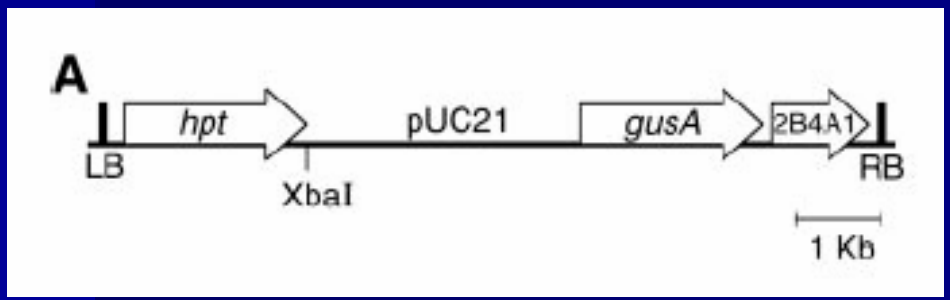
Leslie van der Fits and Johan Memelink*

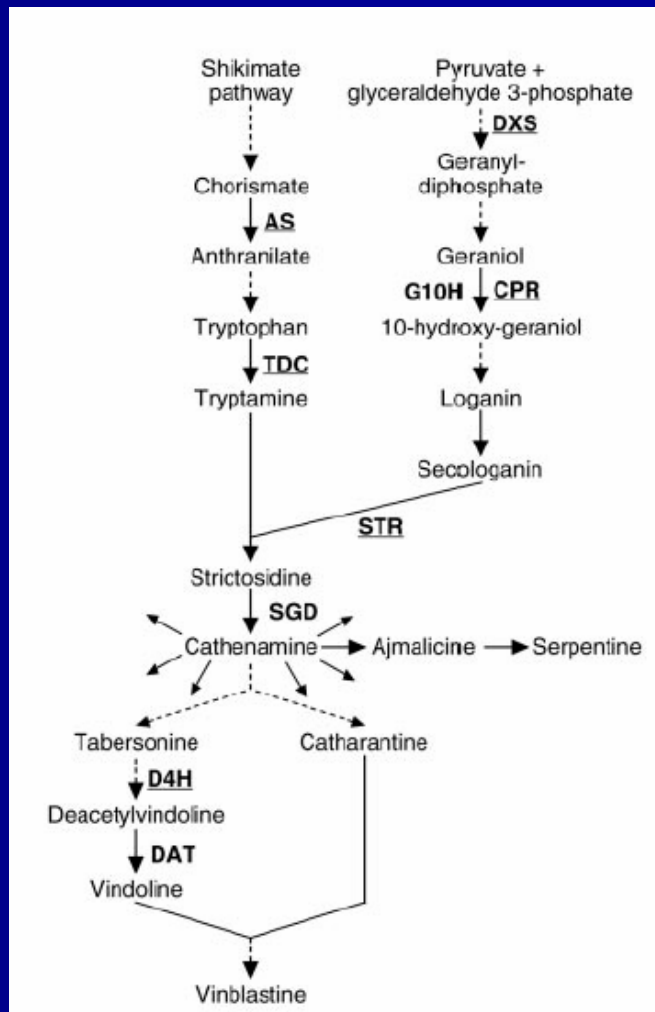
ORCA- Octadecanoid Responsive Cartharanthus AP2/ERF domain protein

www.sciencemag.org SCIENCE VOL 289 14 JULY 2000

Regulation of Monoterpenoid Indole Alkaloids in Catharanthus

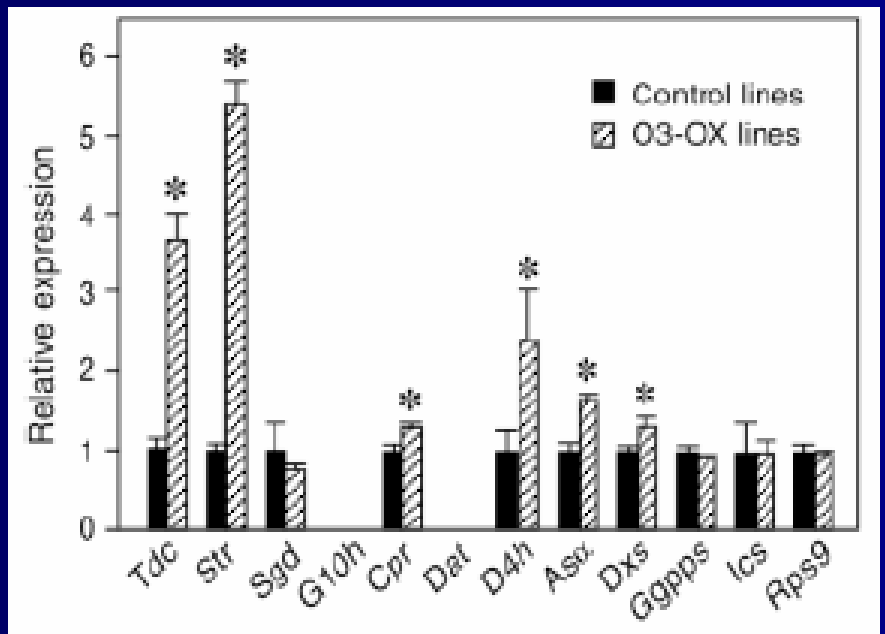
Activation tagging in Catharanthus cell cultures. Screen on toxic 4-methyl tryptophan (TDC can detoxify it)



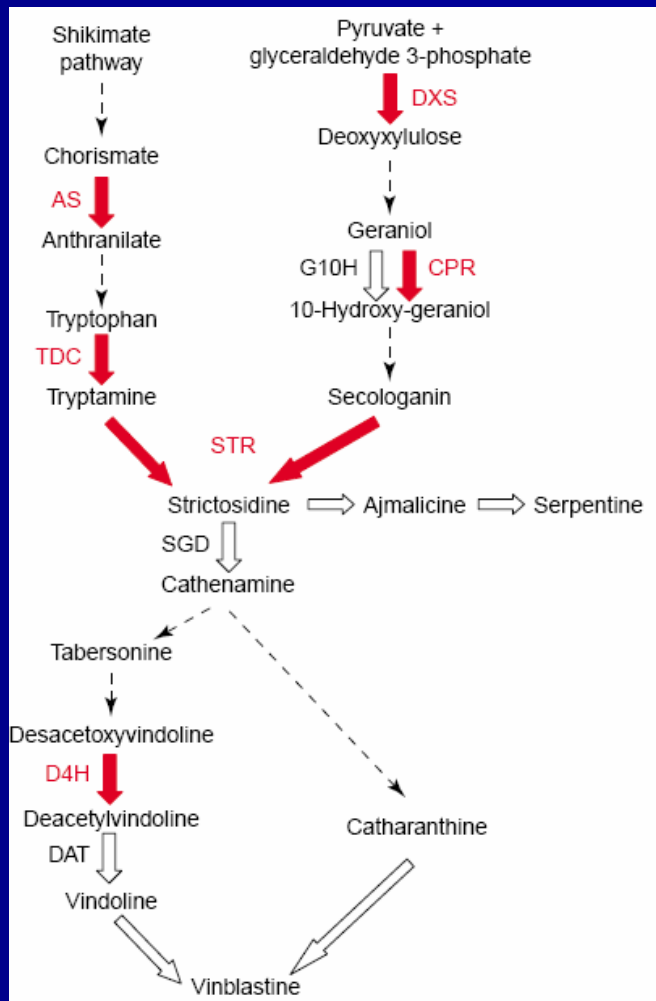


Regulation of Monoterpenoid Indole Alkaloids in Catharanthus

ORCA3 can activate promoters of both primary and secondary metabolic pathways in the TIA pathway of Catharanthus

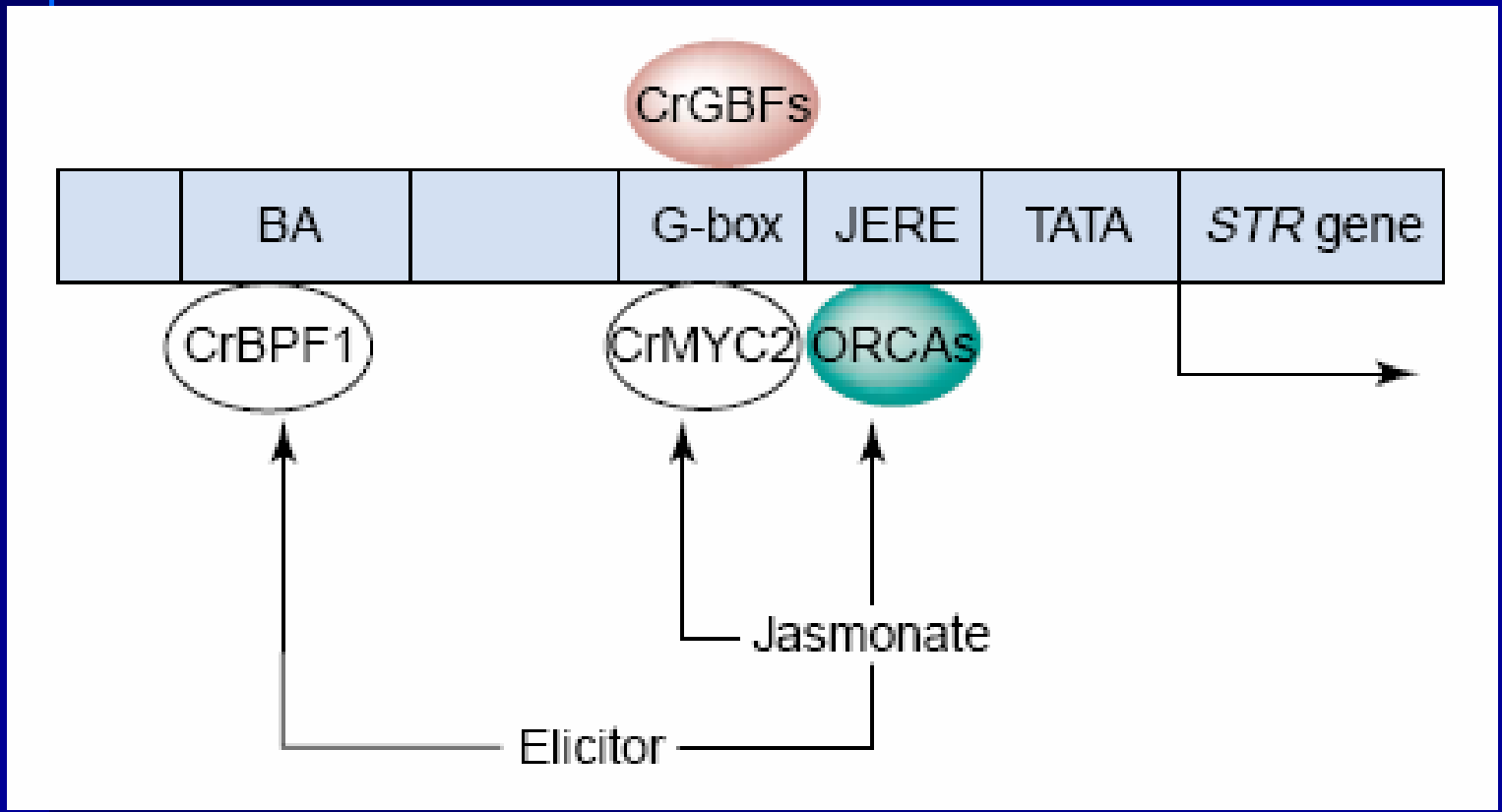


Gantet & Memlink, 2002



Regulation of Monoterpenoid Indole Alkaloids in Catharanthus

Other Factors Involved

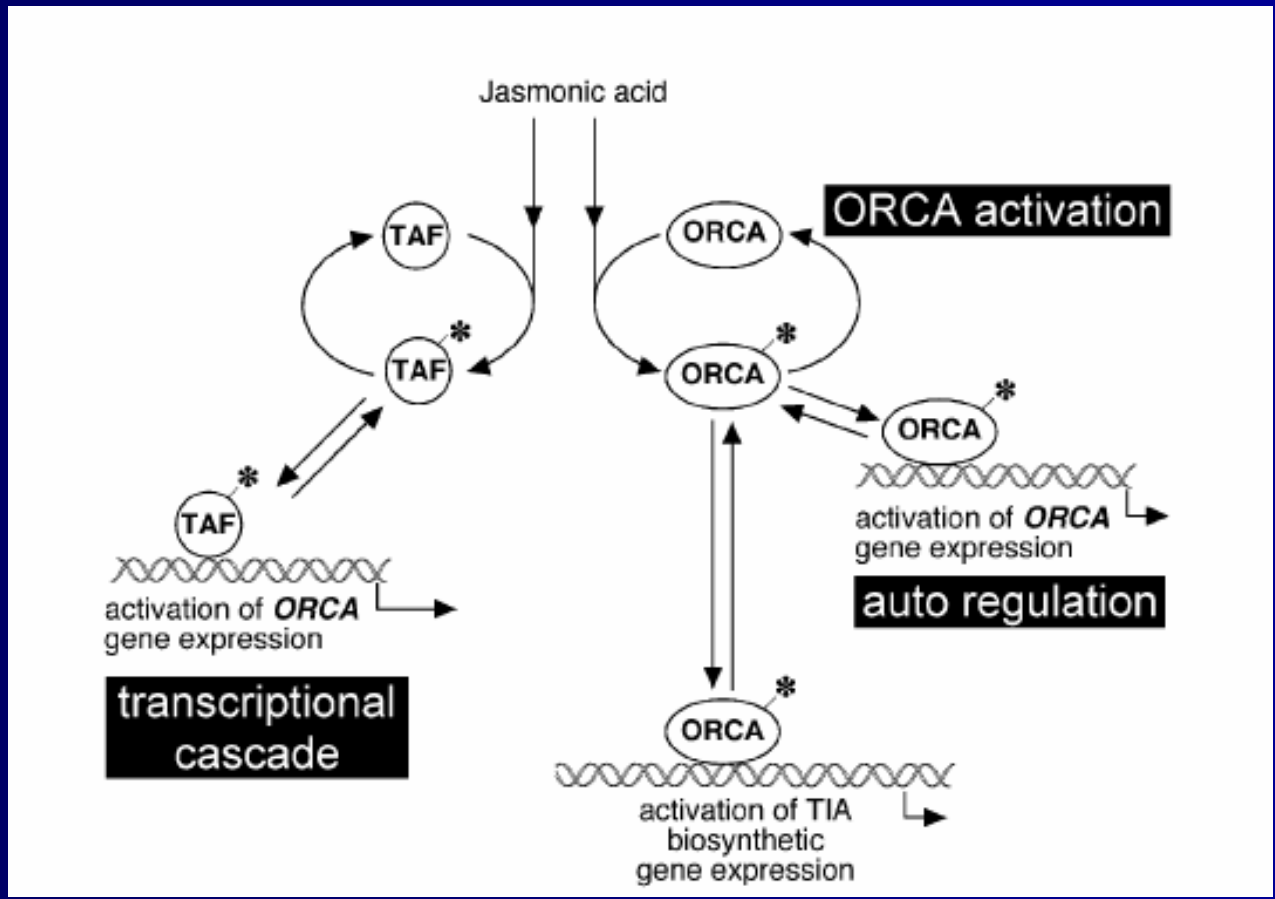


Activator

Repressor

Gantet & Memlink, 2002

Regulation of the TIA Pathway in Catharanthus (autoacivation of ORCAs)



Endt et al, 2002

Regulation of Terpenoid Indole Alkaloids in Catharanthus

Plant transcription factor	Metabolite class	Mammalian homolog[b]	Function in mammals	DNA-binding domain
C1	Anthocyanins	c-MYB	Cell cycle	MYB[c]
P	Phlobaphenes			
TT2	Condensed tannins			
PAP1	Anthocyanins			
AtMYB4	Sinapate esters			
CrBPF1	Alkaloids?			
R	Anthocyanins	c-MYC	Cell cycle	bHLH
TT8	Condensed tannins			
CrMYC2	Alkaloids?			
ORCA2	Alkaloids	None		AP2/ERF
ORCA3	Alkaloids			
CrGBF1	Alkaloids?	CREB	Long-term memory, T-cell development, blood pressure	bZIP
CrGBF2	Alkaloids?			

Gantet & Memlink, 2002

Regulation of Monoterpenoid Indole Alkaloids in Catharanthus by Jasmonate

ORCAnization of jasmonate-responsive gene expression in alkaloid metabolism

Johan Memelink, Rob Verpoorte and Jan W. Kijne

Review

TRENDS in Plant Science Vol.6 No.5 May 2001

Regulation of Monoterpenoid Indole Alkaloids in Catharanthus by Jasmonate

- Plants lack an immune system (as in animals), but possess mechanisms that recognize potential pathogens and initiate defense responses

- Various types of oxygenated fatty acids, termed ‘oxylipins’ or ‘octadecanoids’, are involved in responses to physical damage by animals or insects, stress and attack by pathogens

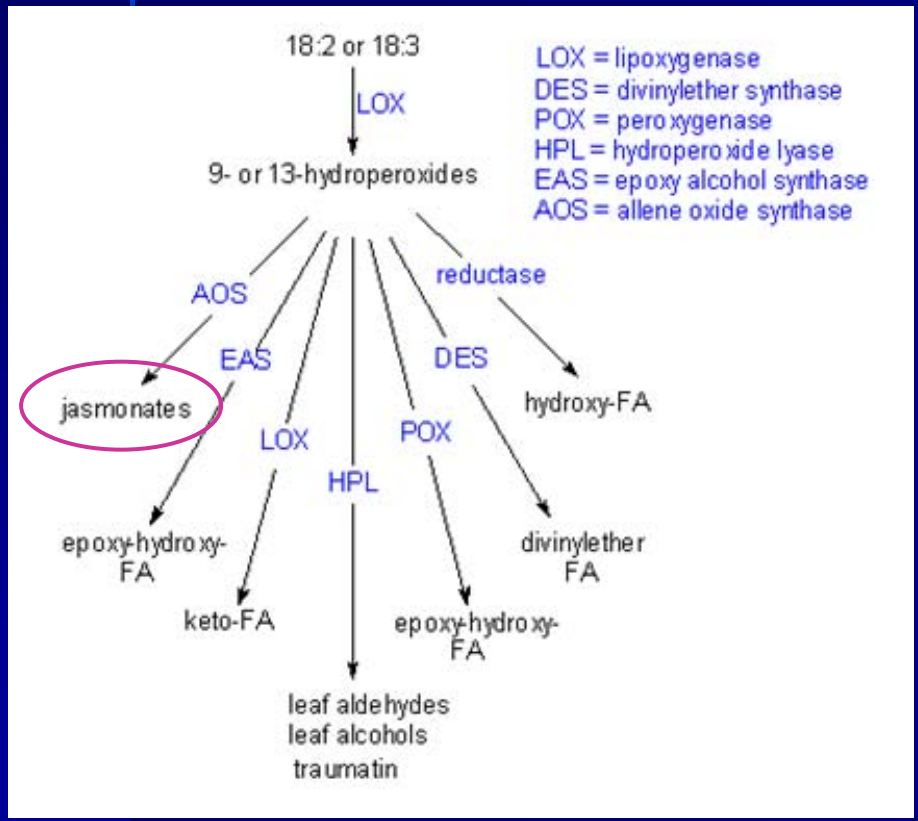
Regulation of Monoterpenoid Indole Alkaloids in Catharanthus by Jasmonate

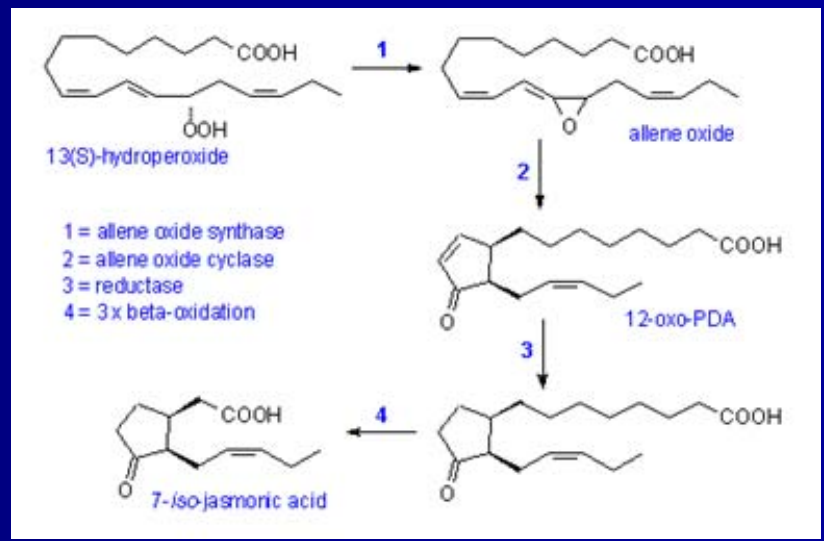
- These compounds are similar to the eicosanoids derived from arachidonate in animals (important in the inflammatory process)

- Oxylipins are derived from linoleic and α-linolenic acids with a first key step being the action of lipoxygenases (LOX)

- Such compounds are highly reactive, and quickly metabolized by various enzymes into series of oxylipins, including Jasmonates with a range of distinct activities.

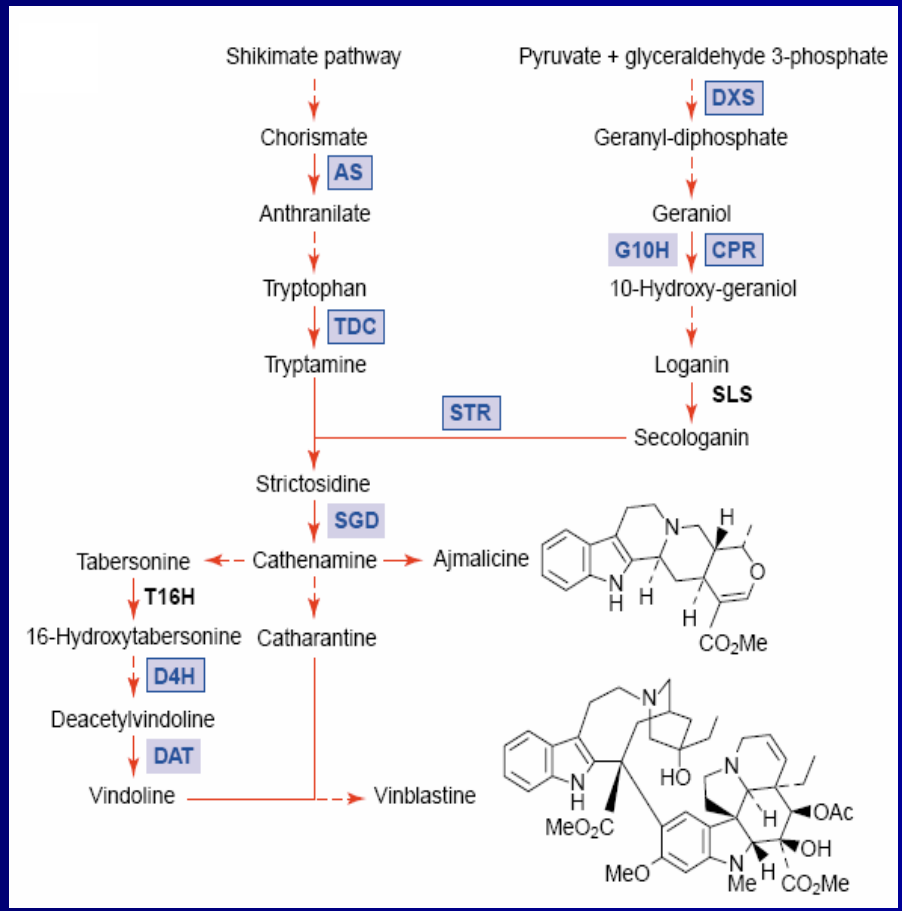
Regulation of Monoterpenoid Indole Alkaloids in Catharanthus by Jasmonate





Regulation of Monoterpenoid Indole Alkaloids in Catharanthus by Jasmonate

ORCA3 and other genes induced by Jasmonate



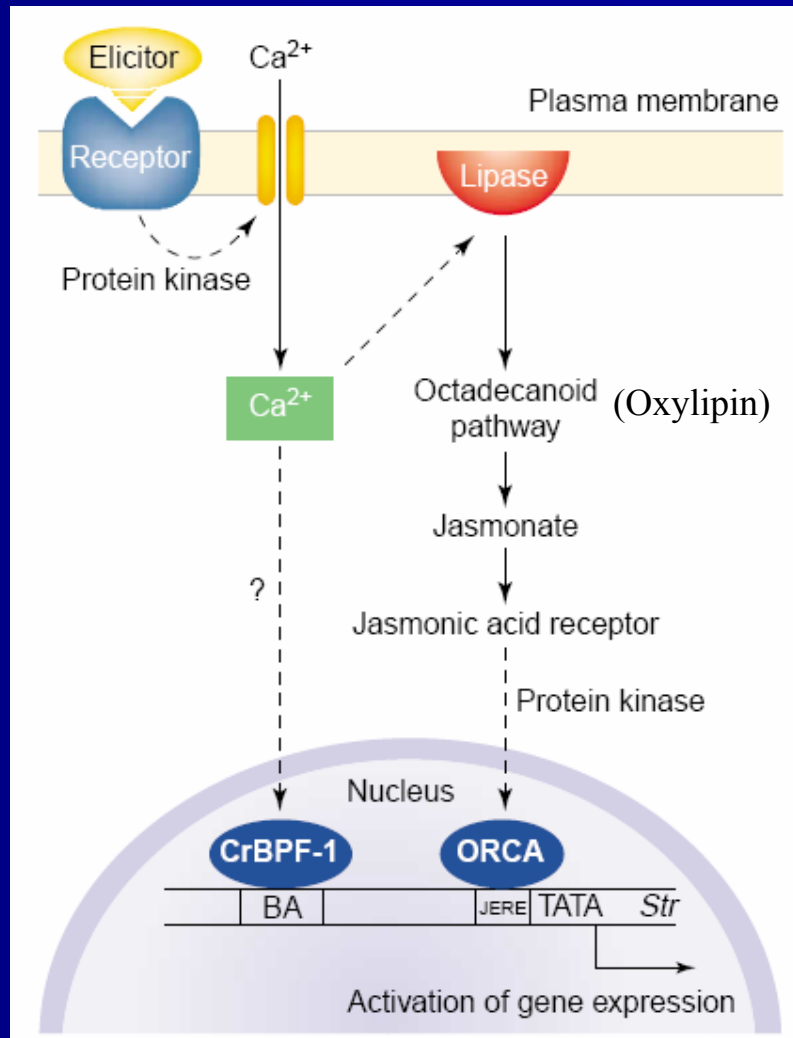
ORCAs act in a Jasmonic Acid Dependant Elicitor Signal Transduction Pathway

Memlink et al, 2001

- Elicitor (any compound inducing a plant defense reaction (either from microorganism, plant and abiotic such as heavy metals)

-Protein phosphorylation and calcium efflux are required for elicitor induced Jasmonate biosynthesis

- CrBF-1 acts in a signal transduction pathway independent of Jasmonic acid



Plant Secondary Metabolites Induced by Jasmonates

Metabolite class and subclass	Plant species
Polyamines	
	Hyoscyamus muticus
Coumaroyl-conjugated polyamines	
	Hordeum vulgare
Anthraquinones	
	Rubia tinctorum
Naphthoquinones	
	Lithospermum erythrorhizon
Gum (polysaccharide)	
	Prunus persica
	Tulipa gesneriana
Terpenoids	
Diterpenes (taxol)	*Taxus* spp.
Sesquiterpenes	*Lactuca sativa*
	Phaseolus lunatus
	Zea mays
Triterpenes	*Scutellaria baicalensis*
Alkaloids	
Acridone	*Ruta chalepensis*
Nicotine	*Nicotiana* spp.
Tropane	*Datura stramonium*
Benzylisoquinoline	*Eschscholzia californica*
	Papaver somniferum
	Thalictrum tuberosum
Terpenoid indole	*Catharanthus roseus*
	Cinchona ledgeriana
	Rauvolfia spp.
Phenylpropanoids	
Rosmarinic acid	*Coleus blumei*
	Lithospermum erythrorhizon
Coumarins	*Nicotiana tabacum*
Furanocoumarins	*Petroselinum crispum*
Flavonoids	*Arabidopsis thaliana*
	Crotalaria cobalticola
	Glycine max
	Oryza sativa
	Petroselinum crispum
	Prunus persica
	Tulipa gesneriana

Benzylisoquinoline Alkaloids

- A large and diverse class present in a range of plant families

- The first biosynthetic step is decarboxylation of Tyrosine by Tyrosine Decarboxylase (TYDC) to form Tyramine

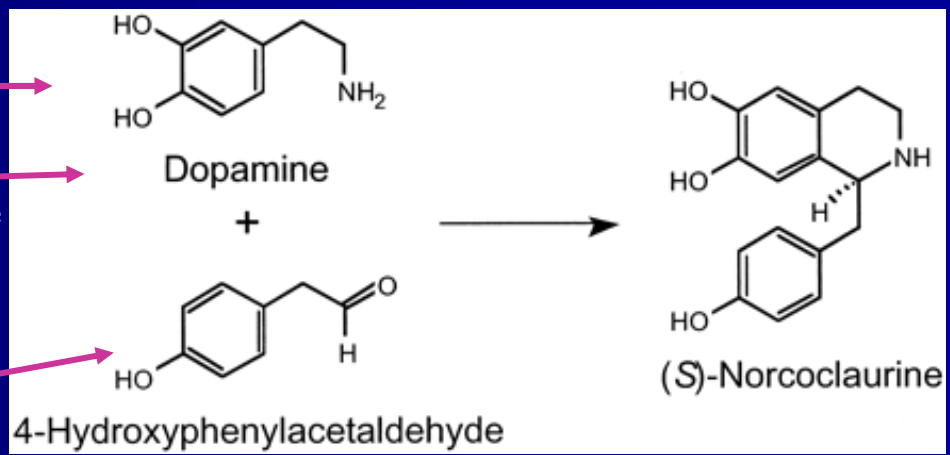
Benzylisoquinoline Alkaloids

- Coupling of two Tyramine derivatives yields (*S*)-Norcoclaurine
- Norcoclaurine is the precursor of several thousand benzylquinoline alkaloids

Tyrosine- Tyramine

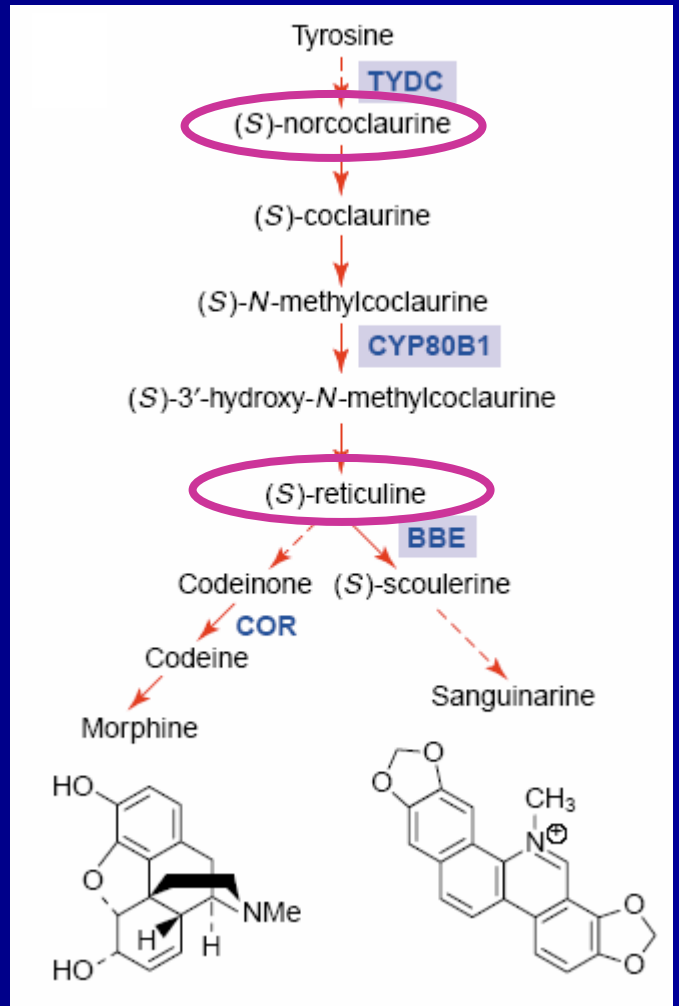
Tyrosine- L-DOPA (phenol oxidase)

Tyrosine- p-Hydroxy-phenylpyruvate (transaminase)



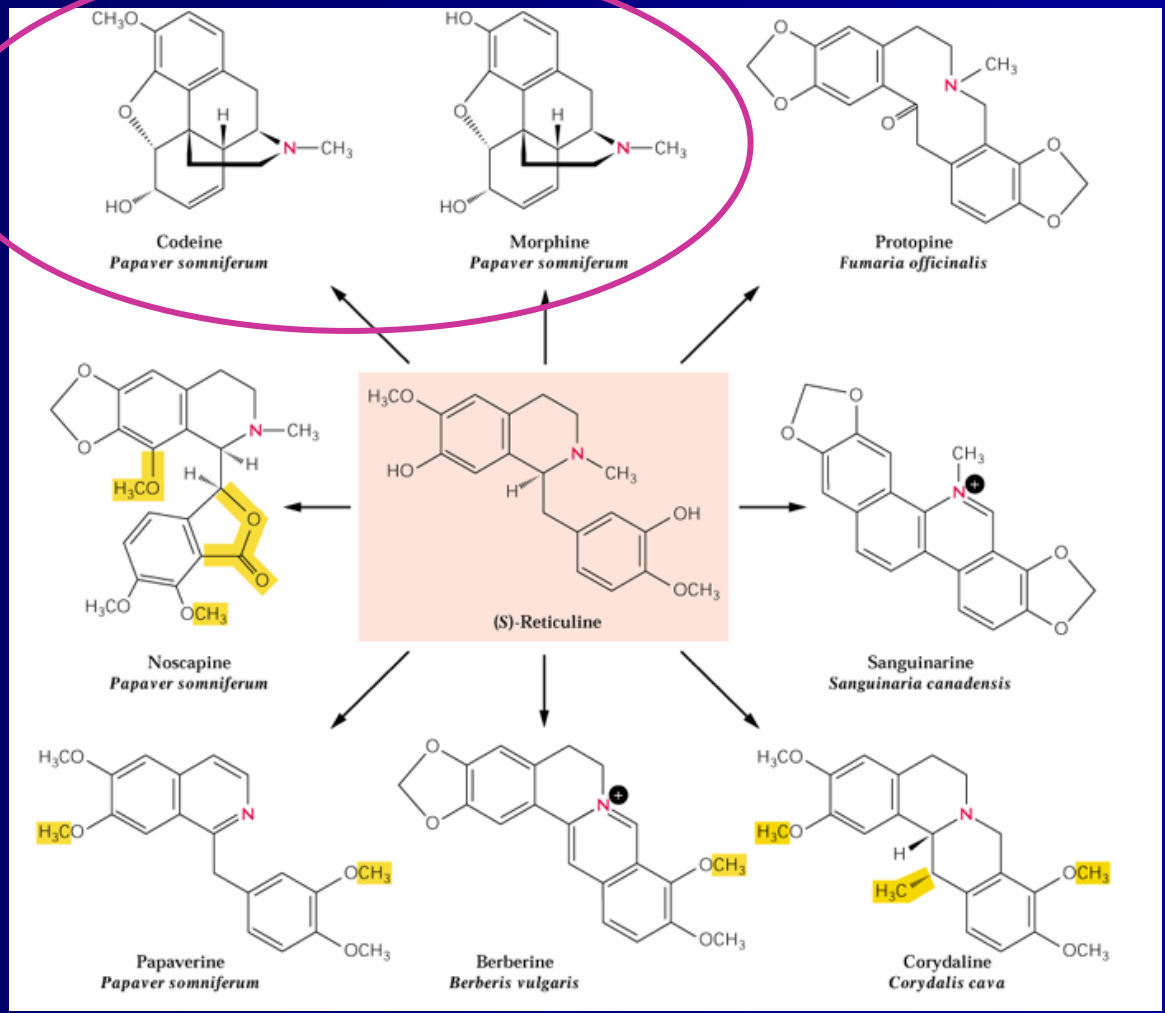
Benzylisoquinoline Alkaloids

A series of methylation and oxidation reactions yield the branch point intermediate of BIA biosynthesis, (*S*)-Reticuline



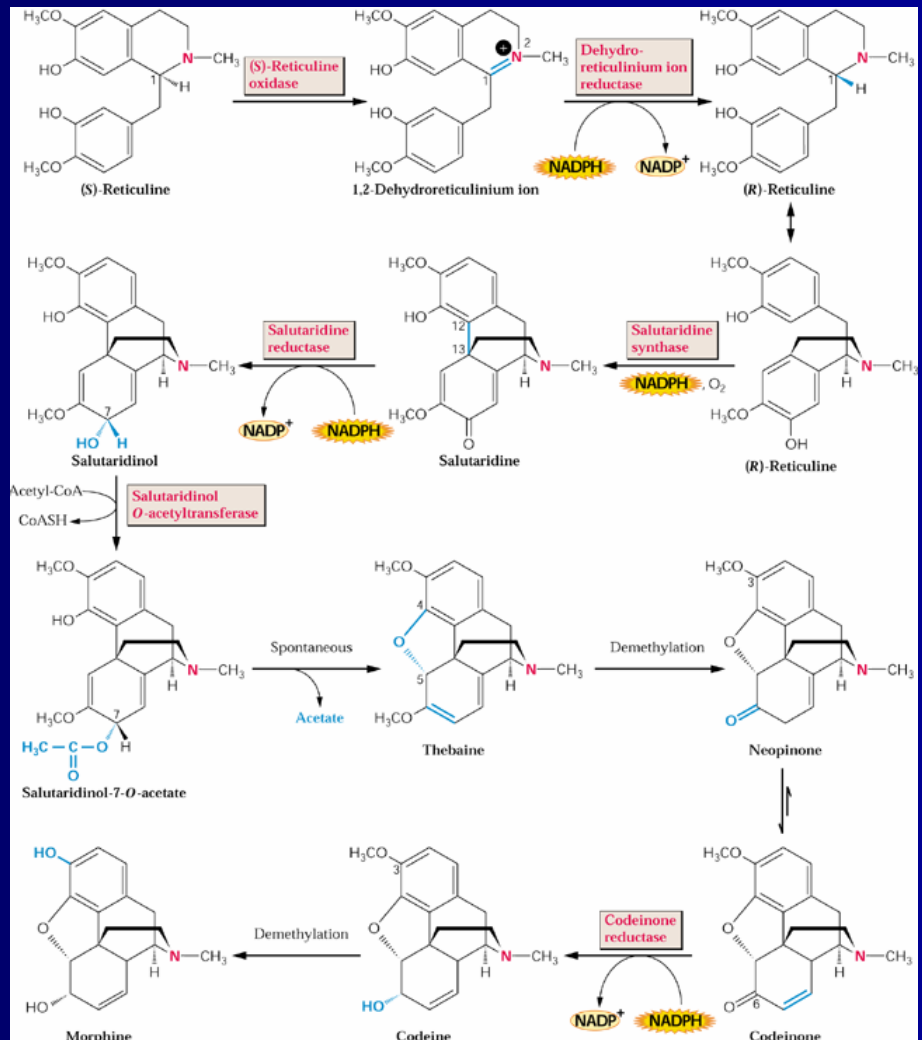
(S)- Reticulin: the Chemical Cameleon

(twisted and turned before being oxidized to generate different structures)



Benzylisoquinoline Alkaloids

Morphine and Codeine biosynthesis in Opium Poppy

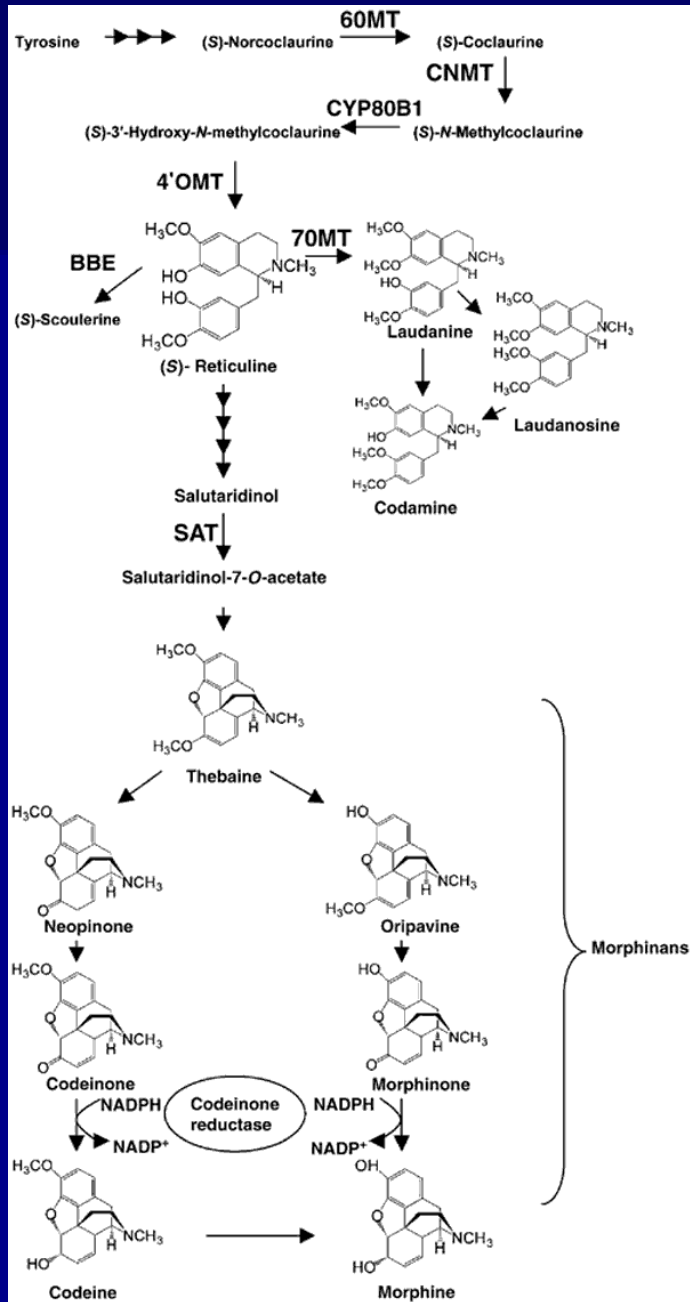


Benzylisoquinoline Alkaloids

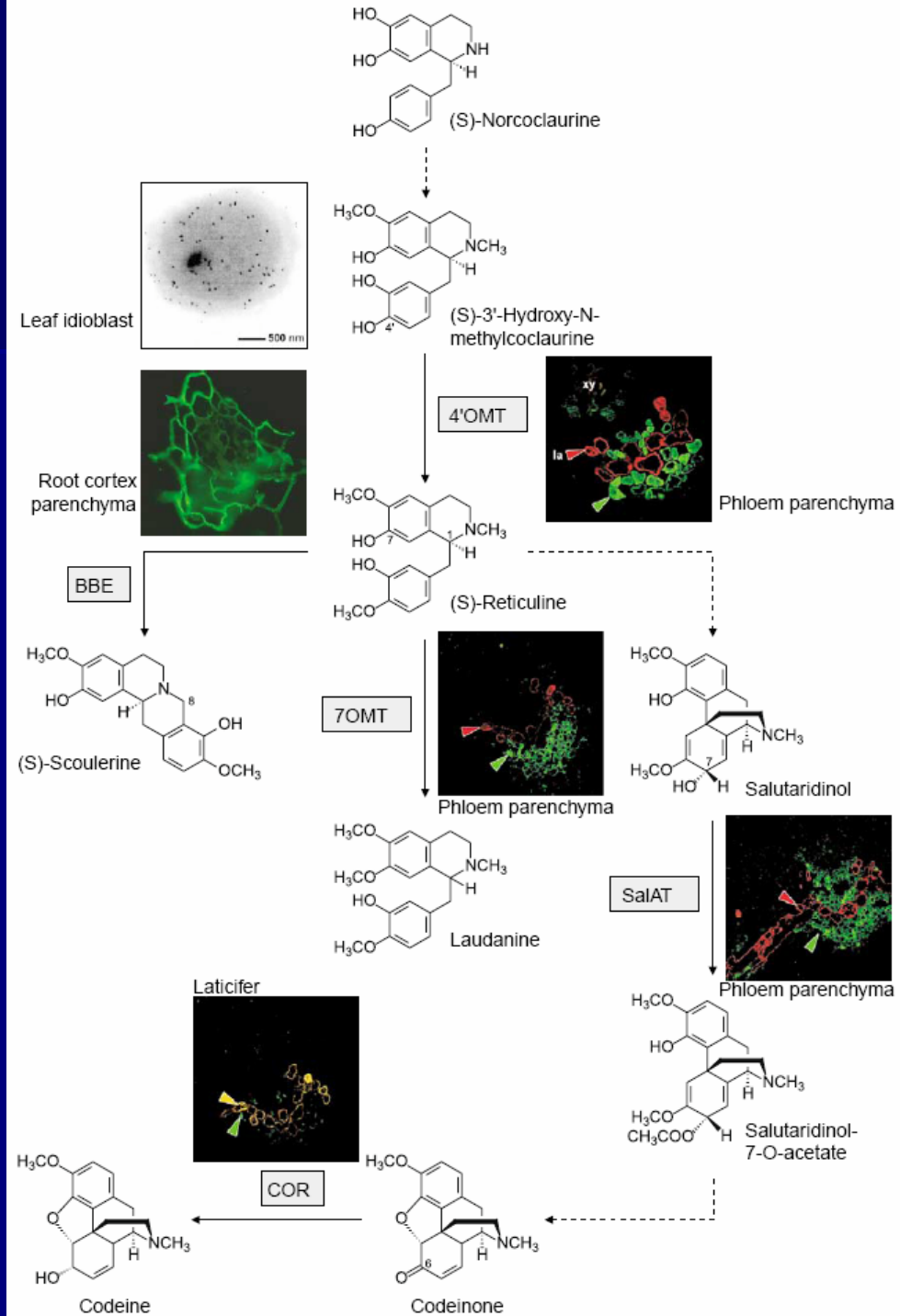
Morphine and Codeine Biosynthesis in Opium Poppy

- After 190 years since its discovery the characterization of the enzymes is nearly complete

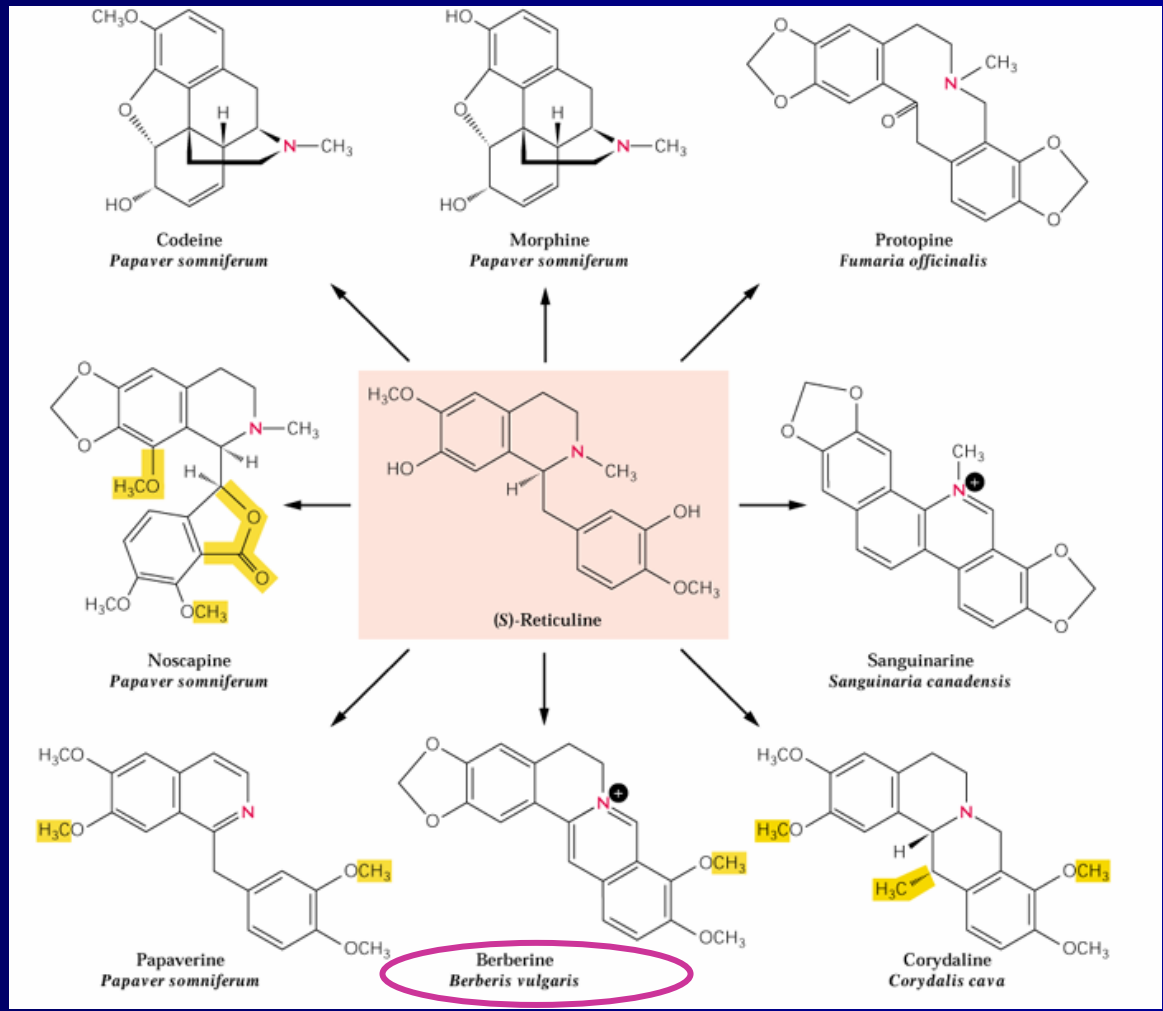
- Genes cloned but not all the pathway



Morphine and Codeine Biosynthesis in Opium Poppy (cell localization)



Biosynthesis of BERBERINE

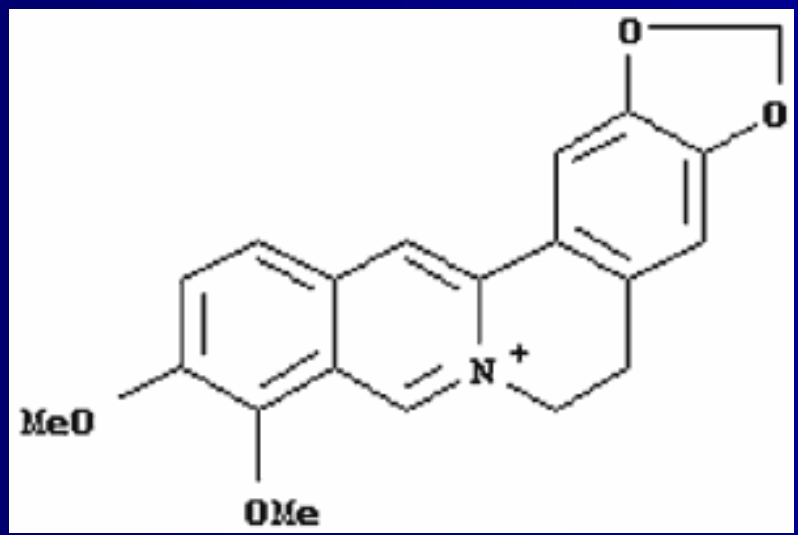


Benzylisoquinoline Alkaloids

Biosynthesis of BERBERINE

- A pigment with bright yellow color

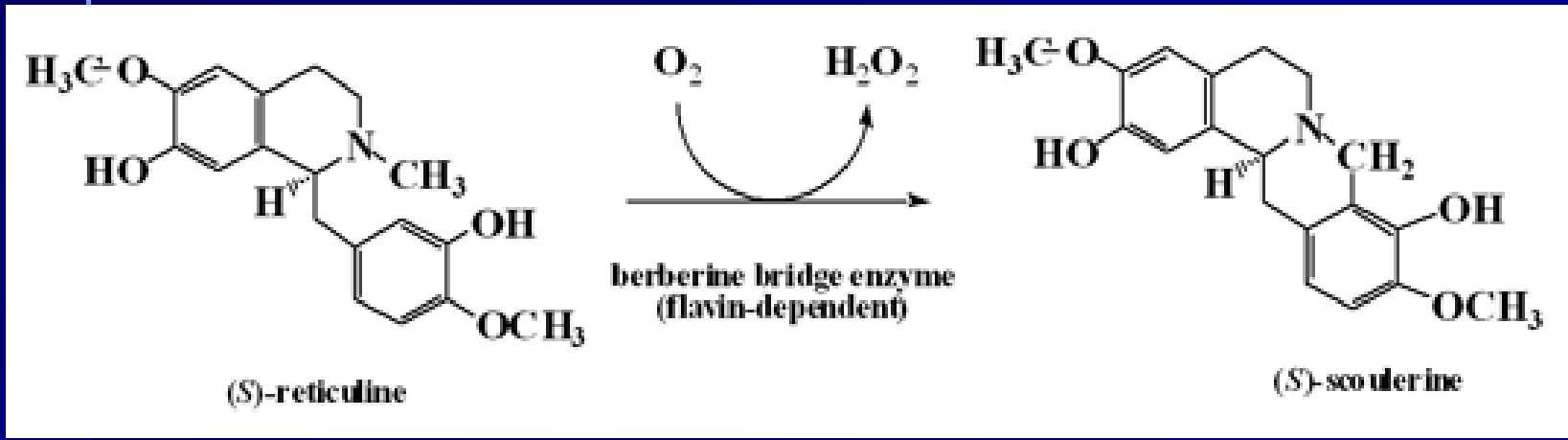
- Native American used to dye cloth, reduce inflammation, stimulate digestion, treat infections and induce abortions



Biosynthesis of BERBERINE

- A major alkaloid in Goldenseal (*Hydrastis canadensis*)
- Used to prevent colds and flu
- The third most popular herbal in the US

Biosynthesis of BERBERINE (Berberine bridge enzyme)



- The enzyme possesses a covalently attached FAD moiety, which is essential for catalysis

- The reaction involves the oxidation of the *N*-methyl group of the substrate (*S*)-reticuline by the enzyme-bound flavin and concomitant formation of a carbon-carbon bond (the "bridge")

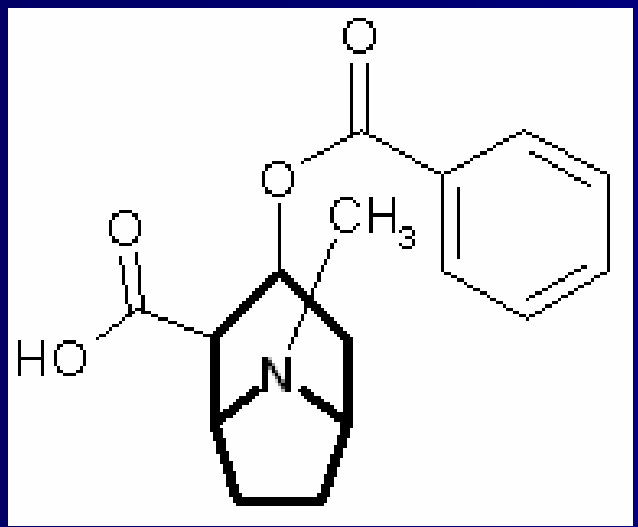
Tropane Alkaloids

- Plants containing these alkaloids have been used throughout history as poisons, but many of the alkaloids do have valuable pharmaceutical properties

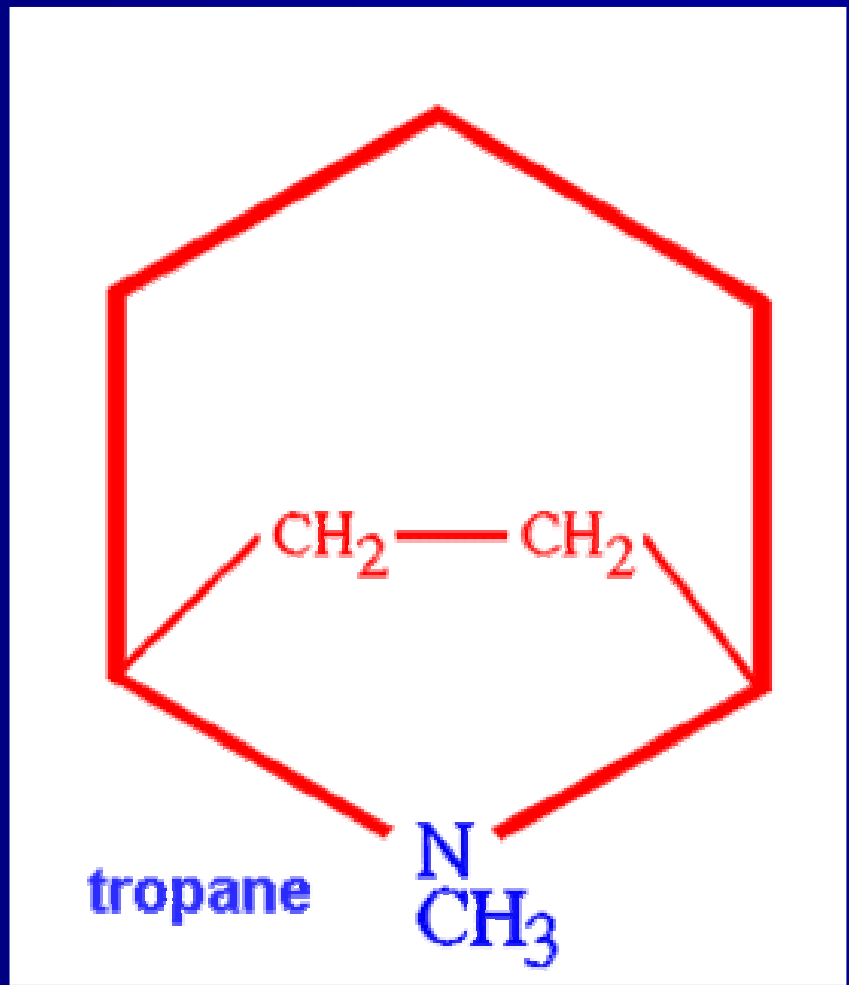
- Known to be present in the *Solanaceae* family

- The TA Cocaine was found in very small amounts in the original Coca-Cola formula, but was not the main concern of the USDA at the time. Caffeine was considered to be the major problem with the drink.

Tropane Alkaloids

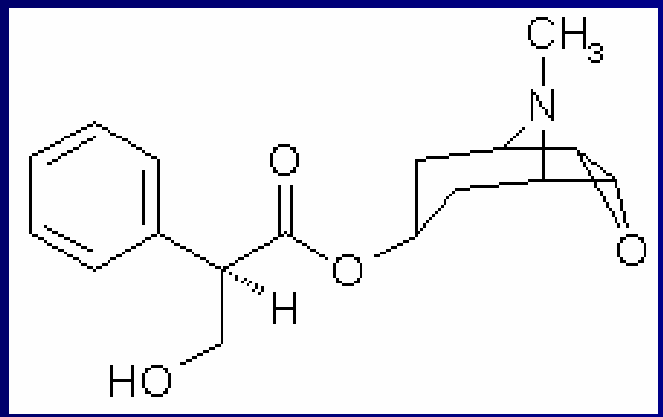


Cocaine



Tropane Alkaloids- Datura

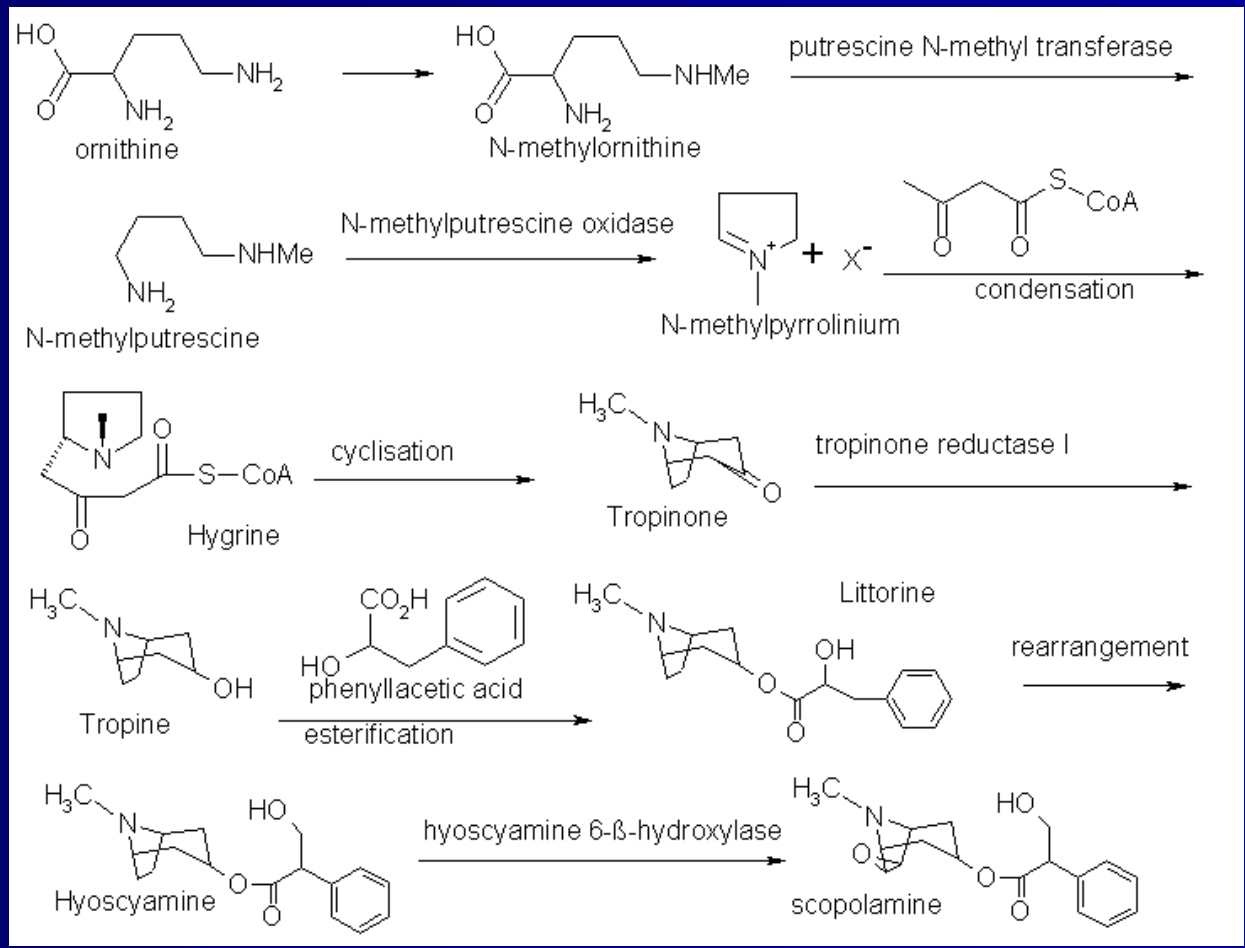
- Datura, a rich source of scopolamine and hyoscyamine used as a sedative
- Scopolamine can cause death in infants



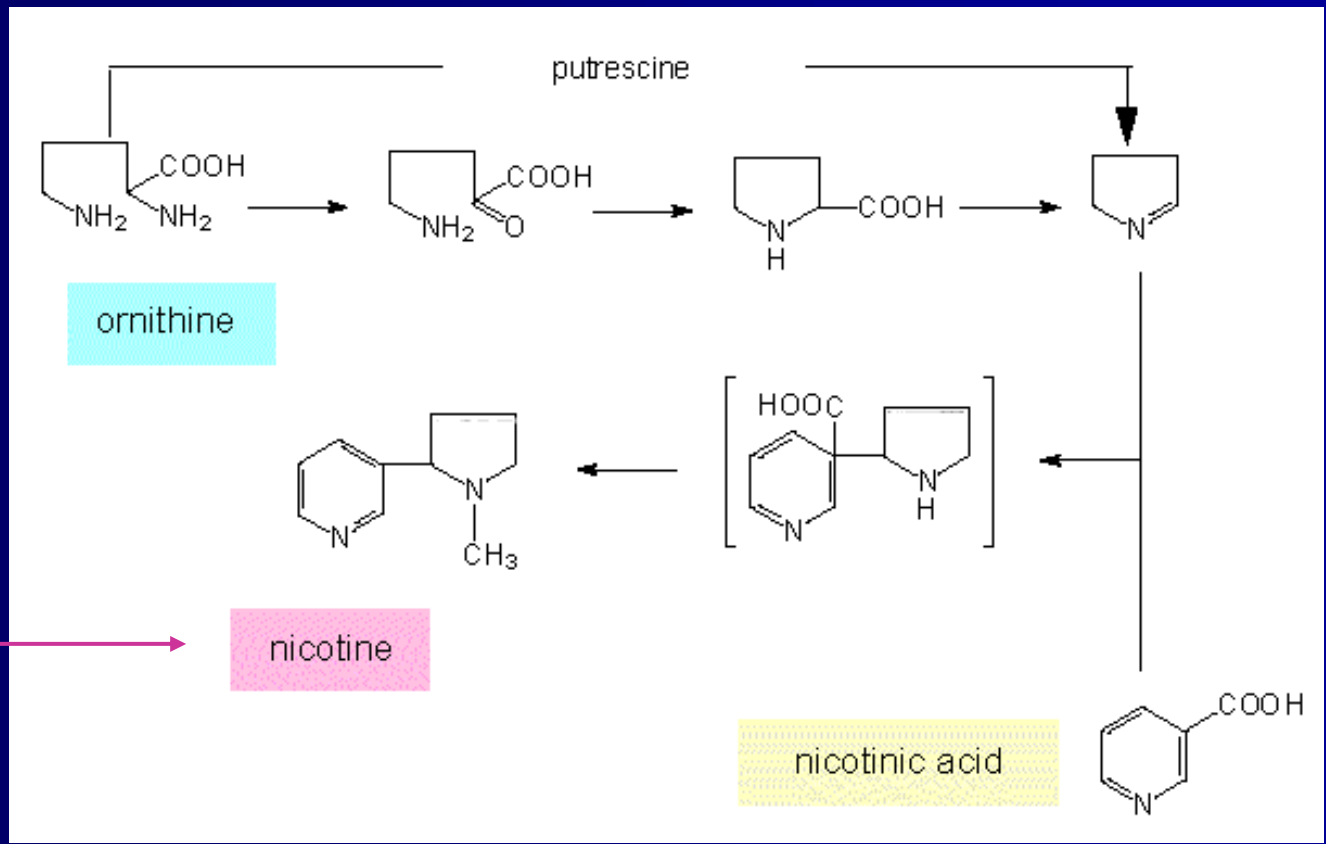
Scopolamine

Tropane Alkaloids- Biosynthesis

- Methylation of putrescine is the start point for biosynthesis



Tropane Alkaloids- Biosynthesis



A pyridine alkaloid

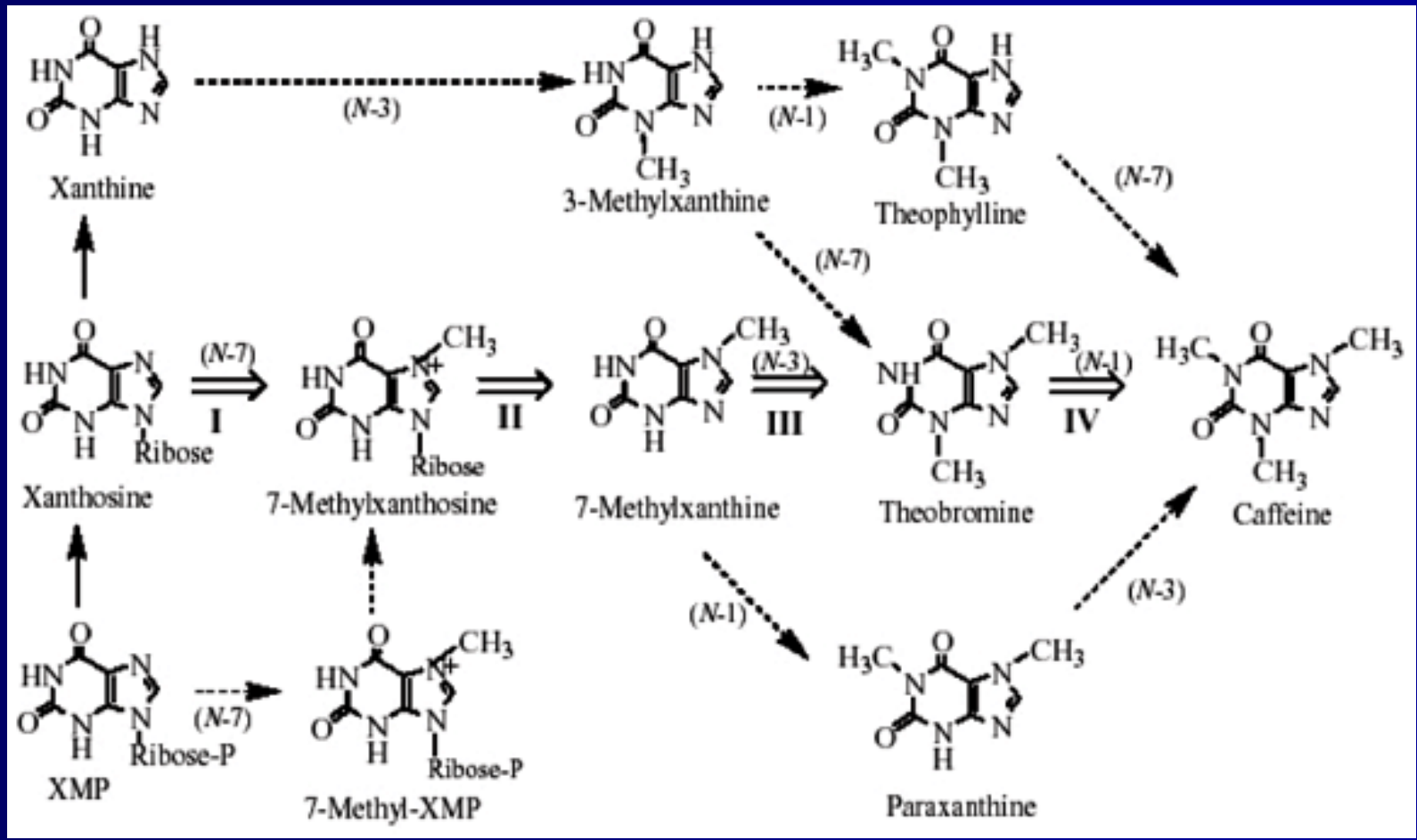
Purine Alkaloids

- Caffeine the most important example

- In, coffee, tea, mate', cacao, camellia

- Purin alkaloid biosynthesis starts with xanthosine, a nucleotide degradation product

Purine Alkaloids- Caffeine Biosynthesis

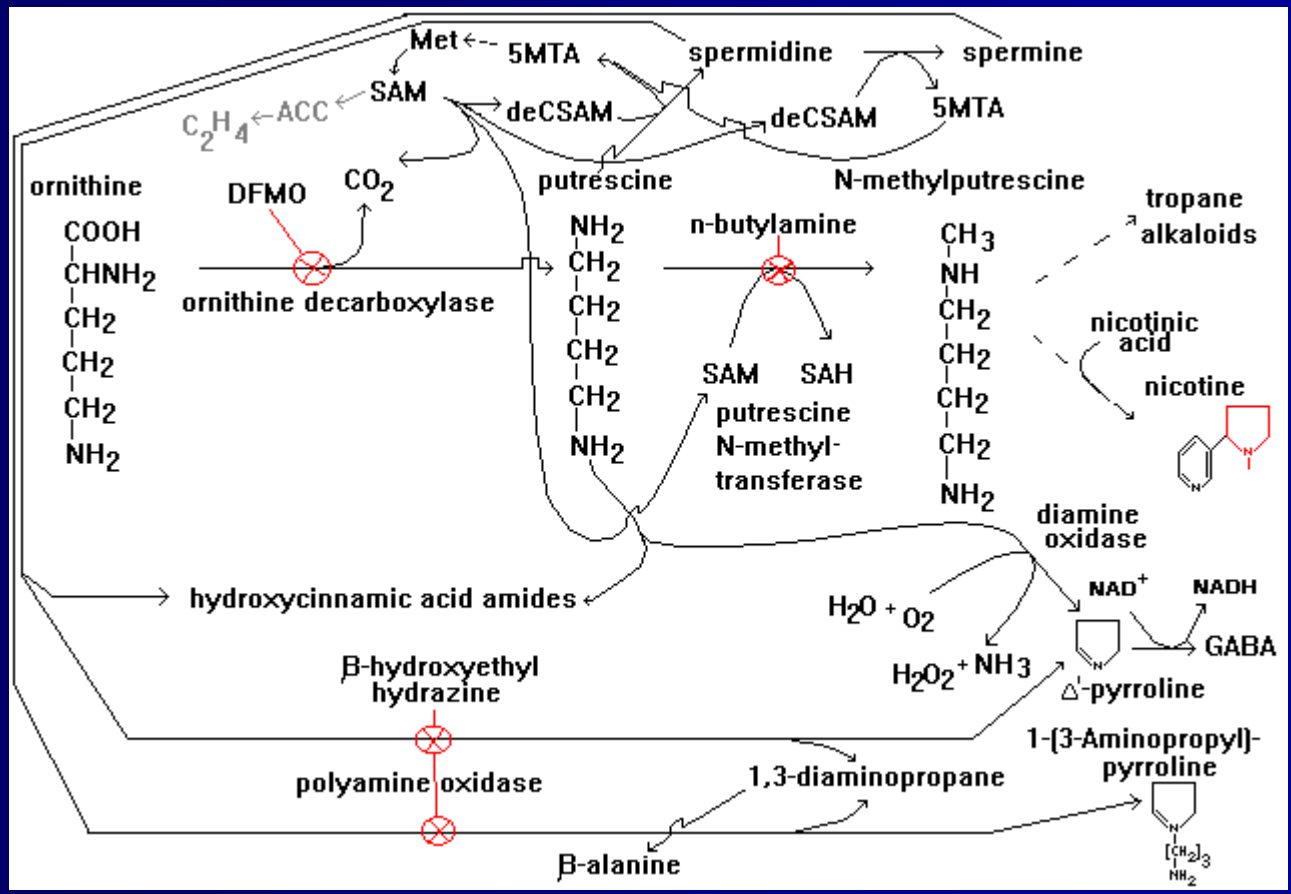


Pyrrolizidine Alkaloids

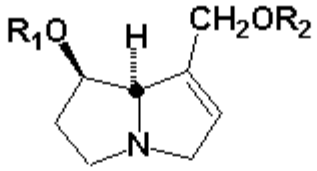
- The leading plant toxins
- Over 360 different structures, found in 3% of the world flowering plants
- Primarily restricted to; Boraginaceae, Asteraceae, Fabaceae, and Orchidaceae
- Most of them are esters of basic alcohols known as necine bases

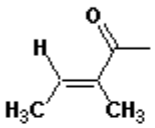
Pyrrolizidine Alkaloids

- Mostly derived from either the polyamines putrescine and spermidine

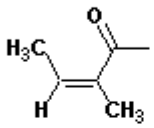


Pyrrolizidine Alkaloids

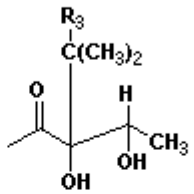




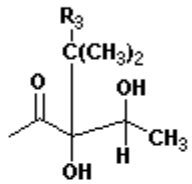
I



II

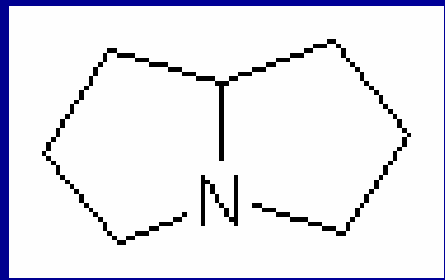


III



IV

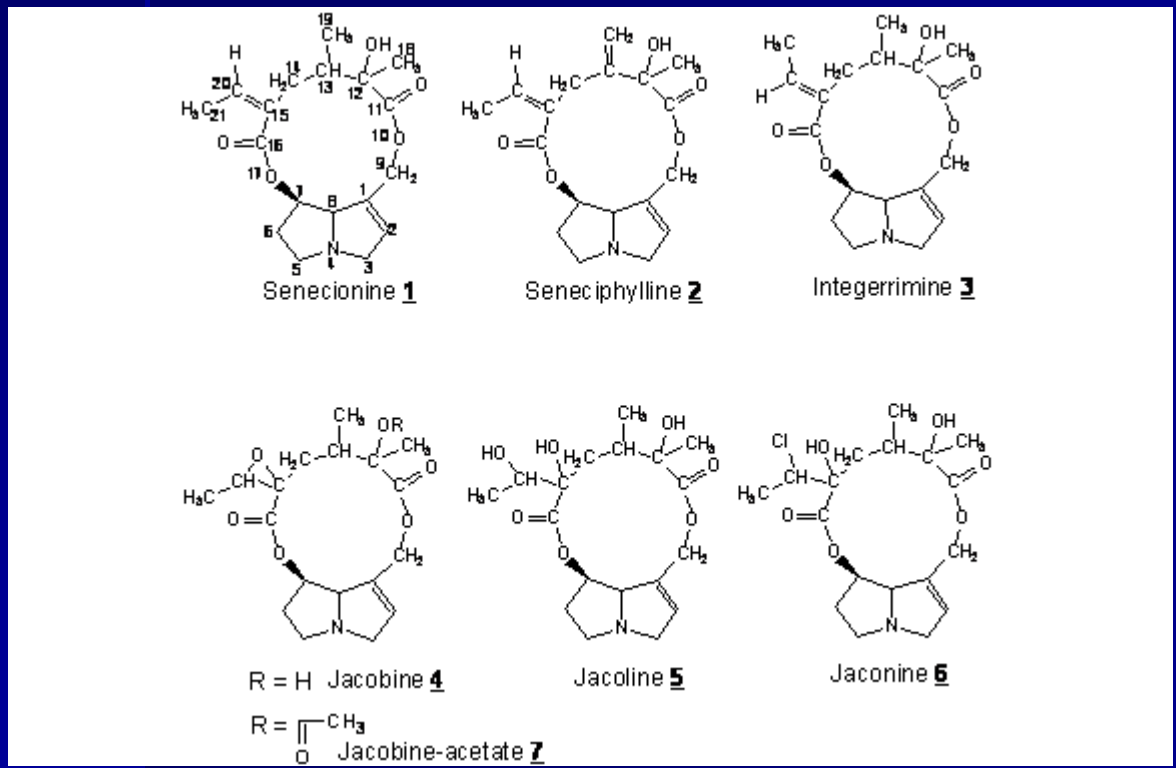
Name	R_1	R_2	R_3
retronecine	H	H	H
lycopsamine	H	III	H
intermedine	H	IV	H
7-acetyllycopsamine	CH_3CO	III	H
7-acetylintermedine	CH_3CO	IV	H
symphytine	I	III	H
symlandine	II	III	H
echimidine	II	III or IV	OH
uplandicine	CH_3CO	III or IV	OH



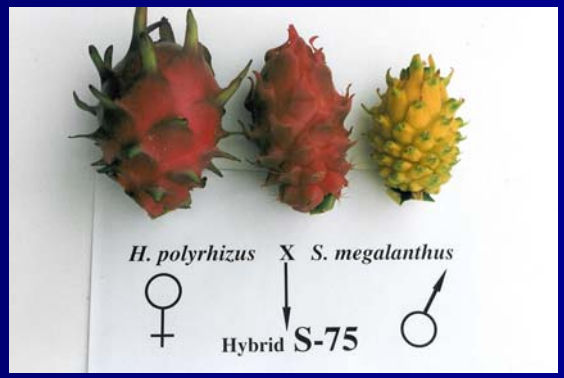
A necine base

Pyrrolizidine Alkaloids- Jacobine and others from *Senecio jacobae*

Jacobine occurs in flowers



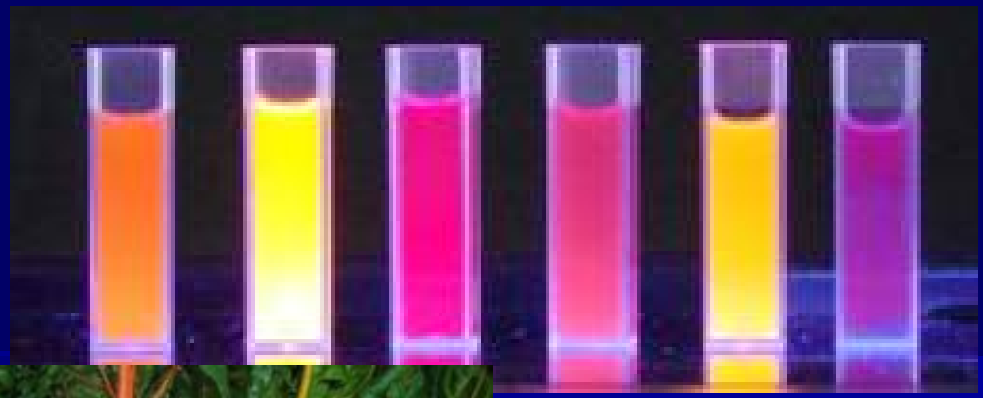
Other Alkaloids- Betalains



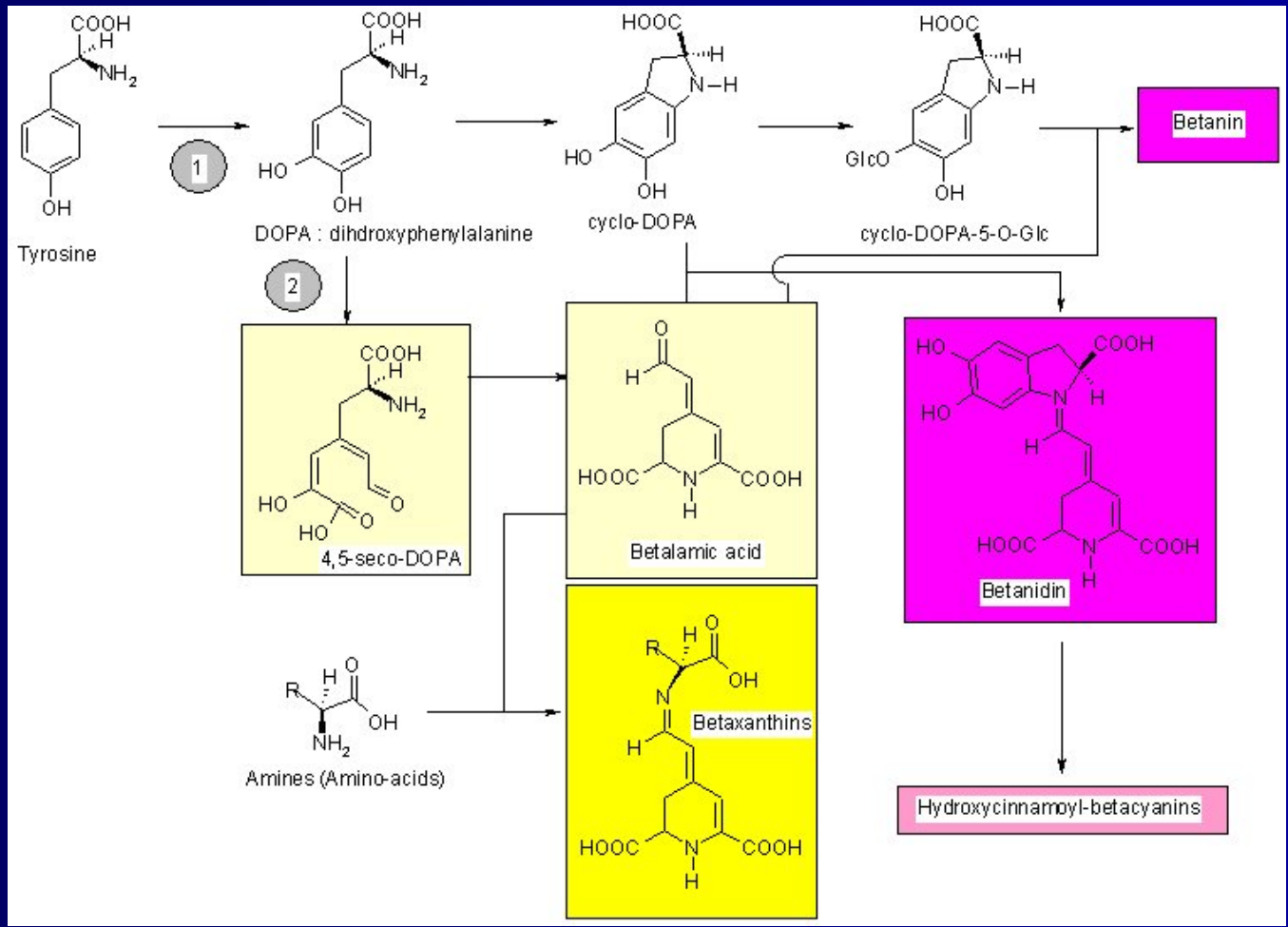
Antioxidants, stable in heat, stable in pH 3 and 7, different colors (bogonvilia, portulaca, celosia, red beet, sabres).

Red Pitaya (Y.Sitrit, BGU)

Other Alkaloids- Betalains

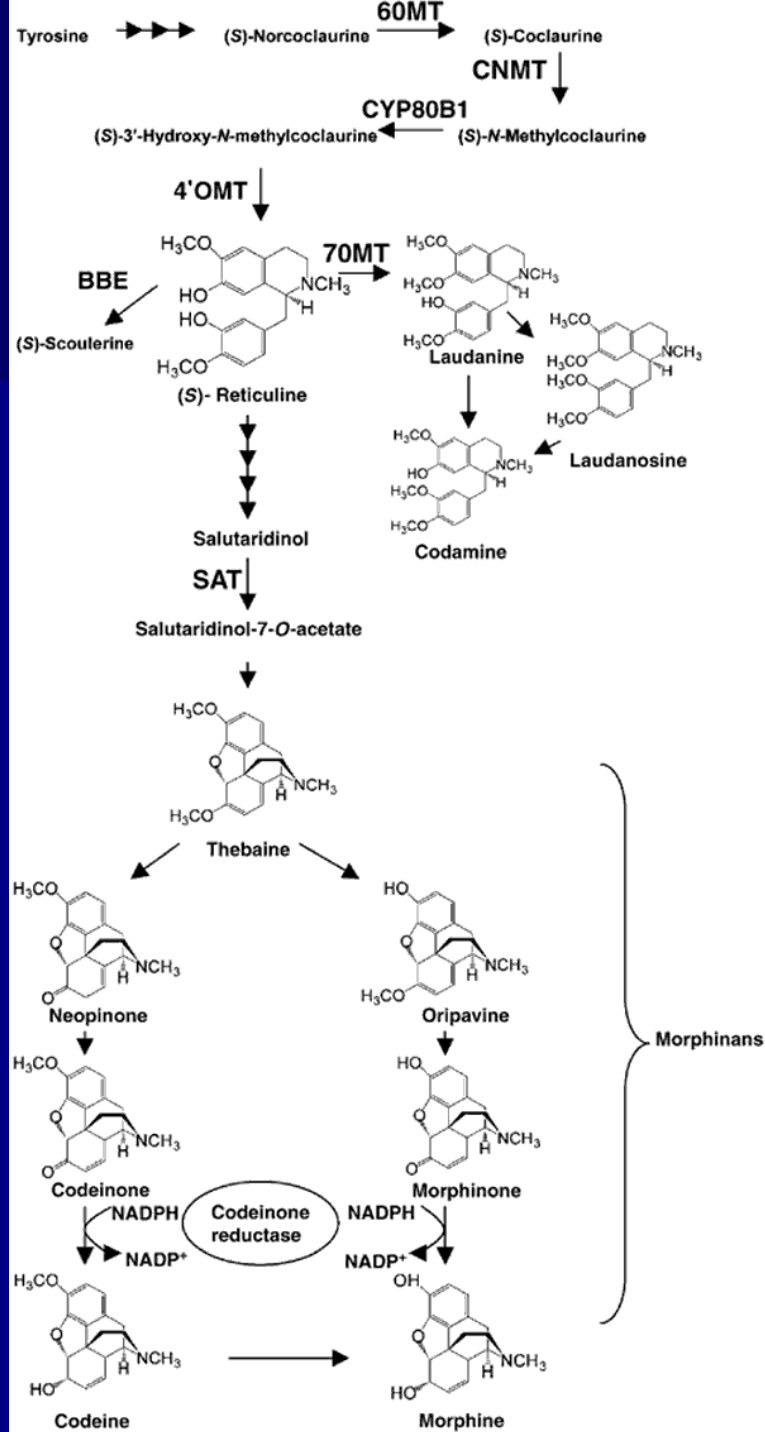


Betalains Biosynthesis

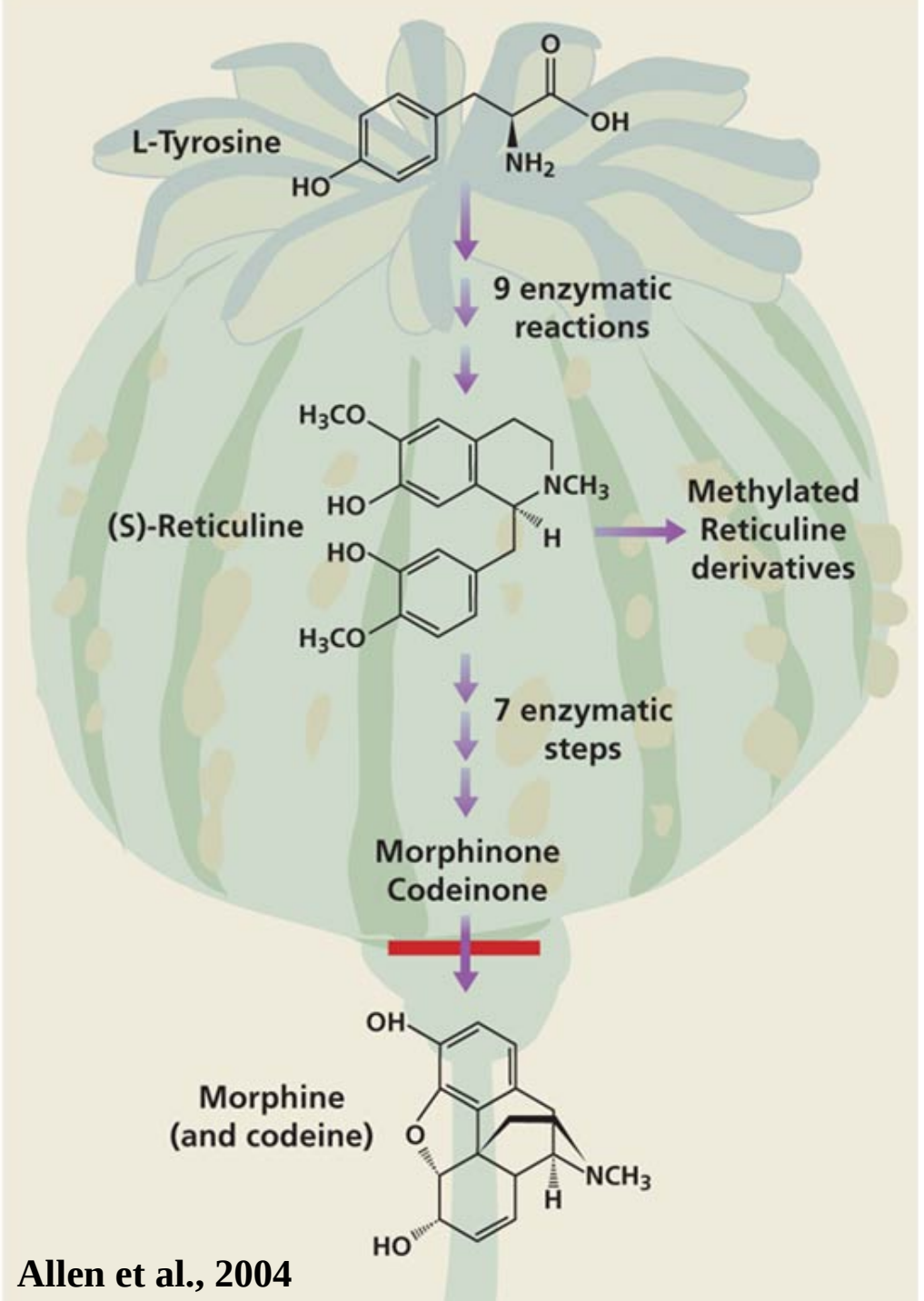


Metabolic Engineering of Opium Poppy

- Block of Codeinone reductase (COR) by RNAi
- Surprise, no Morphinans
- Yes, Reticuline and derivatives.
- Reticulin is non narcotic



Metabolic Engineering of Opium Poppy



Allen et al., 2004