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Chapter 6-2
The Mevalonic Acid Pathway
The Terpenes

Elisabeth Jacobsen and Lucas Boquin, NTNU

Spring 2022

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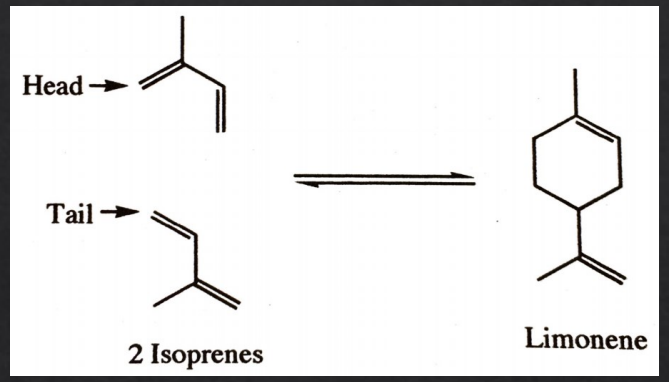
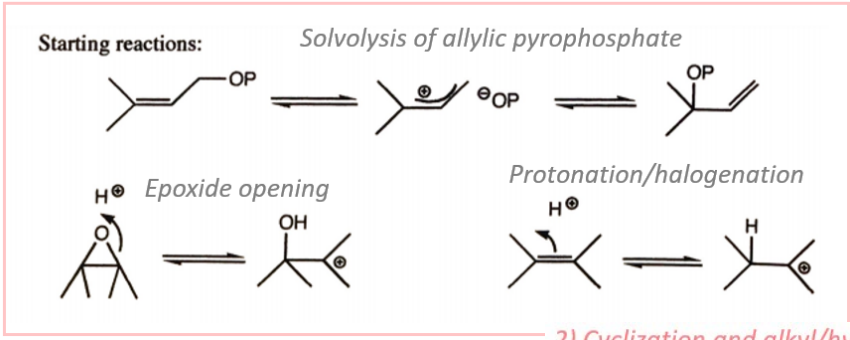
Previously…

- Terpenes can be broken down into C_5 units called isoprenes

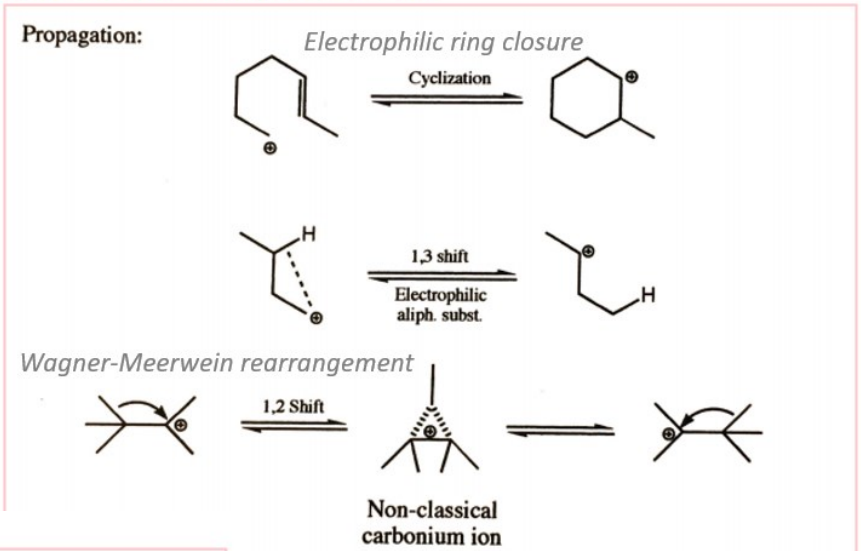


Hemiterpenes	C_5
Monoterpenes	C_{10}
Sesquiterpenes	C_{15}
Diterpenes	C_{20}
Sesterterpenes	C_{25}
Triterpenes	C_{30}
Tetraterpenes	C_{40}
Polyterpenes	C_5 x2000

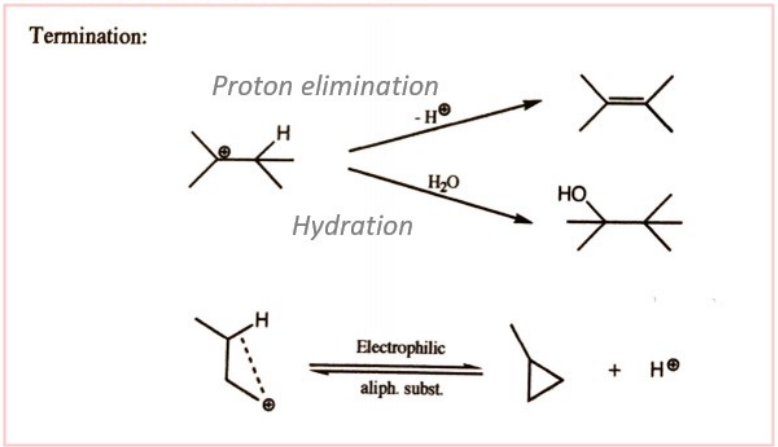
1) Generation of carbenium ion



2) Cyclization and alkyl/hydride shifts

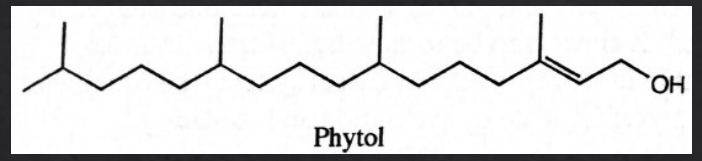


3) Removal of the positive charge



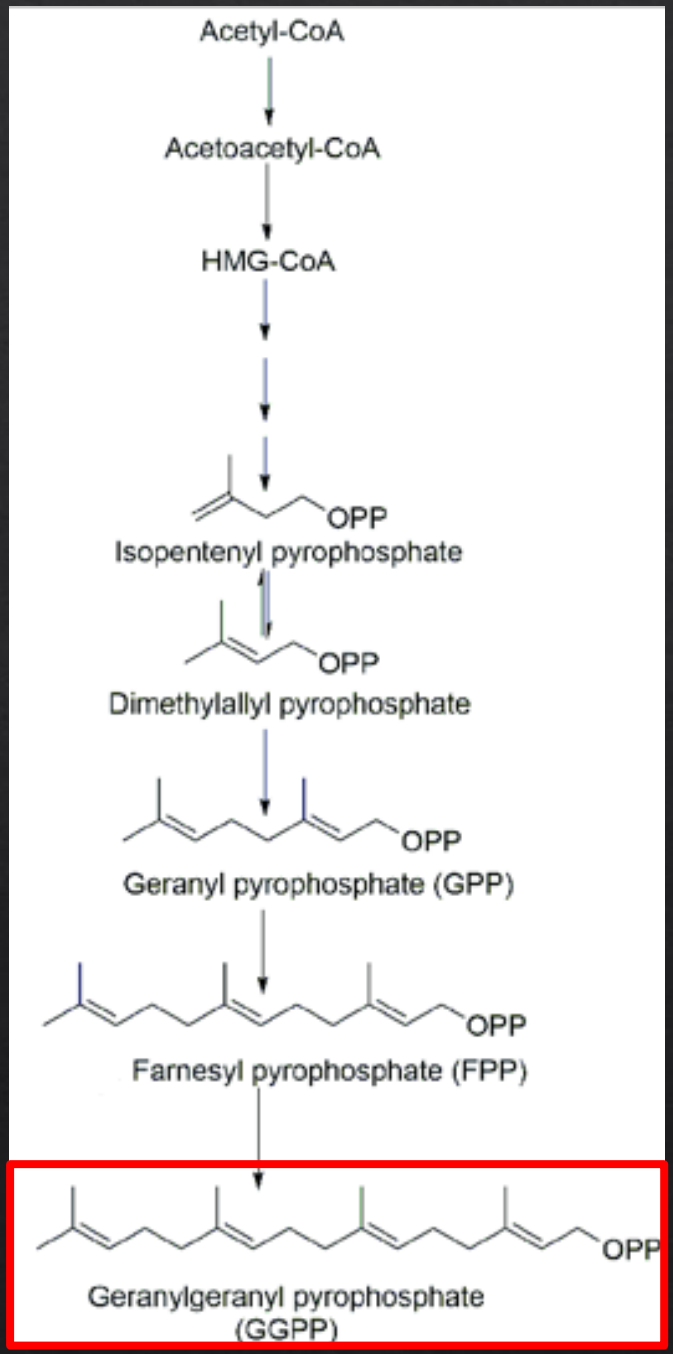
Diterpenes (C_{20})

- Formed from geranylgeranyl pyrophosphate (GGPP)
- Some linear diterpenes exist:



Phytol

Phytol forms the lipophilic side chain of chlorophyll in plants.



Diterpenes (C_{20})

- Formed from geranylgeranyl pyrophosphate (GGPP)
- Some linear diterpenes exist, but most of the diterpenes are mono- (rare), di-, tri- and tetracyclic derivatives.

Sclareol is present in *Salvia sclarea*. It is used in the perfume industry, and in the treatment of leukemia

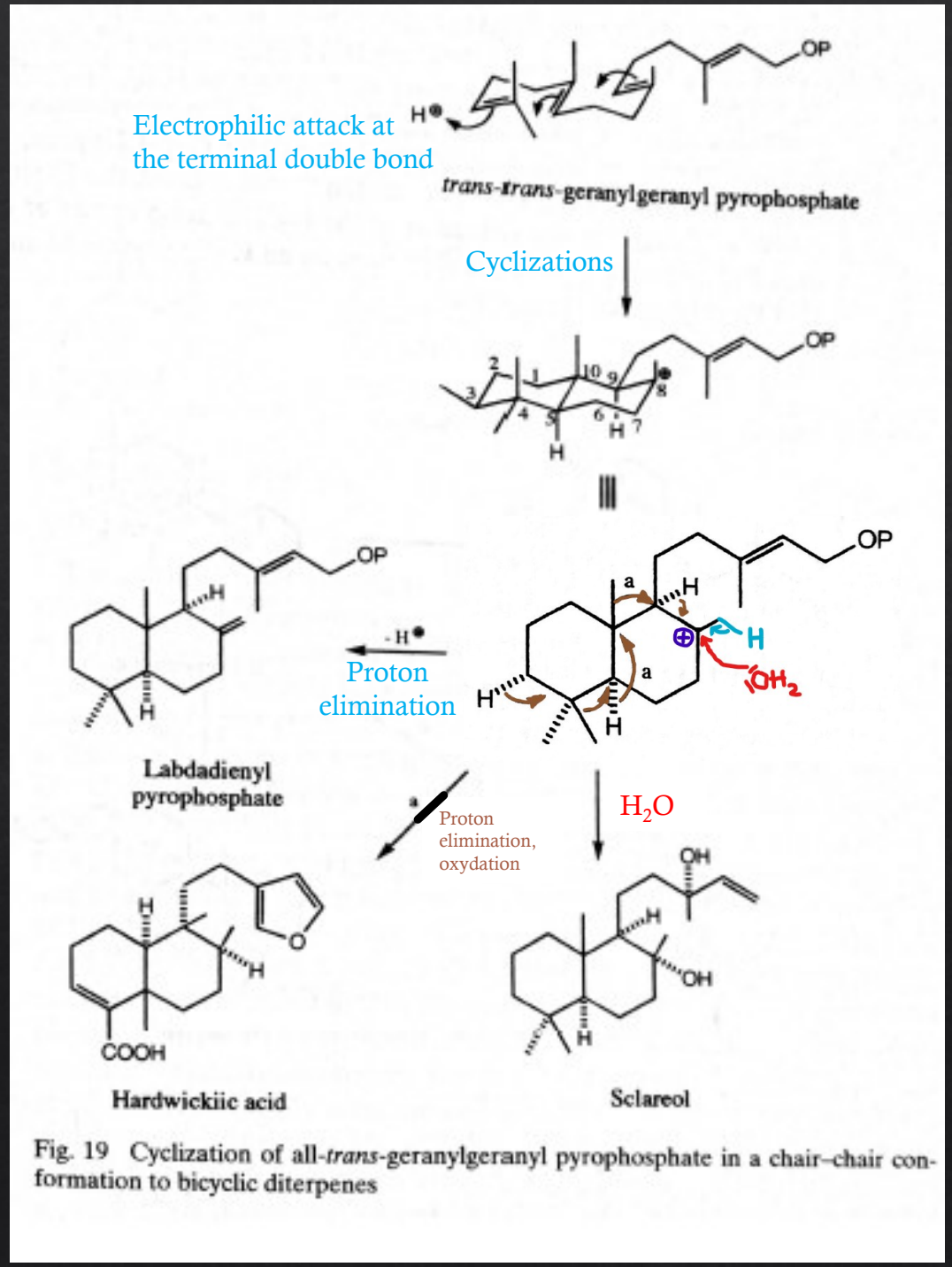


Fig. 19 Cyclization of all-*trans*-geranylgeranyl pyrophosphate in a chair–chair conformation to bicyclic diterpenes

Diterpenes (C_{20})

- A lot of tricyclic diterpenes are formed from the solvolysis of labdadienyl pyrophosphate.

Abietic acid is a widely distributed molecule in *Coniferae*. It is used, among others, in soaps and in lacks.

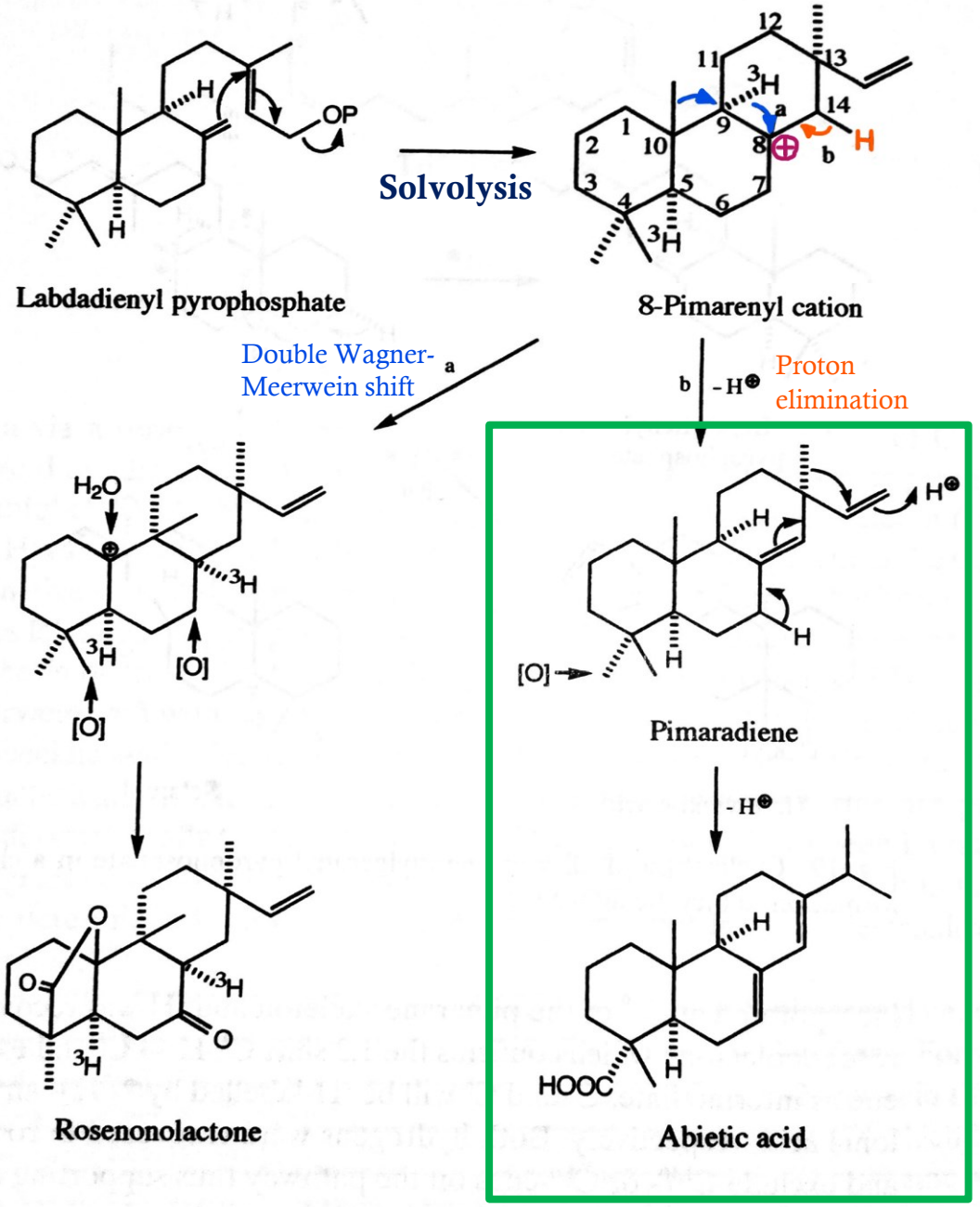


Fig. 20 Biosynthesis of tricyclic diterpenes

Diterpenes (C_{20})

- Tetracyclic diterpenes can also be formed from labdadienyl pyrophosphate. A lot of them are used as hormones.
- Finally, macrocyclic diterpenes also exist.
  - In nasutiterme termites, cambrenes serve as scent-trail pheromones.
  - Certain cambrenes have tumor inhibitor effects

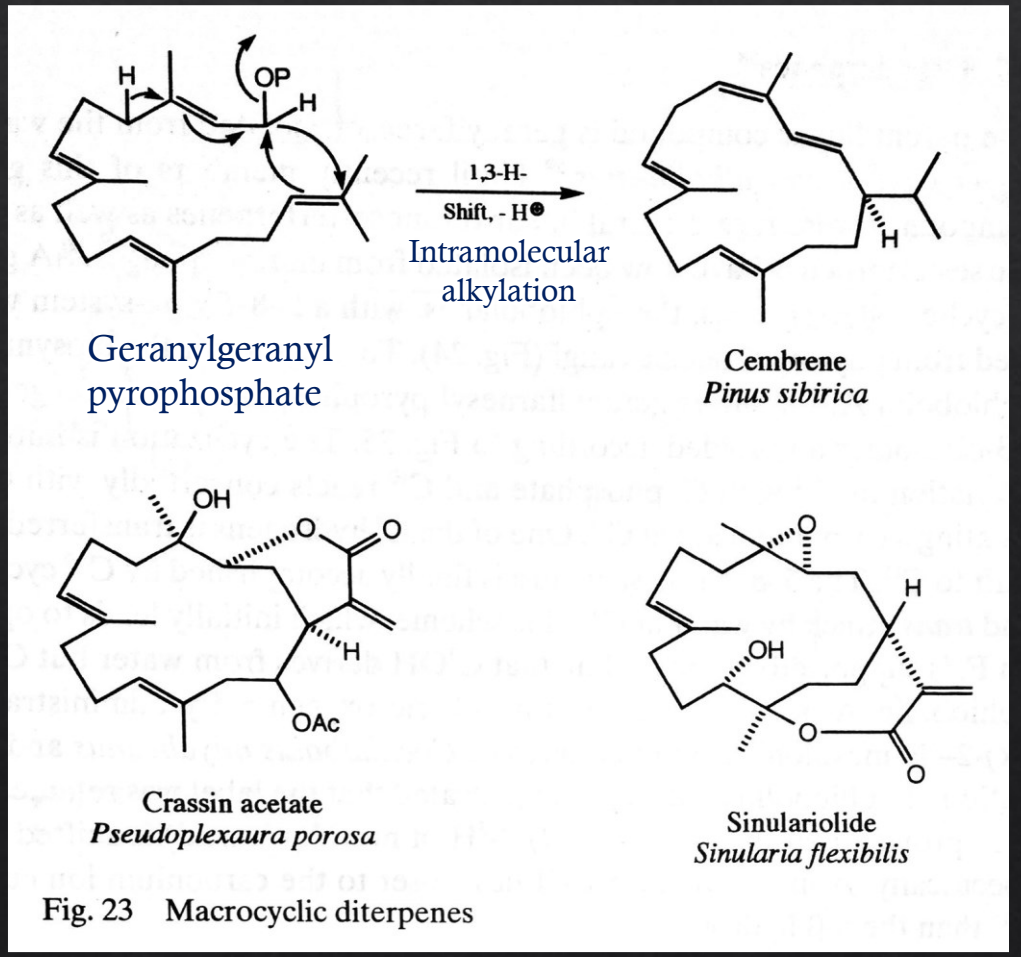
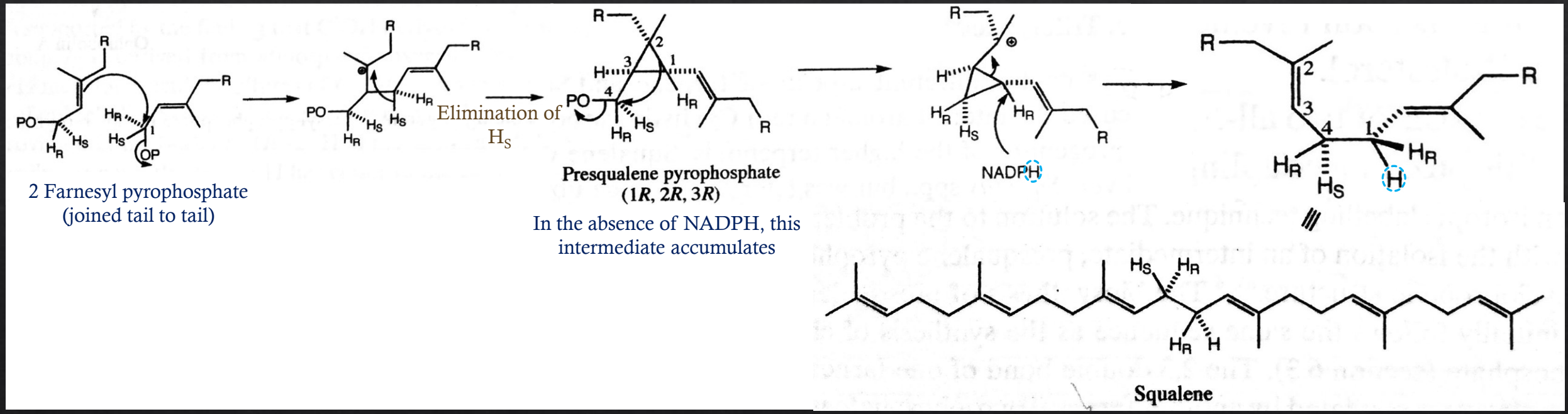


Fig. 23 Macrocyclic diterpenes

Squalene (Triterpenes, C_{30})

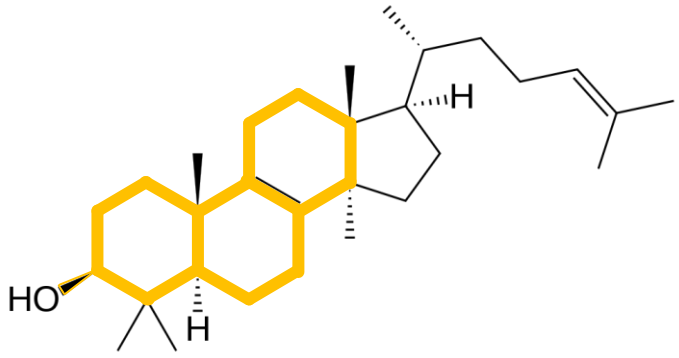
- Squalene is a rare C_{30} hydrocarbon that is thought to be the precursor of a lot of higher terpenoids. It is, for instance, an intermediate for the biosynthesis of cholesterol.



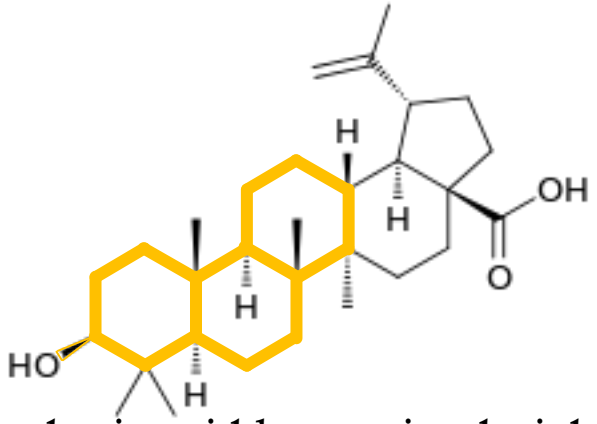
- Fording squalene then allow the formation of a lot of triterpenes.

Triterpenes, C_{30}

- Triterpenes have few skeletal variations; they have usually three 6-membered rings, and an equatorial hydroxy group on the first ring.
- They can be divided into two groups: tetracyclic triterpenes and pentacyclique triterpenes.



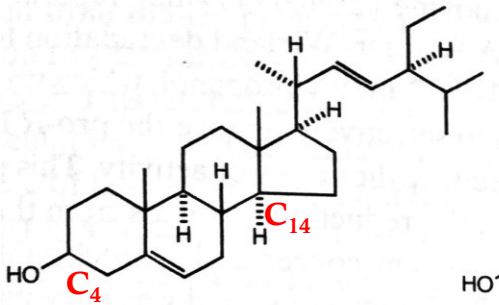
Lanosterol is the precursor of all steroids produced by animals and fungi



Butelonic acid has antimalarial, anti-inflammatory, and potentially anticancer properties

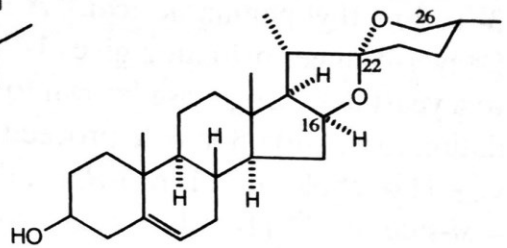
Steroids

- Most of steroids come from squalene, but have lost the carachteristic squeleton of terpenes because of extensive degradation (oxidative cleavage, ring openings, wagner-meerwein shifts, introduction of additional hydroxyl and olefinic groups,…)
- The fundamental secondary modification leading to steroids is selective C_4 and C_{14} demethylation.



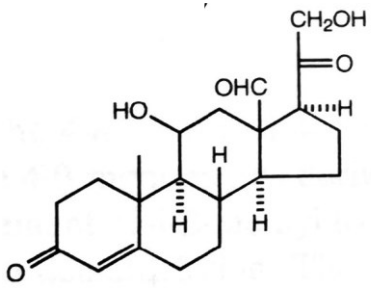
Stigmasterol (Phytosterol)

Sterols are essential structural component of cell membranes (like cholesterol in animals)



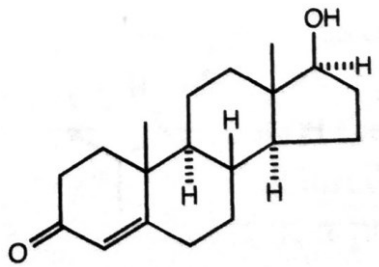
Diosgenin
(Sapogenin)

Sapogenin can protect plants against microbes, fungi, and other organisms



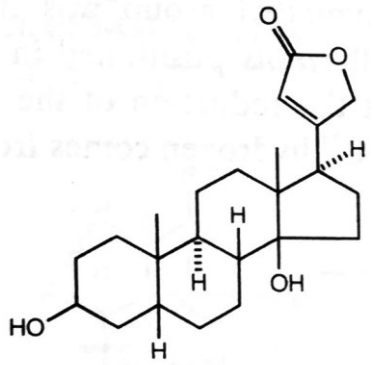
Aldosterone (Adrenal steroid)

Adrenal steroids are produced by the adrenal glands. They regulate the metabolism of sugars and proteins, and the water level in the kidney. A lot of them are involved in stress response processes.



Testosterone
(Male sex hormone)

In both sexes, testosterone plays an important role in health and well-being. Women have less of this hormone than men, but are more sensitive to it.



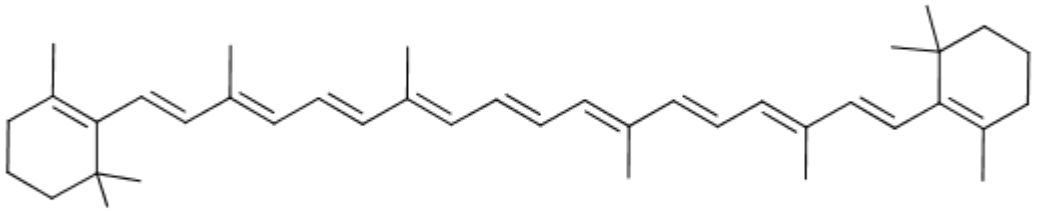
Digitoxigenin
(Cardiac aglycone)

Cardiac glycosides have powerful and specific actions on the heart muscle. They are used in heart ailment treatments.

Carotenes (C_{40})

- Their biosynthesis is similar to the one of squalene: Tail-to-tail coupling of two pyrophosphate (here: geranylgeranyl pyrophosphate).
- They are yellow-red conjugated polyene pigments, and are present in egg yolks, carrots, tomatoes, yellow automn leaves, algae,…

During the autumn, the first pigment in leaves to degrade is chlorophyll. The main remaining pigments (carotenes), give the characteristic yellow color to the leaves.



β-Carotene is transformed through oxidative fission into vitamin A (retinol) and retinal, which play important role in vision.

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Chapter 6-1
The Mevalonic Acid Pathway
The Terpenes

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

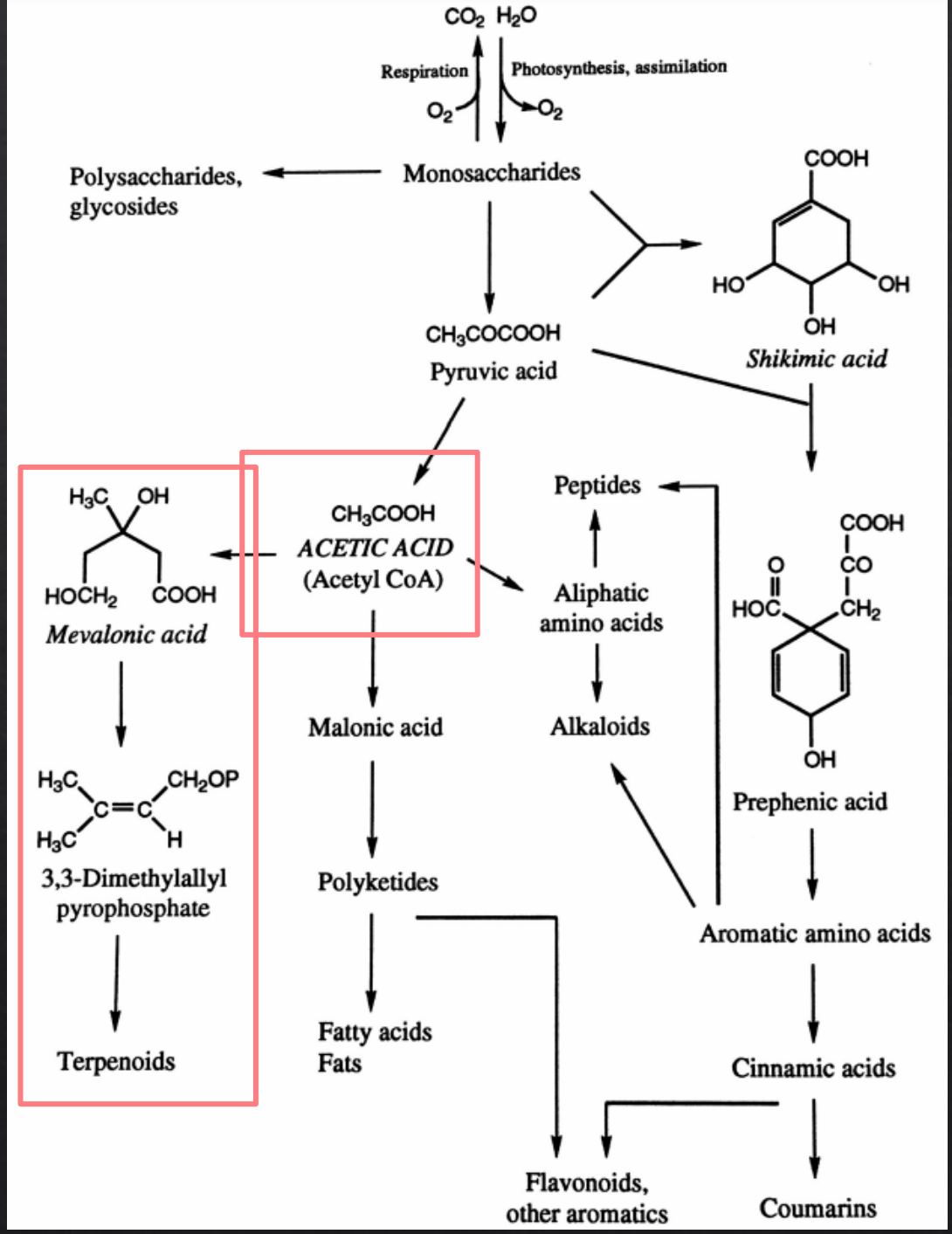
Spring 2022

Mevalonic acid pathway is present in most organisms

Gives the precursors for different end products in each organism

- Animals (steroid hormones, cholesterol)
- Plants (terpenes, terpenoids)

Most organisms produce terpenes through the Mevalonic acid pathway, but there is also an alternative non-mevalonic acid pathway (that we will not discuss).



Terpenes

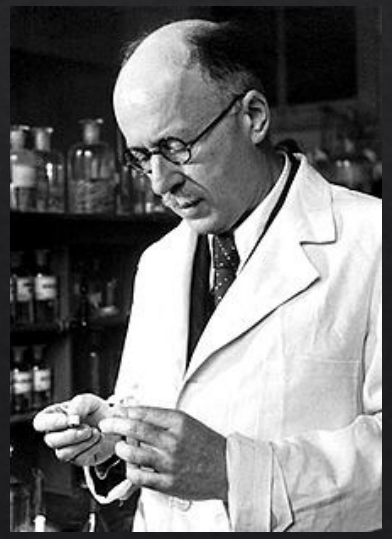
- Terpenes are secondary metabolites (primarily from plants)
  - $(C_5H_8)_n$ - isoprene (isopentenyl) units
  - Terpenoids (aka. isoprenoids) are terpenes with additional functional groups (typically oxygen)

The name «terpene» comes from the terebinth tree (*Pistacia terebinthus*)

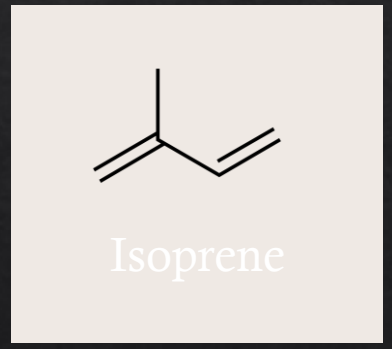
The isoprene rule

Hemiterpenes	C_5
Monoterpenes	C_{10}
Sesquiterpenes	C_{15}
Diterpenes	C_{20}
Sesterterpenes	C_{25}
Triterpenes	C_{30}
Tetraterpenes	C_{40}
Polyterpenes	C_5 x2000

Steroids (C_{27}) do not follow the isoprene rule



Leopold Ruzicka: The Nobel Prize in Chemistry 1939, for his work on terpenes and being the first to synthesize male sex hormones



Applications o[illegible]es

Terpenes function as protective or attractive substances in plants and some animals

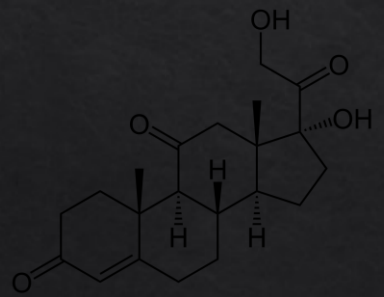
Perfume industry
Essential oils

Painting industry
Turpentine/terpentine

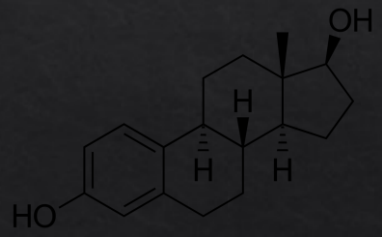
Natural rubber
Polyisoprene

Some thermites fire glue-like, toxic terpene mixtures at predators from «guns» on their head

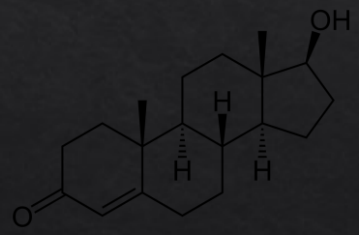
J. Sobotník et al. (2010) Journal of Insect Physiology 56 1012–1021



Cortisone

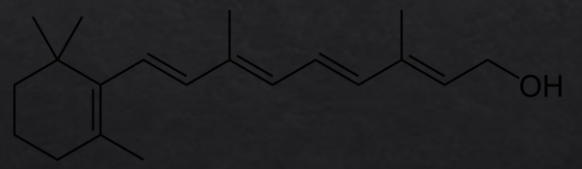


Estrogen

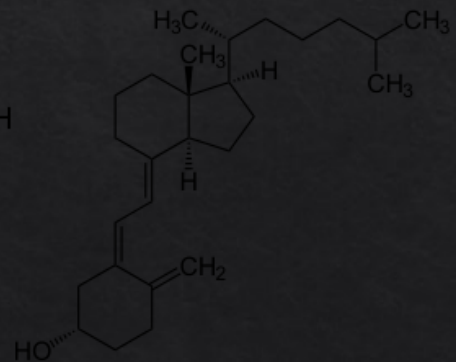


Testosterone

Hormones



Retinol (vitamin A)



Cholecalciferol (vitamin D3)

Vitamins

SYNTHESIS

ACS Meeting News: Taking Cues From Nature En Route To Taxol

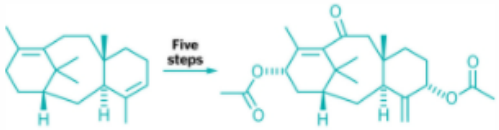
Synthesis of an intermediate along the way to the cancer drug opens avenues to novel analogs

by **Bethany Halford**

MARCH 19, 2014

No one is better than nature at making the cancer drug paclitaxel (Taxol). Cumulatively, chemists have been able to produce only small amounts of the stuff, but a plant cell fermentation process can churn out metric tons of the compound.

[+]Enlarge

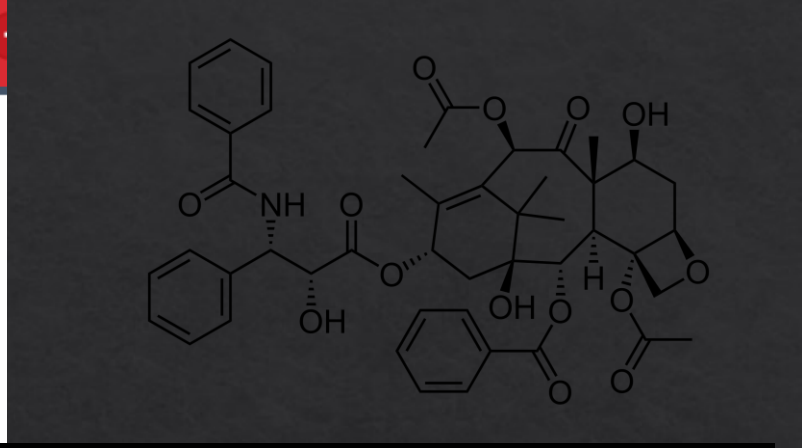


Synthetic chemists would like to learn from nature. By mimicking the early steps in paclitaxel's biosynthesis, a team at Scripps Research Institute, La Jolla, Calif., has potentially come up with a way to create analogs of paclitaxel that are unavailable via bioengineering. These could turn out to be powerful drugs as well. **Phil S. Baran**, who spearheaded the research, spoke about the work on Tuesday at the American Chemical Society meeting in Dallas, in the Division of Organic Chemistry.

First, Baran's team synthesized the natural product (−)-taxuyunnanine D in just five steps from taxadiene. The transformation mimics the first three of eight oxidations that occur biosynthetically when taxadiene is converted to paclitaxel. Taxuyunnanine D, Baran said, could ultimately be used as an intermediate en route to paclitaxel.

The challenge for Baran's group was to control the order of the three oxidations. It's a tough task, Baran explained, because taxadiene is a strained, doubly unsaturated hydrocarbon that is "spring loaded" for oxidation at several spots at once. Through computational modeling, developing a seldom-used chromium reagent, and conducting hundreds of reactions, Baran's team executed the early steps of paclitaxel's biosynthesis (*J. Am. Chem. Soc.* 2014, DOI: **10.1021/ja501782r**).

Chem. & Eng. News March 19., 2014, ISSN 0009-2347



Taxol (paclitaxel) was typically derived from the bark of the tree «Pacific yew».

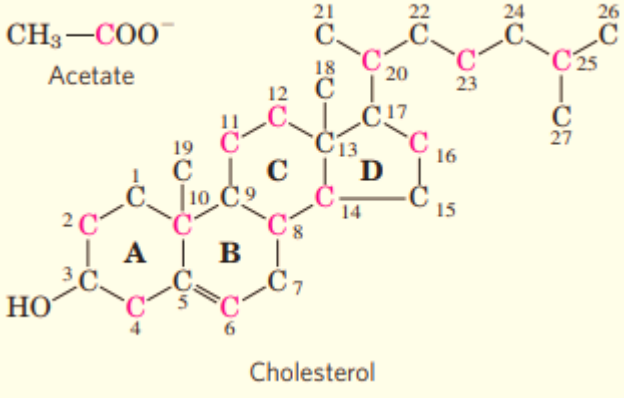
One of the most popular naturally derived cancer drugs, for treating primarily breast cancer.

Desirable to make it from more renewable sources, because the trees die when the bark is harvested.
Now: biosynthetic routes are becoming more popular.

Cholesterol structure

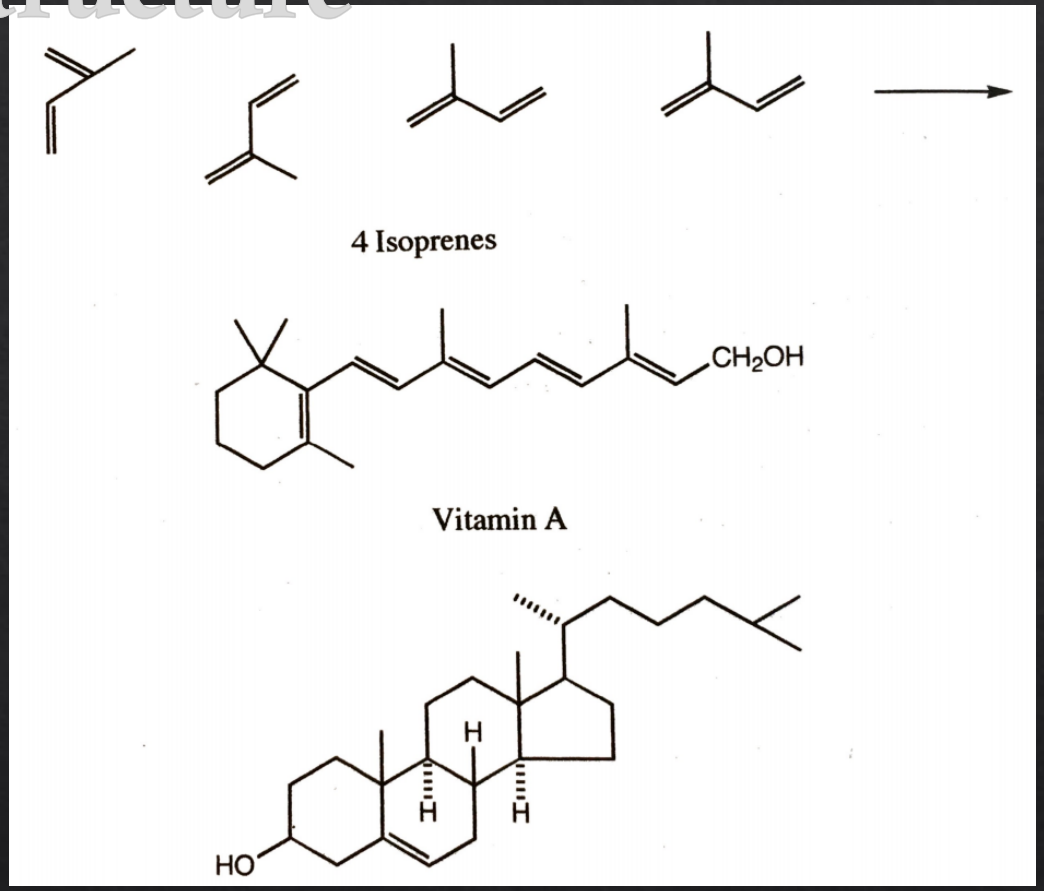
Many terpenes are not UV active
UV spectroscopy did not work well

Cholesterol
- Structure defined in 1932
    - XRD



Lehninger Principles of Biochemistry, 6th ed. 2013

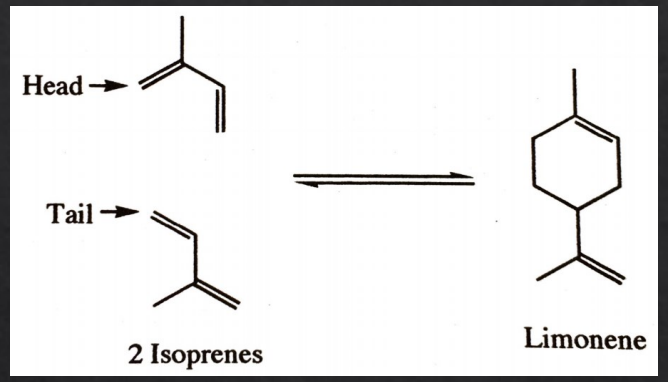
Origin of carbon atoms in cholesterol



Cholesterol

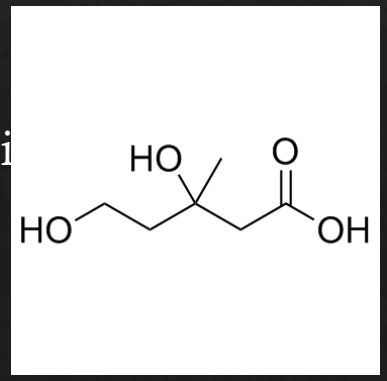
Cholesterol-lowering drugs (statins) inhibit the mevalonic acid pathway

Mevalonic acid p



Limonene can be synthesized by a Diels-Alder reaction of two isoprene units, but isoprene is not the starting material in biosynthesis of limonene and other terpenes.

In 1956, mevalonic acid was isolated, and i was shown how mevalonic acid can be a building block in terpene biosynthesis

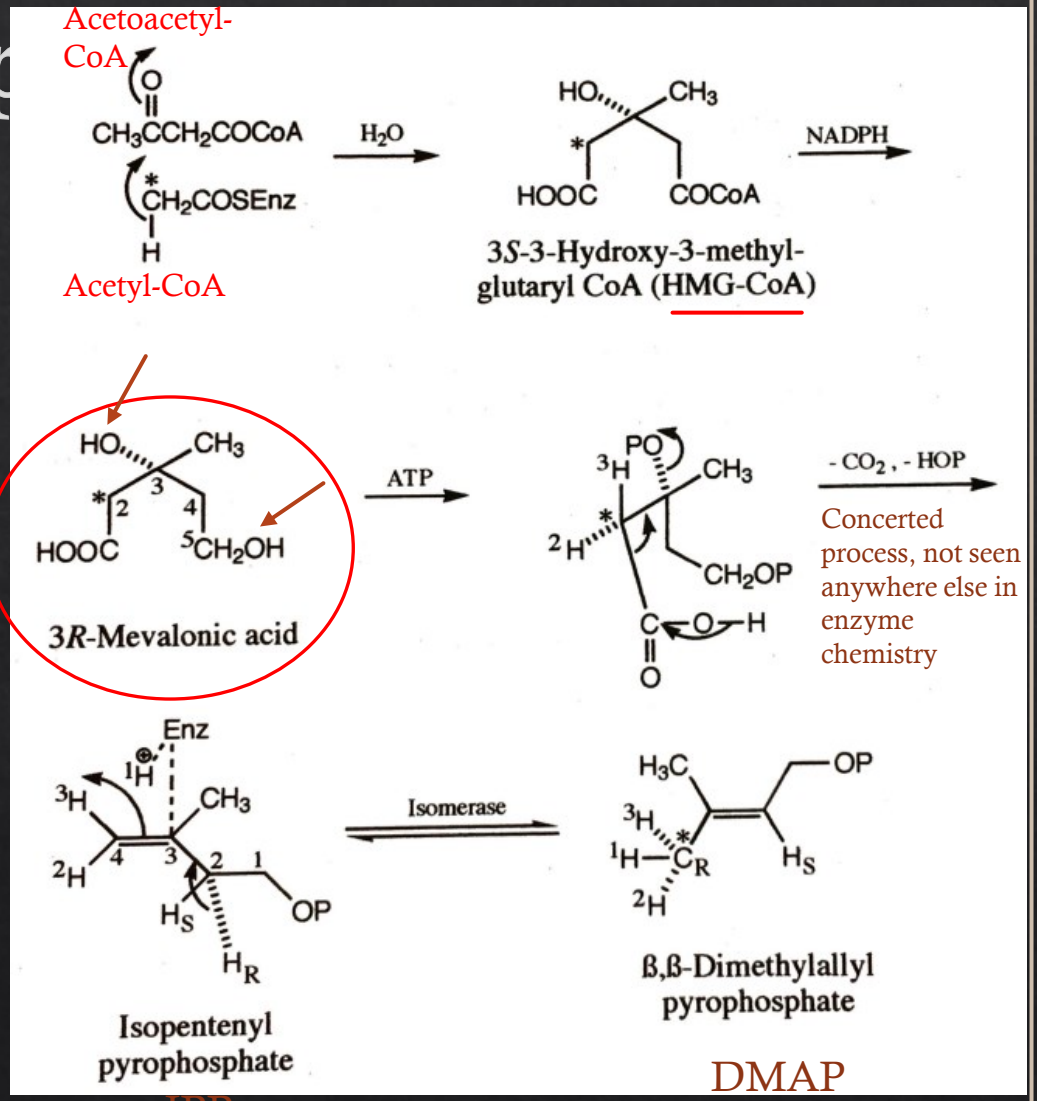


First part is the same in all organisms

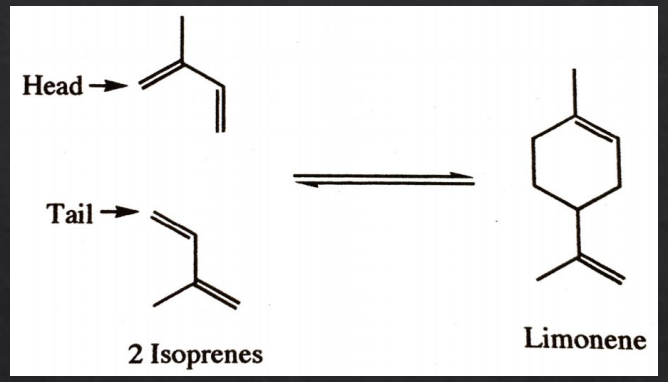
Second part happens differently in eukaryotes and archaea (here: archaea)

End products are the same

Biosynthesis of starter units of terpenes: DMAP/IPP



Mevalonic acid p



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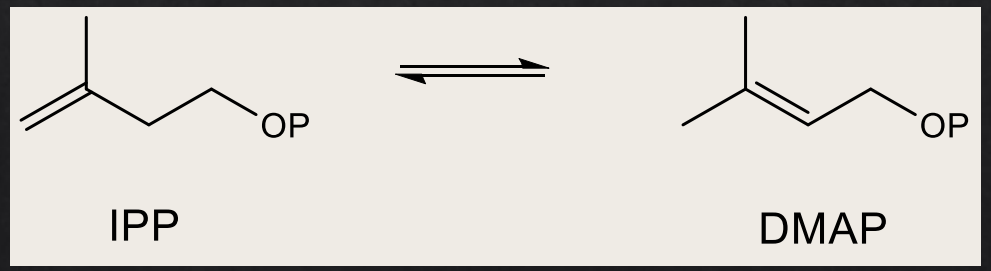
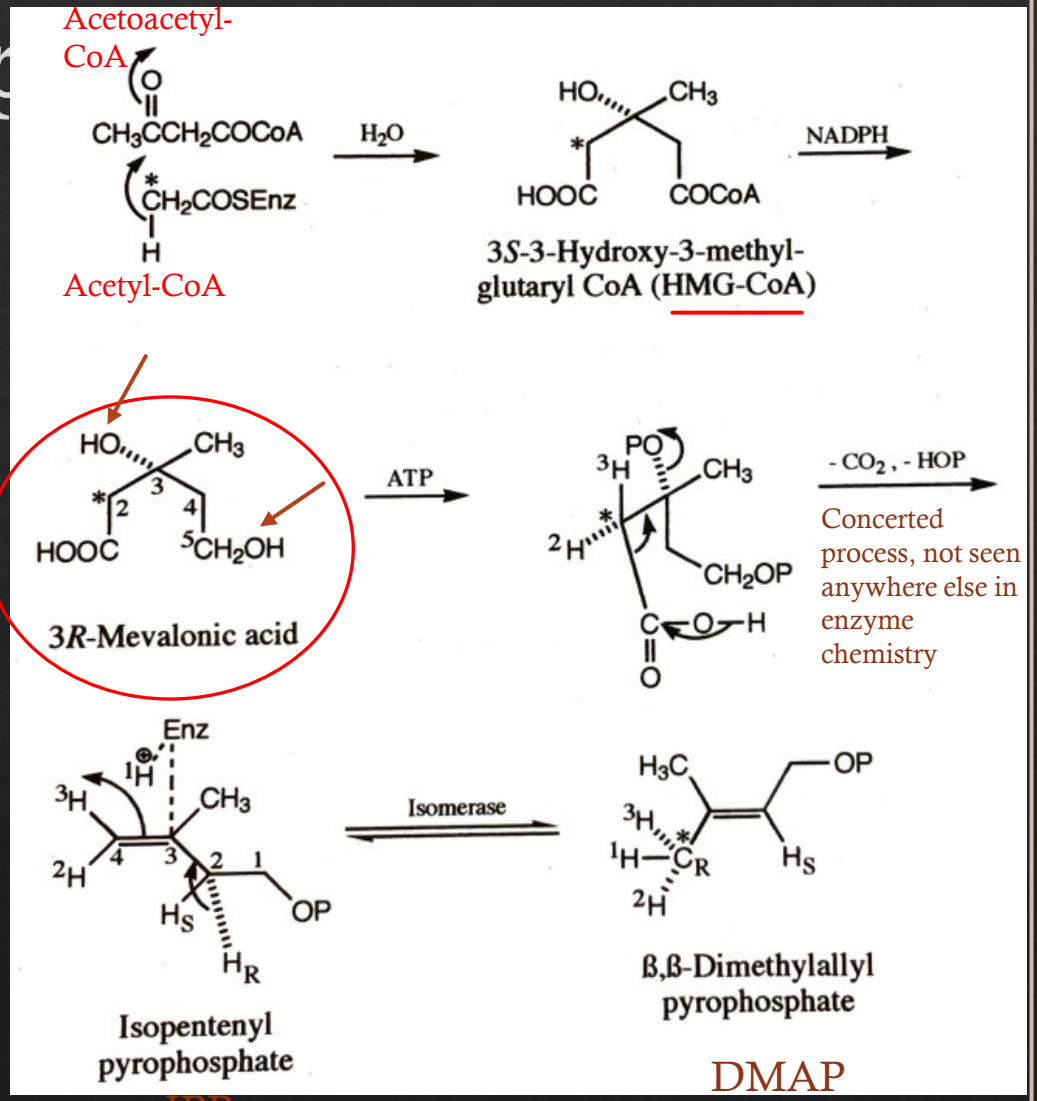
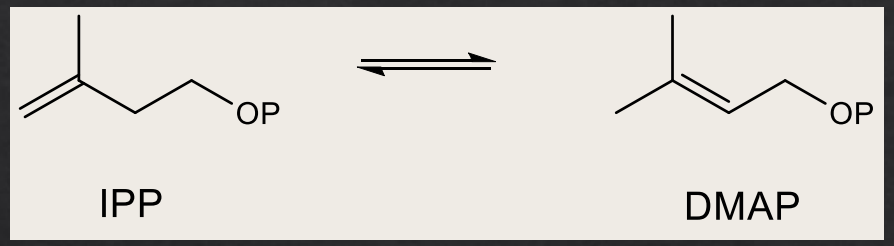


«Activated isoprenes»

Biosynthesis of starter units of terpenes: DMAP/IPP



Biosynthesis of terpenes

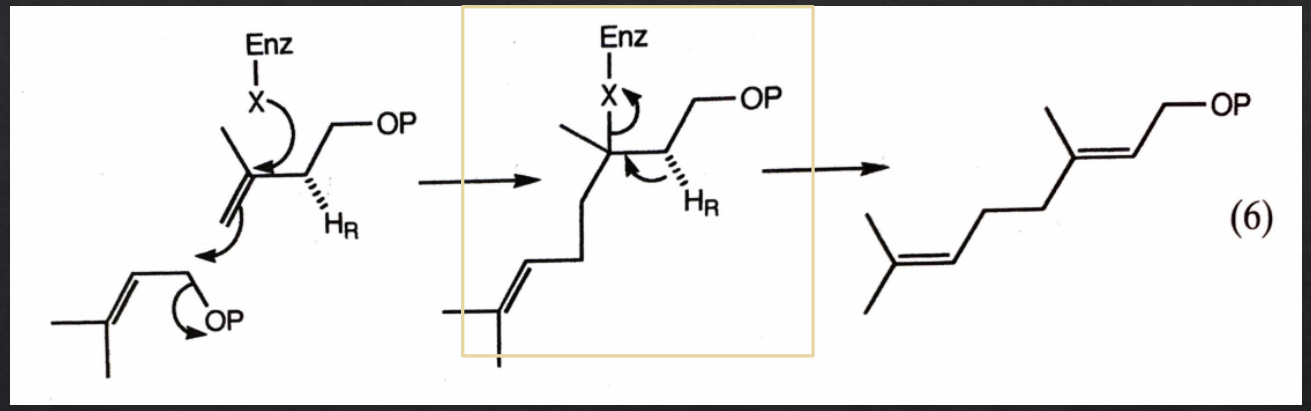


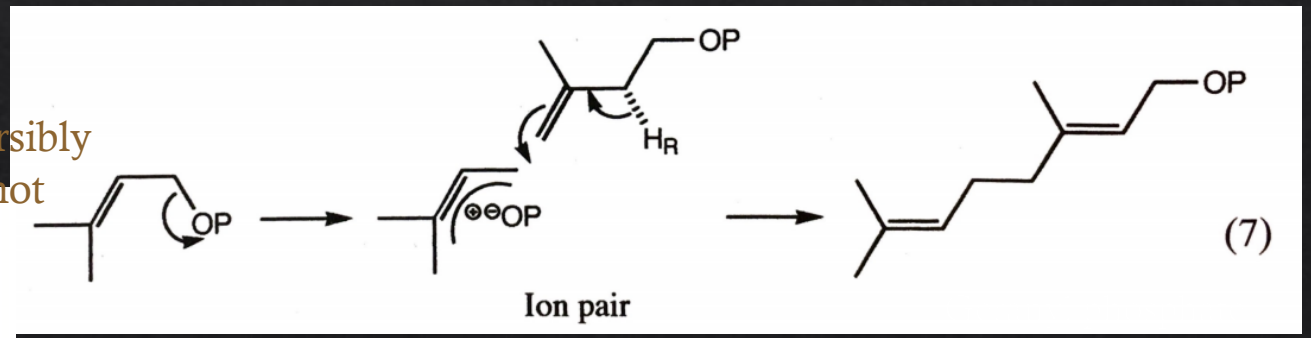
«Activated isoprenes»

Trans-1,2-addition, and *trans*-1,2-elimination – enzyme functions as a nucleophile

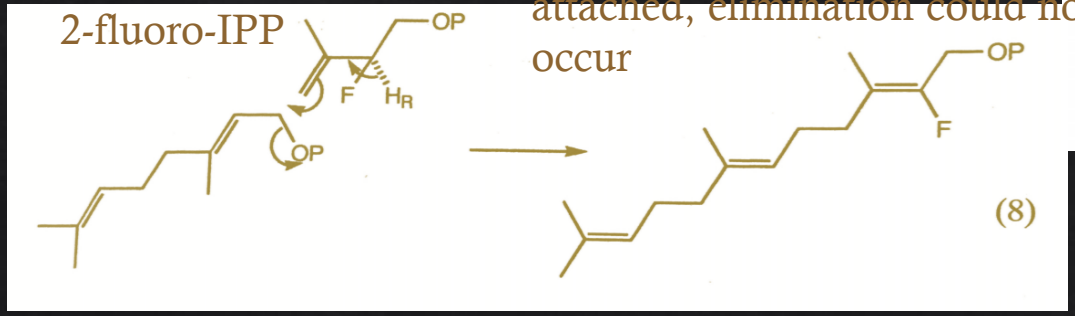
Ionization of DMAP, and addition of IPP to the allylic cation

Two proposed mechanisms for further reaction between IPP and DMAP:





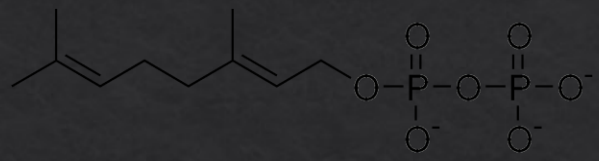
2-fluoro-IPP would be irreversibly attached, elimination could not occur



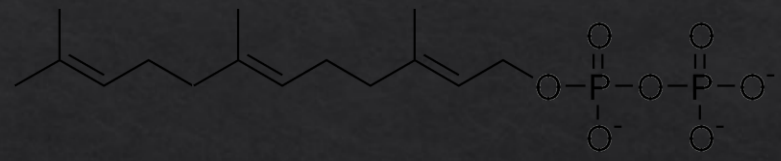
Enzyme activity was not irreversibly lost

4 phases of terpene biosynthesis

1) Synthesis of IPP and DMAP (the mevalonic acid pathway)
2) Linear polymerization of the isoprene units
   - Gives geranyl PP, farnesyl PP, geranylgeranyl PP, etc.
   - (Linear polyprenyl precursors)
3) Folding, cyclization, rearrangement of polyprenyl precursors.
4) Functionalization to create terpenoids

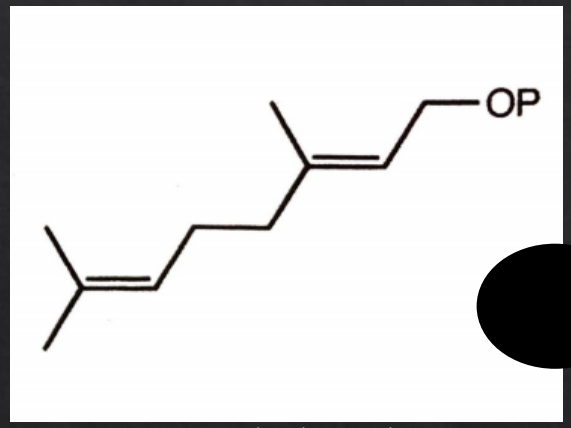


Geranyl pyrophosphate



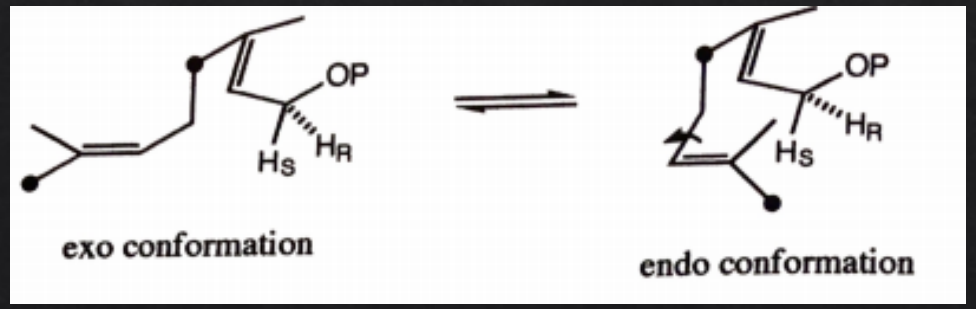
Farnesyl pyrophosphate

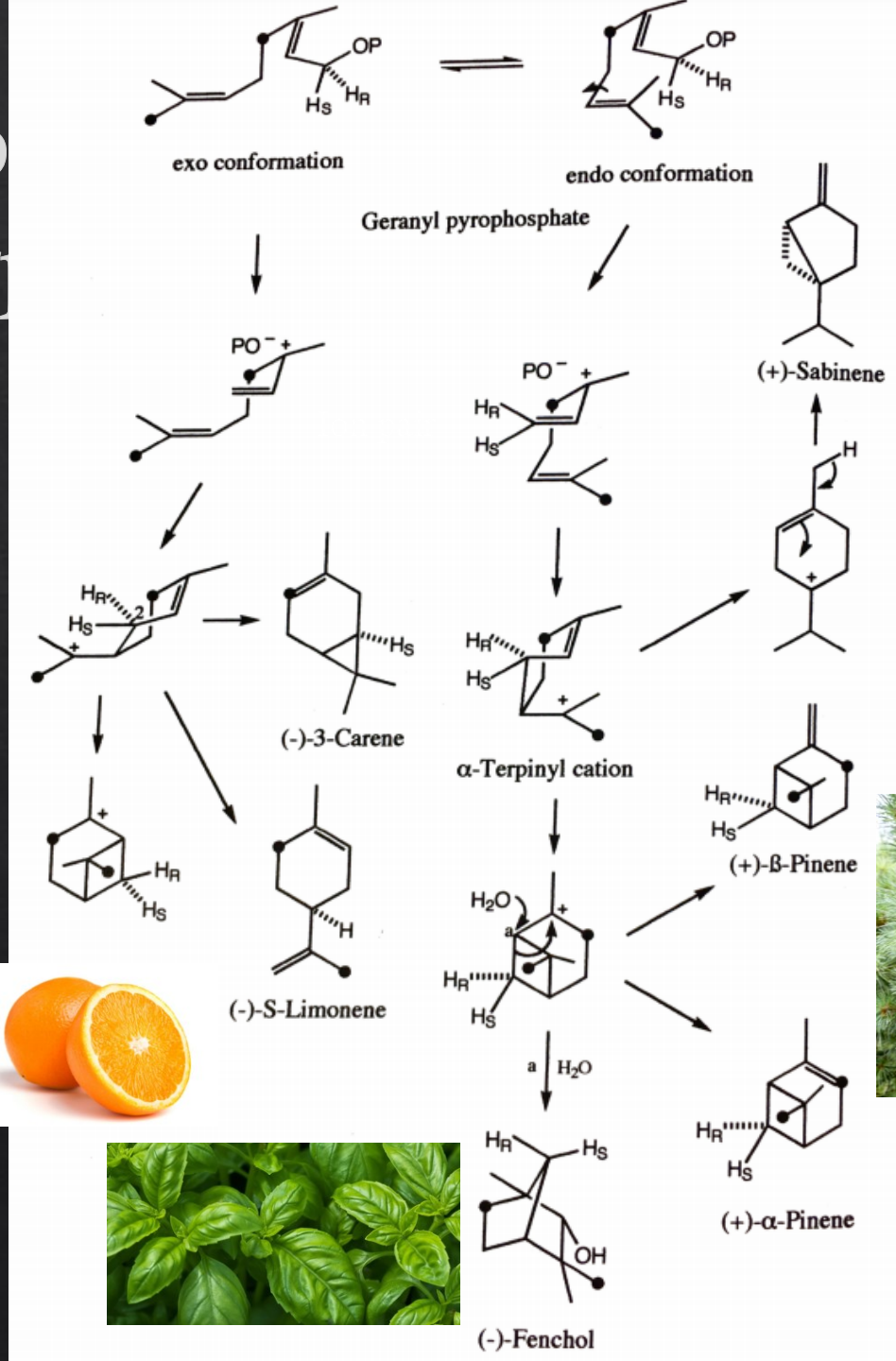
Biosynthesis o
- monoter



Geranyl phosphate
Starting material for monoterpenes

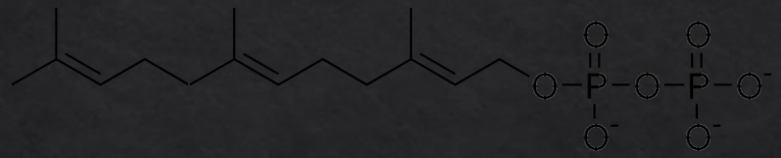
The products from cyclization of geranyl pyrophosphate depend on the enzymes present in the plant



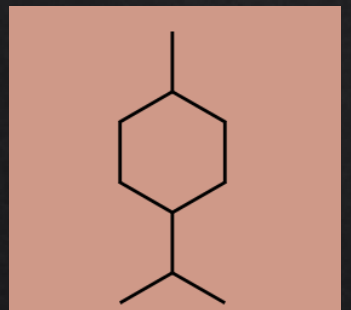


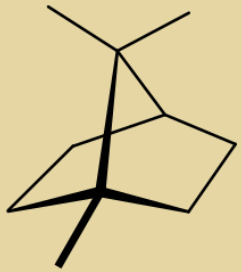
Sesq

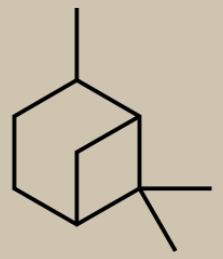
- Longer chain lenght – more structural variety
- Largest group of terpenes
- 50 different carbon skeletons



Farnesyl pyrophosphate = starting material for sesquiterpenes


menthane

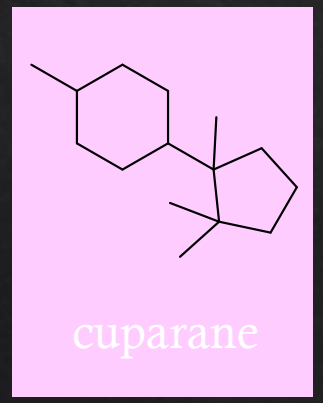

bornane

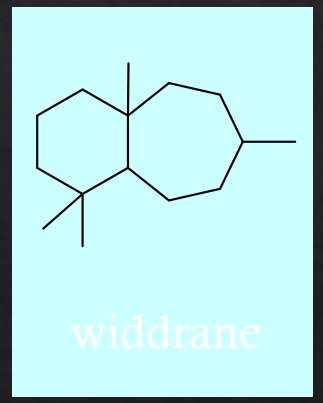

pinane

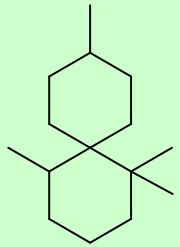

carane

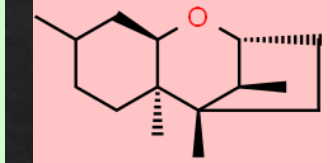
Sesquiterpe

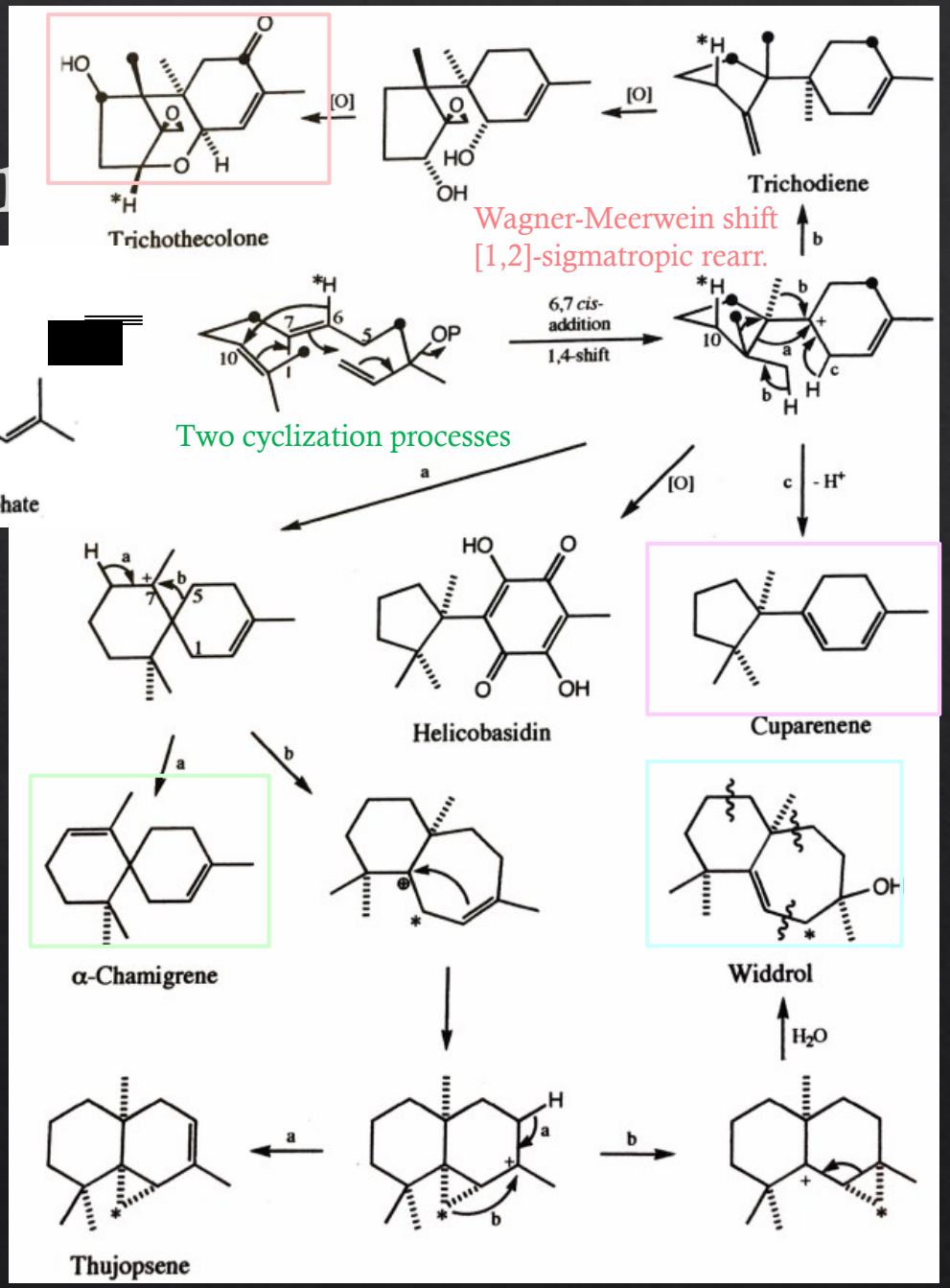
- Hydride and alkyl shifts
  - via carbocation intermediates
  - Gives even more structure variation


cuparane


widdrane


chamigrane


trichothecane



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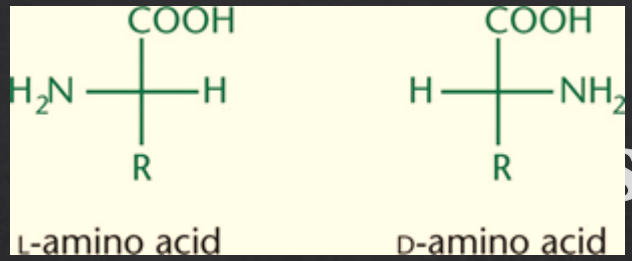
Chapter 7
Amino Acids, Peptides and Proteins

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022



Amino acids contain:
- amino group (-NH_2)
- carboxyl group (-COOH)

Amino acids in proteins are L-α-amino acids

- 20 common amino acids

10 of these are essential t humans

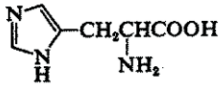
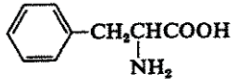
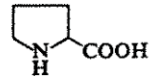
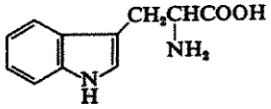
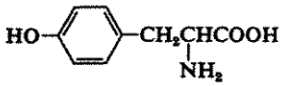
- More complex biosynthesis

300 non-protein amino acids

Table 1 Structure of the commonest amino acids derived from proteins. Name of amine derived by decarboxylation

Amino acid	Structure	Abbreviated symbol	Amine
Alanine N,1	$CH_3CHCOOH$ | NH_2	Ala	Ethylamine
Arginine E,3	$H_2N^{\oplus}=CNH(CH_2)_3CHCOOH$ | NH_2 | NH_2	Arg	Agmaline (4-Guanidobutyl-amine)
Aspartic acid N,4	$^{\ominus}OOCCH_2CHCOOH$ | NH_2	Asp	β-Alanine
Asparagine N,2	$NH_2COCH_2CHCOOH$ | NH_2	Asn	β-Alanyl amide
Cysteine N,2	$HSCH_2CHCOOH$ | NH_2	Cys	2-Mercaptoethyl amine
Glutamic acid N,4	$^{\ominus}OOCCH_2CH_2CHCOOH$ | NH_2	Glu	γ-Aminobutyric acid (GABA)
Glutamine N,2	$H_2NCOCH_2CH_2CHCOOH$ | NH_2	Gln	γ-Aminobutyr-amide
Glycine N,2	CH_2COOH | NH_2	Gly	Methylamine

Table 1 (*continued*)

Amino acid	Structure	Abbreviated symbol	Amine
Histidine E,3		His	Histamine
Isoleucine E,1	$C_2H_5CHCHCOOH$ | | H_3C NH_2	Ile	2-Methylbutyl-amine
Leucine E,1	$(CH_3)_2CHCH_2CHCOOH$ | NH_2	Leu	3-Methylbutyl-amine
Lysine E,3	$H_2N(CH_2)_4CHCOOH$ | NH_2	Lys	Cadaverine, 1,5-Diamino-pentane
Methionine E,1	$CH_sS(CH_2)_2CHCOOH$ | NH_2	Met	3-Methylmercapto-propylamine
Phenylalanine E,1		Phe	Phenylethylamine
Proline N,1		Pro	Pyrrolidine
Serine N,2	$HOCH_2CHCOOH$ | NH_2	Ser	Ethanolamine
Threonine E,1	$CH_3CHCHCOOH$ | | HO NH_2	Thr	2-Hydroxypropyl-amine
Tryptophan E,1		Try	Tryptamine
Tyrosine N,2		Tyr	Tyramine
Valine E,1	$(CH_3)_2CHCHCOOH$ | NH_2	Val	*i*-Butylamine

Requirement by man: E, essential; N, non-essential. Polarity of the chain; 1, non-polar; 2, neutral polar; 3, positively charged; 4, negatively charged

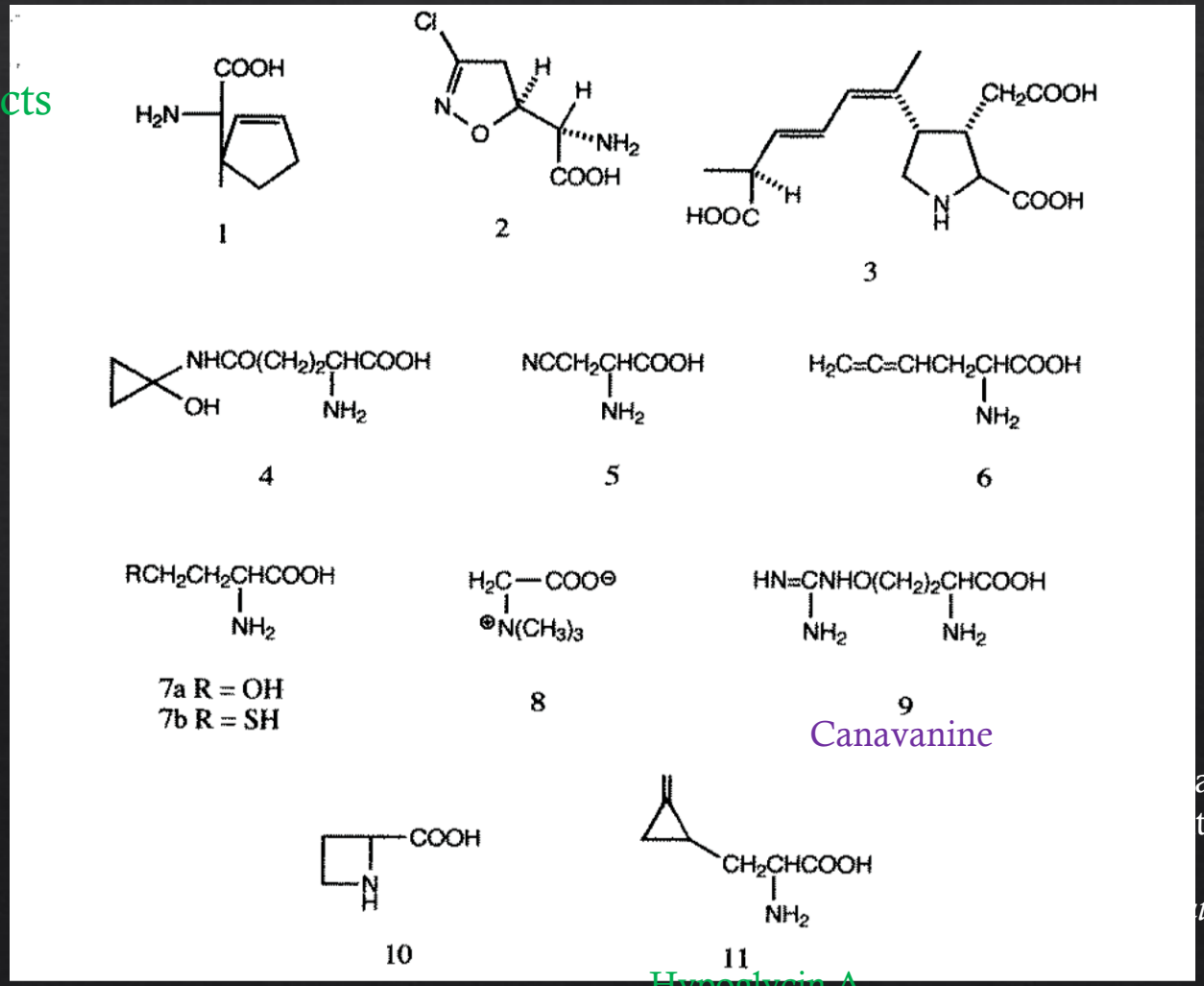
Examples of non-protein amino acids

Blood sugar depressing effects

Blighia sapida
National fruit of Jamaica, poisonous if not cooked properly

Hypoglycin A binds irreversibly to coenzyme A, inhibiting many enzymes

=> Depletes glucose stores



Phytotoxic effects

avanine
ts growth
of
thyrus

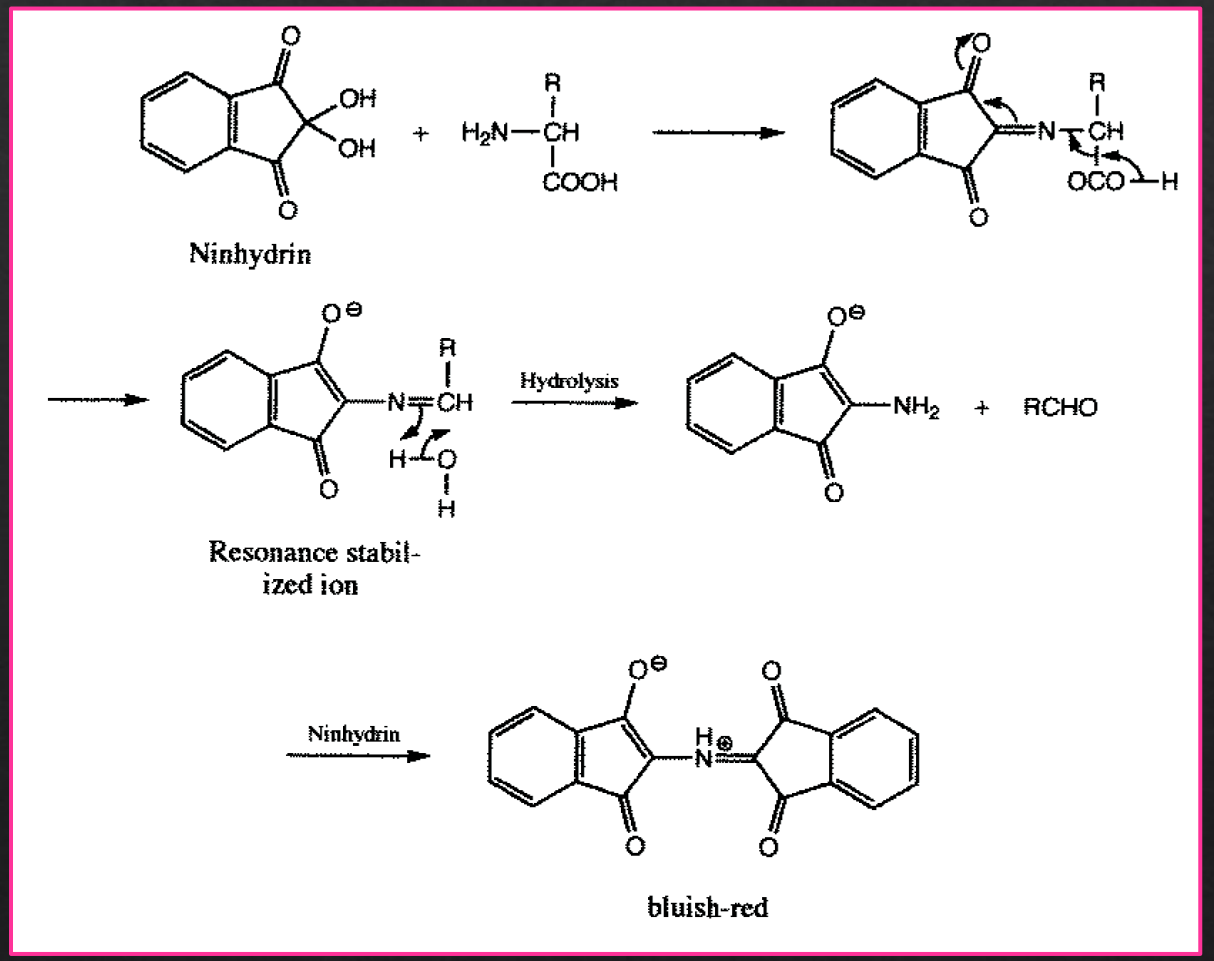
Vicia bengalensis
Produces canavanine

Detection of amino acids

- Forensic chemistry
  - Detection of fingerprints on paper

Ninhydrin can be sprayed on the paper, followed by heating

If amino acids are present, they form a purple complex with ninydrin



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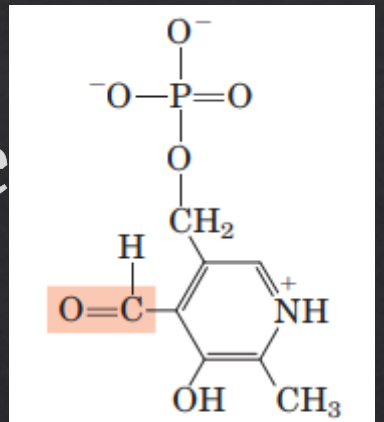
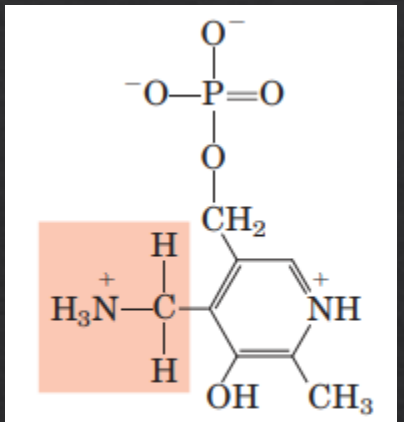
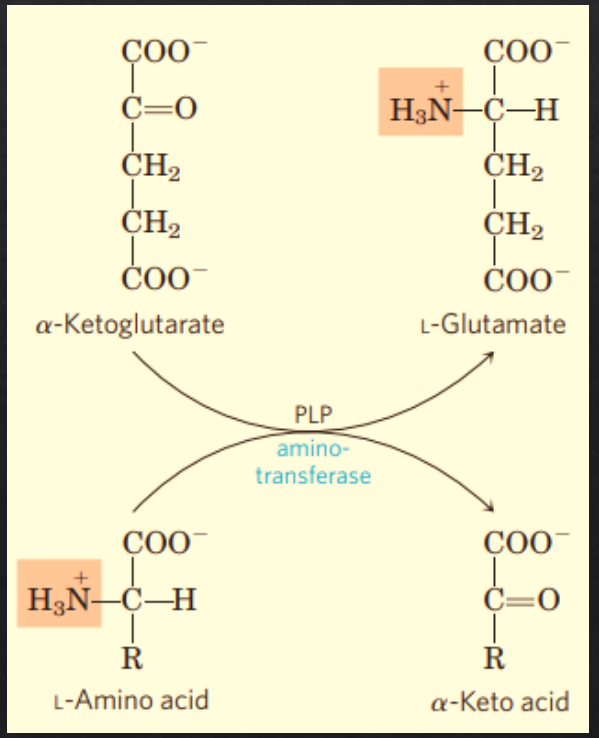
Pyridoxal phosphate

- Cofactor in transamination reactions
  - Temporary carrier of amino groups
- Important for both synthesis and degradation of amino acids
- Glutamic acid is often the amino group donor (or acceptor) in transaminations



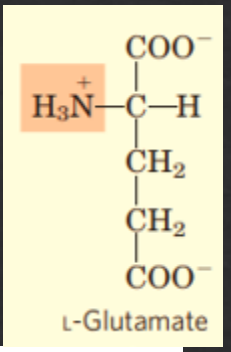


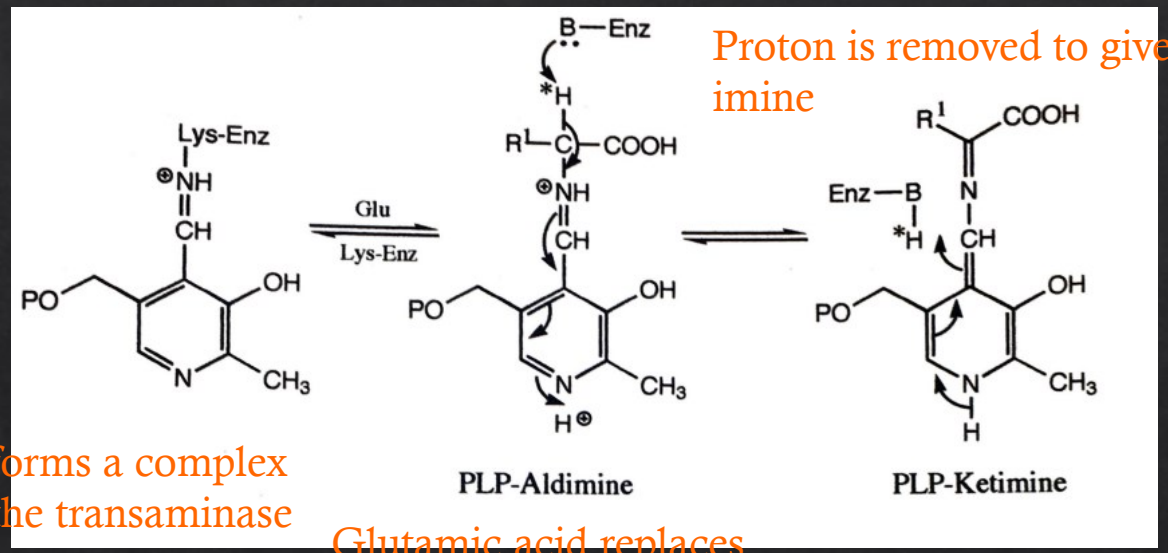


Lehninger Principles of Biochemistry, 6th ed. 201

Pyridoxal phosphate (PLP)

Glutamic acid is the NH_3 donor

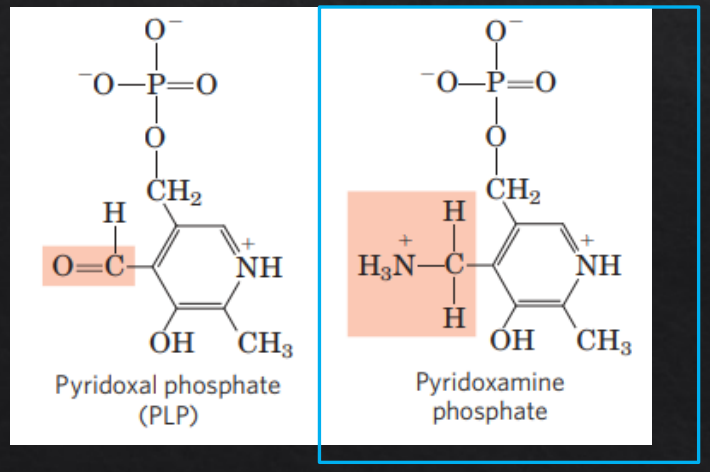




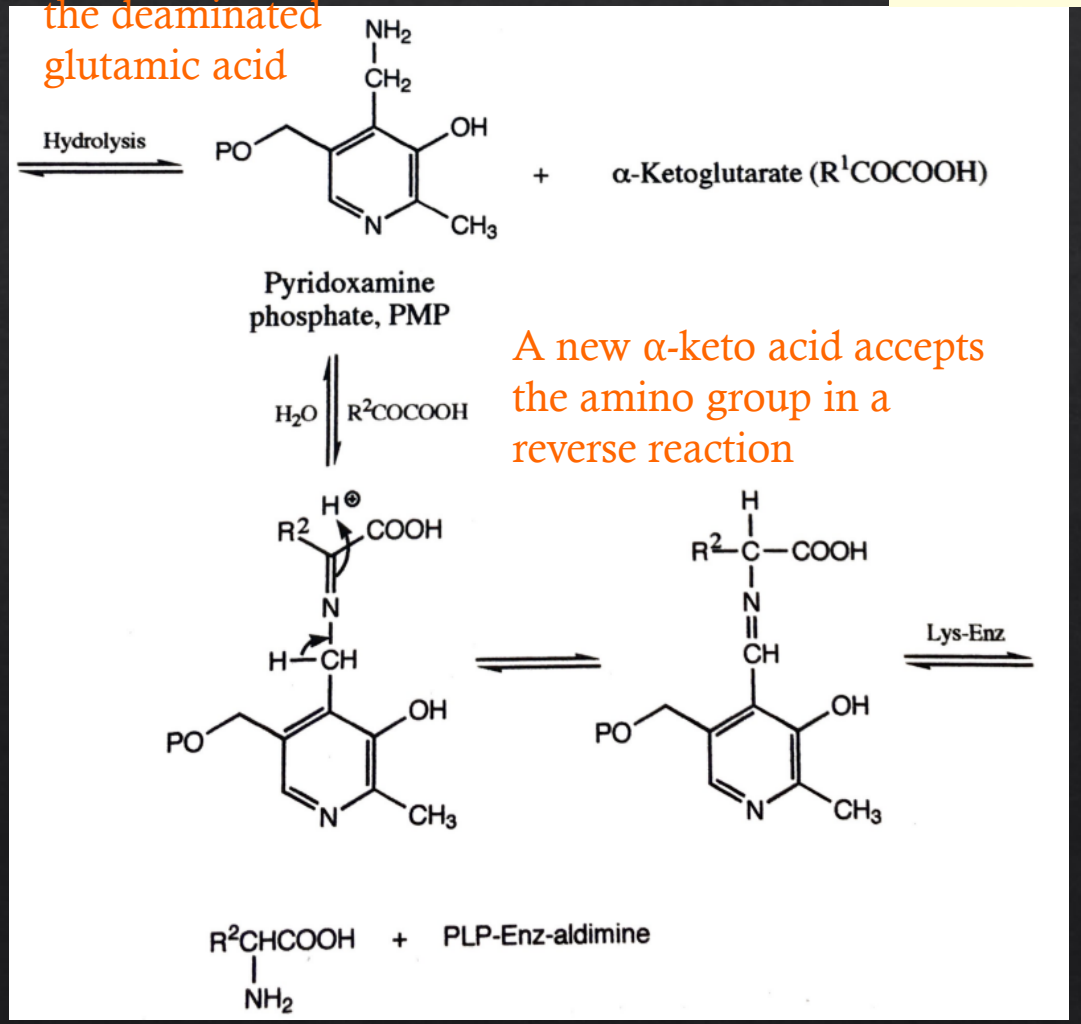
Proton is removed to give imine

PLP forms a complex with the transaminase

Glutamic acid replaces Lys



Hydrolysis removes the deaminated glutamic acid

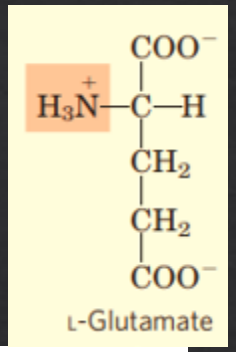


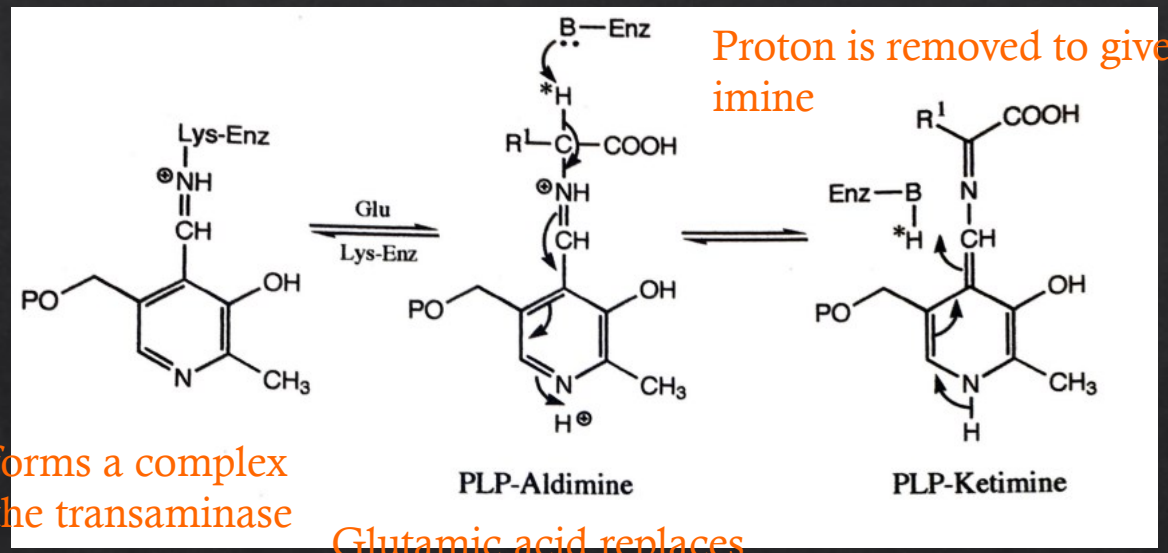
A new α-keto acid accepts the amino group in a reverse reaction

Lehninger Principles of Biochemistry, 6th ed. 2013

Pyridoxal phosphate (PLP)

Glutamic acid is the NH_3 donor





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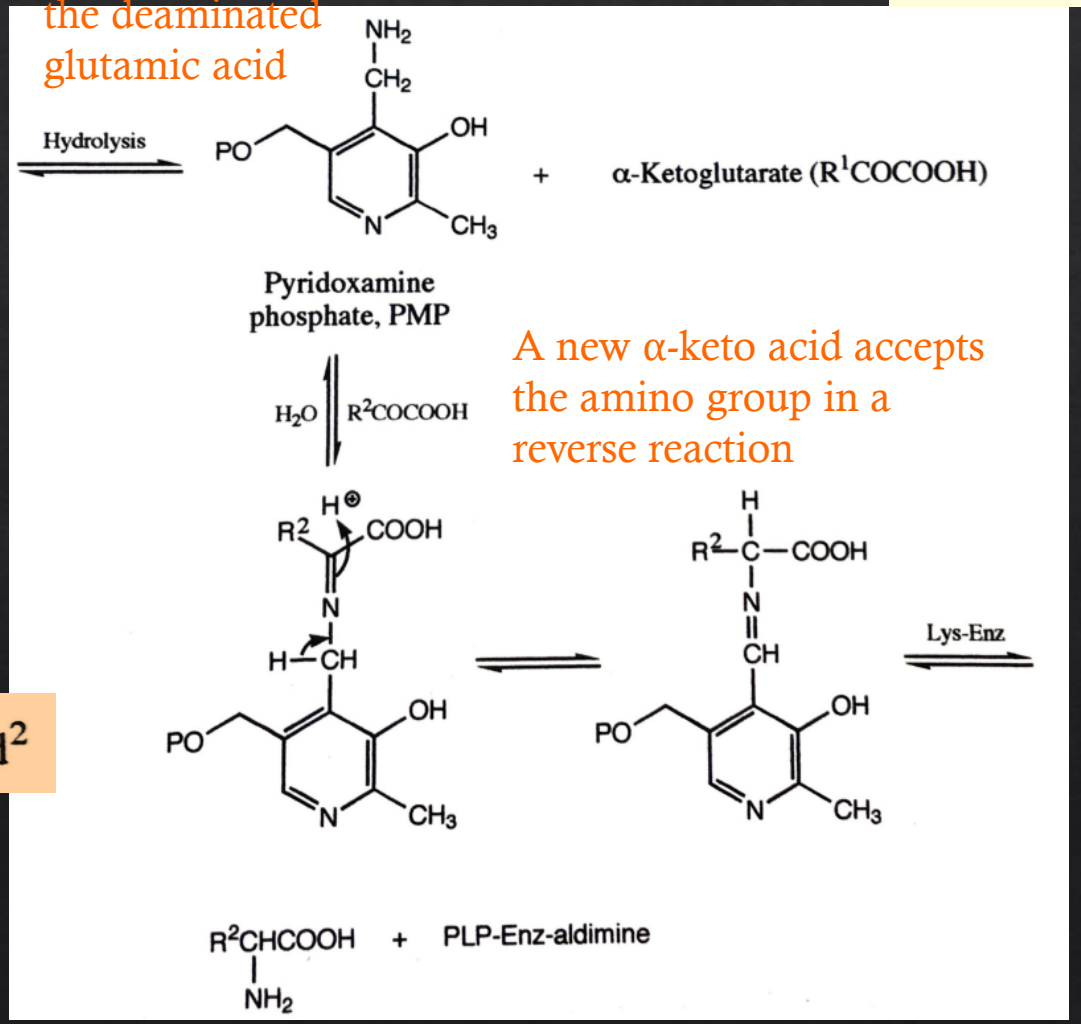
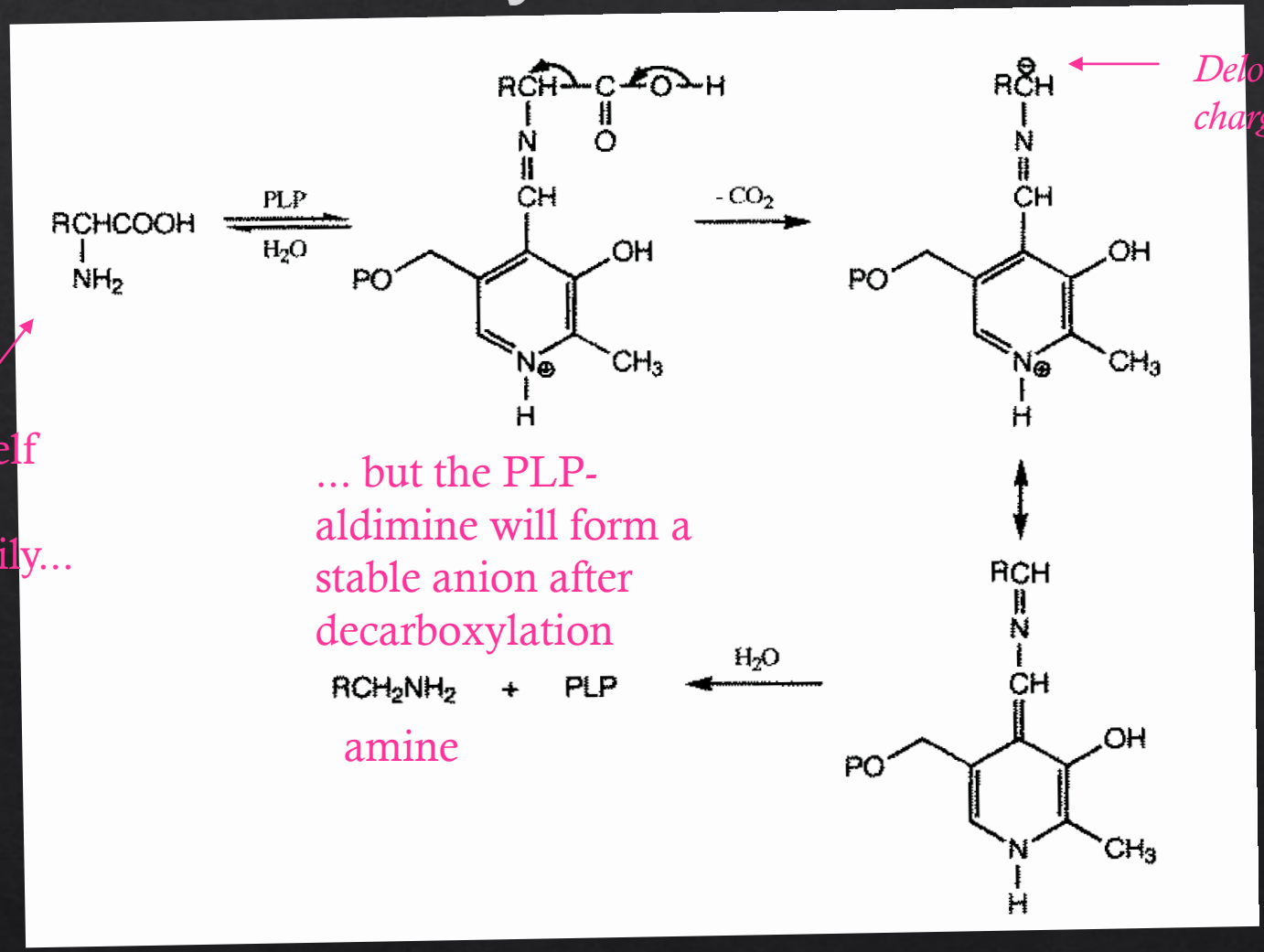


A new α-keto acid accepts the amino group in a reverse reaction

Summary of transamination reactions:

$$\text{Amino acid}^1 + \text{Keto acid}^2 \rightleftharpoons \text{Keto acid}^1 + \text{Amino acid}^2$$

Decarboxylation of amino acids



Delocalized negative charge

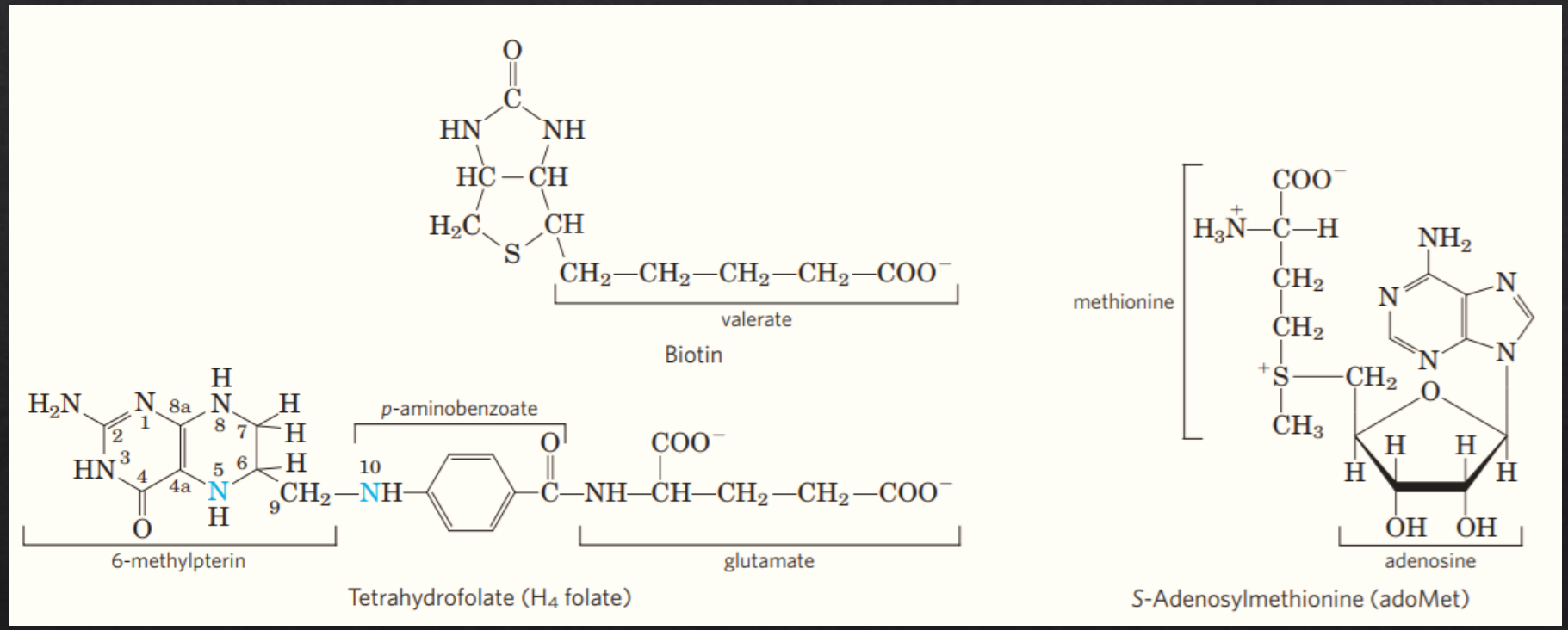
The amino acid itself will not be decarboxylated easily…

… but the PLP-aldimine will form a stable anion after decarboxylation

This is an important biosynthetic route to amines

Cofactors for transfer of one-carbon fragments

- Important cofactors in amino acid catabolism



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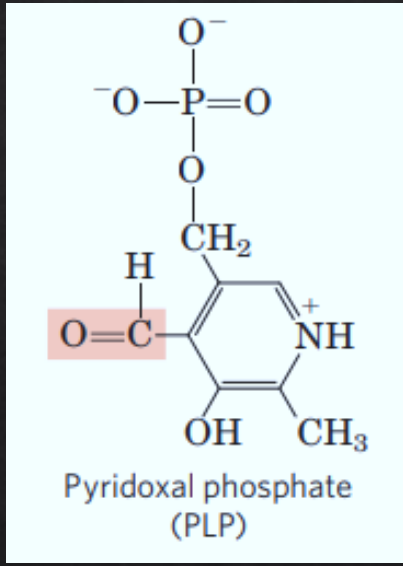
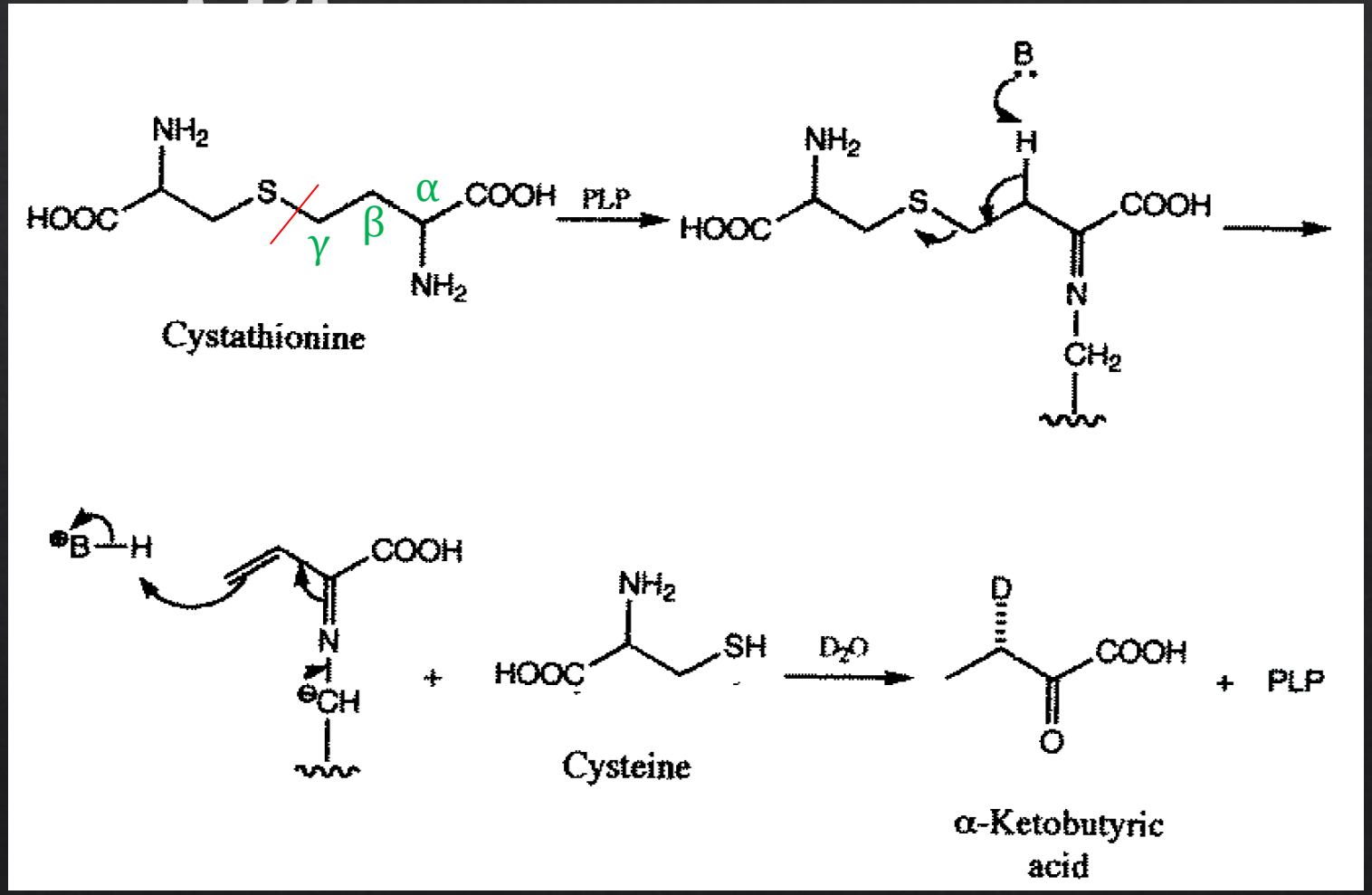
Biosynthesis of cysteine in mammals – role of PLP

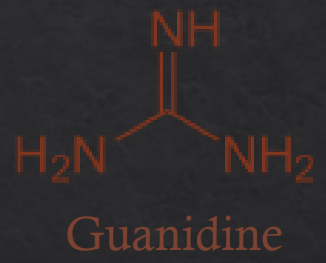
PLP activates cleavage of cystathionine in the γ position





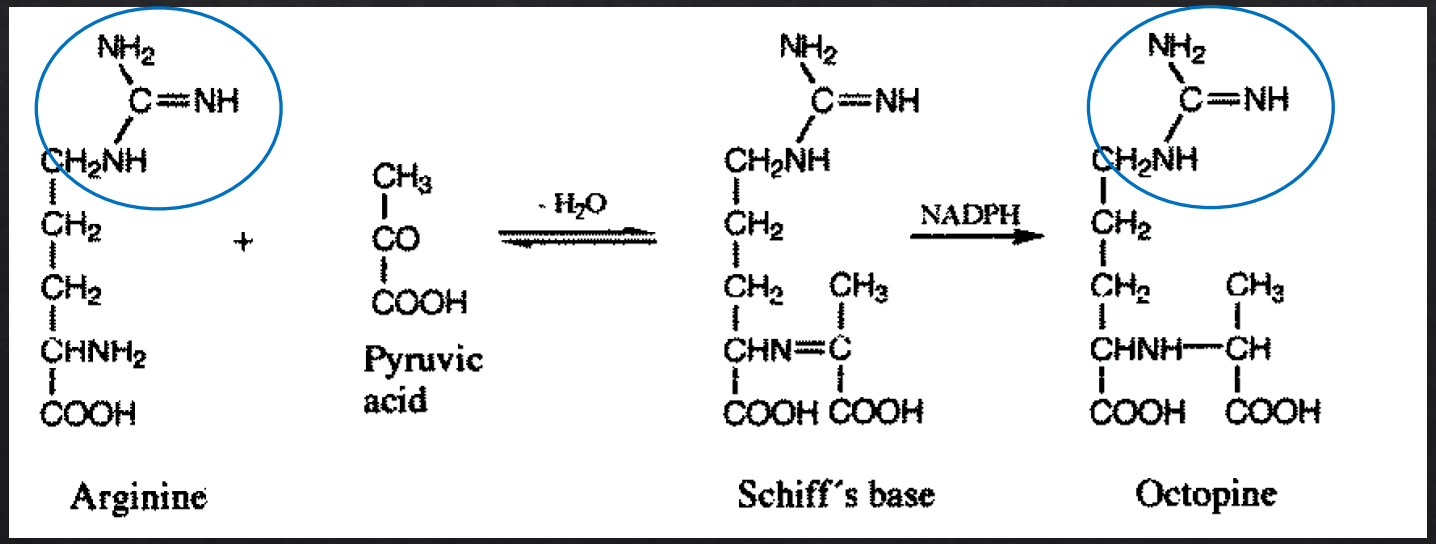
The guanidino functional g

Common functional group in marine metabolites



Guanidine

Example: **Biosynthesis of octopine**



Octopine is an analog of lactic acid in the octopus.

Plants can also produce it if infected with the bacteria *agrobacterium tumifaciens,* which changes the DNA of the plant and induces tumor growth in the infected plant.

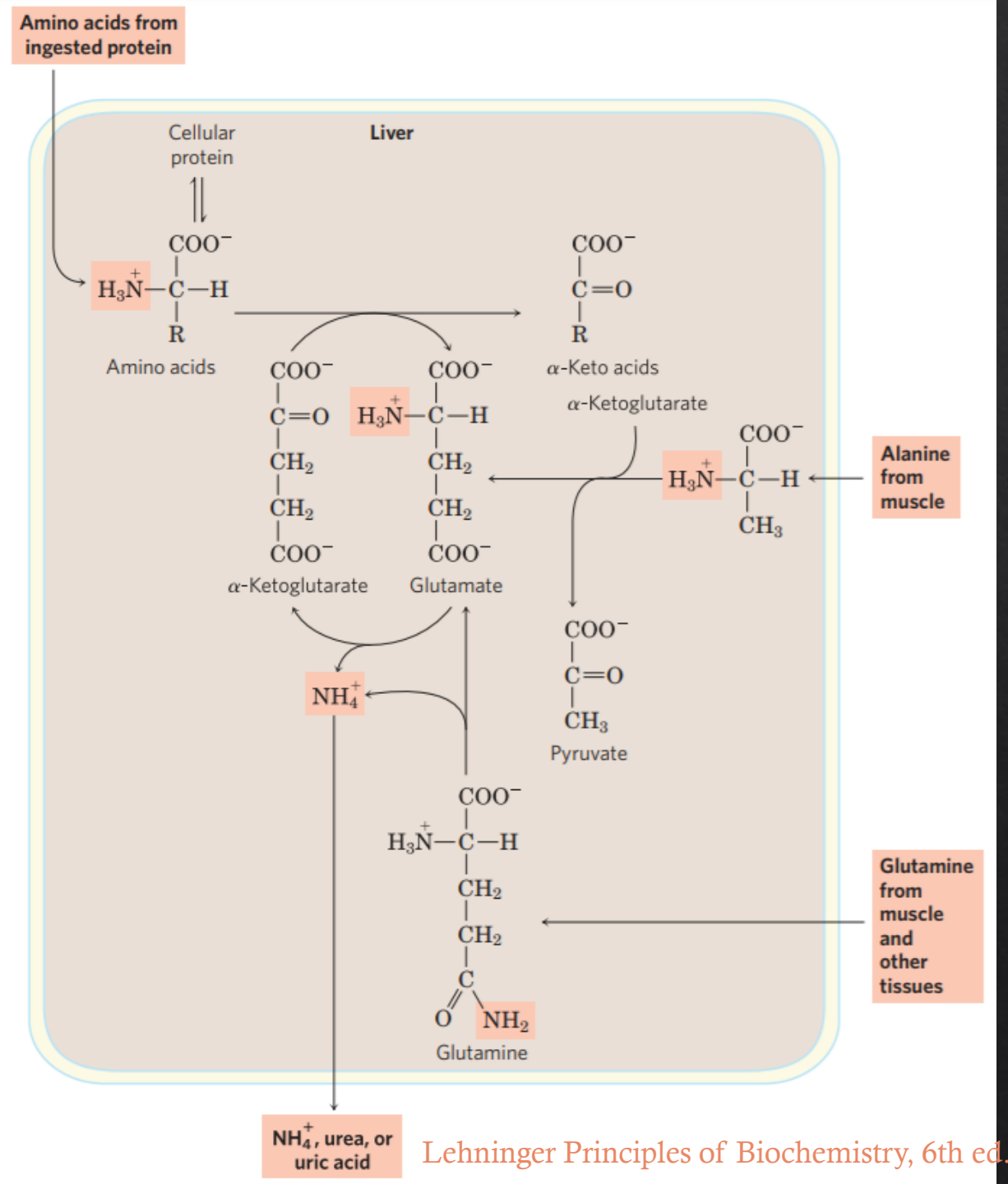
The ure

- NH3 is toxic, removed in urea cycle

1 - NH3 from different tissue in the vertebrates is transferred to the liver as glutamine and alanine

2 - The amino group gets transferred to α-ketoglutarate to form glutamic acid

3 – Glutamic acid releases the amino group which then enters the urea cycle



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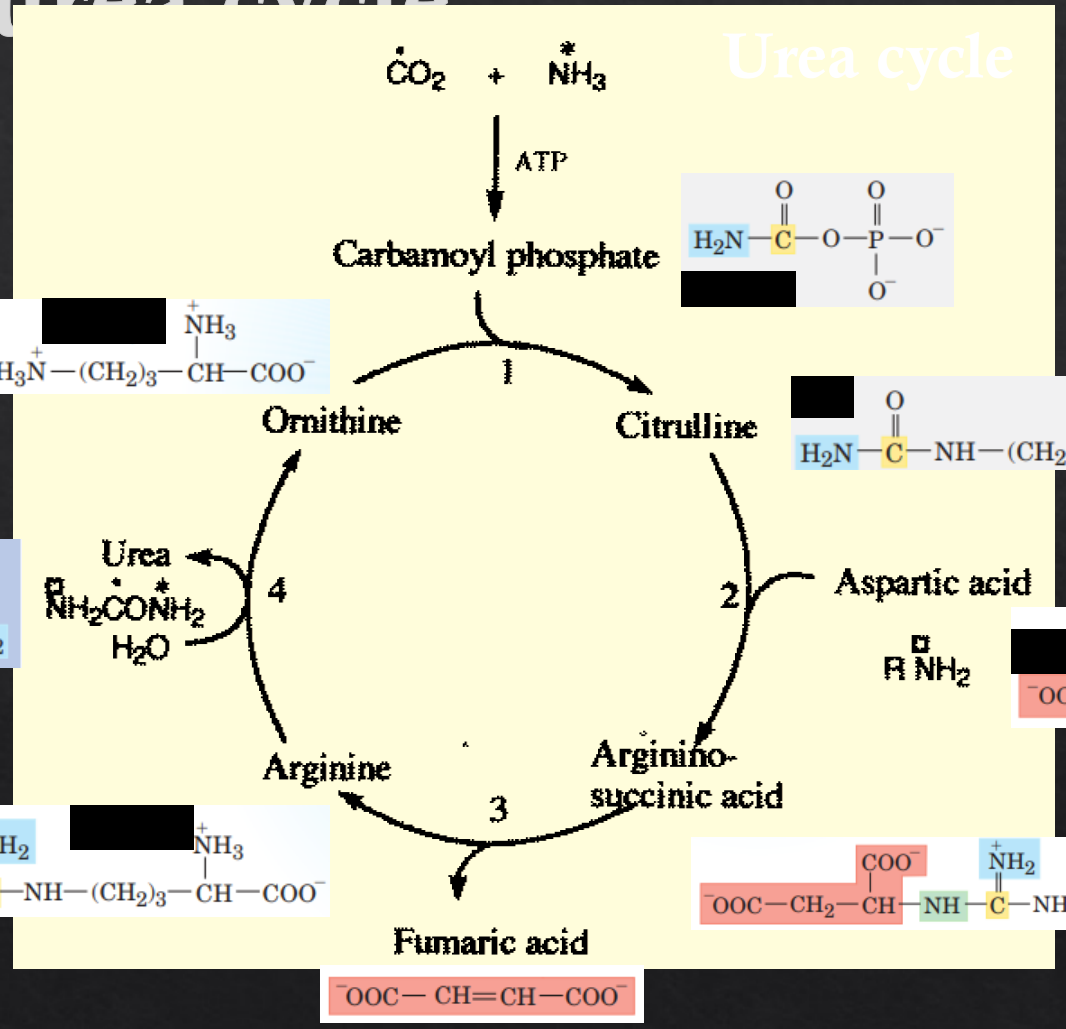
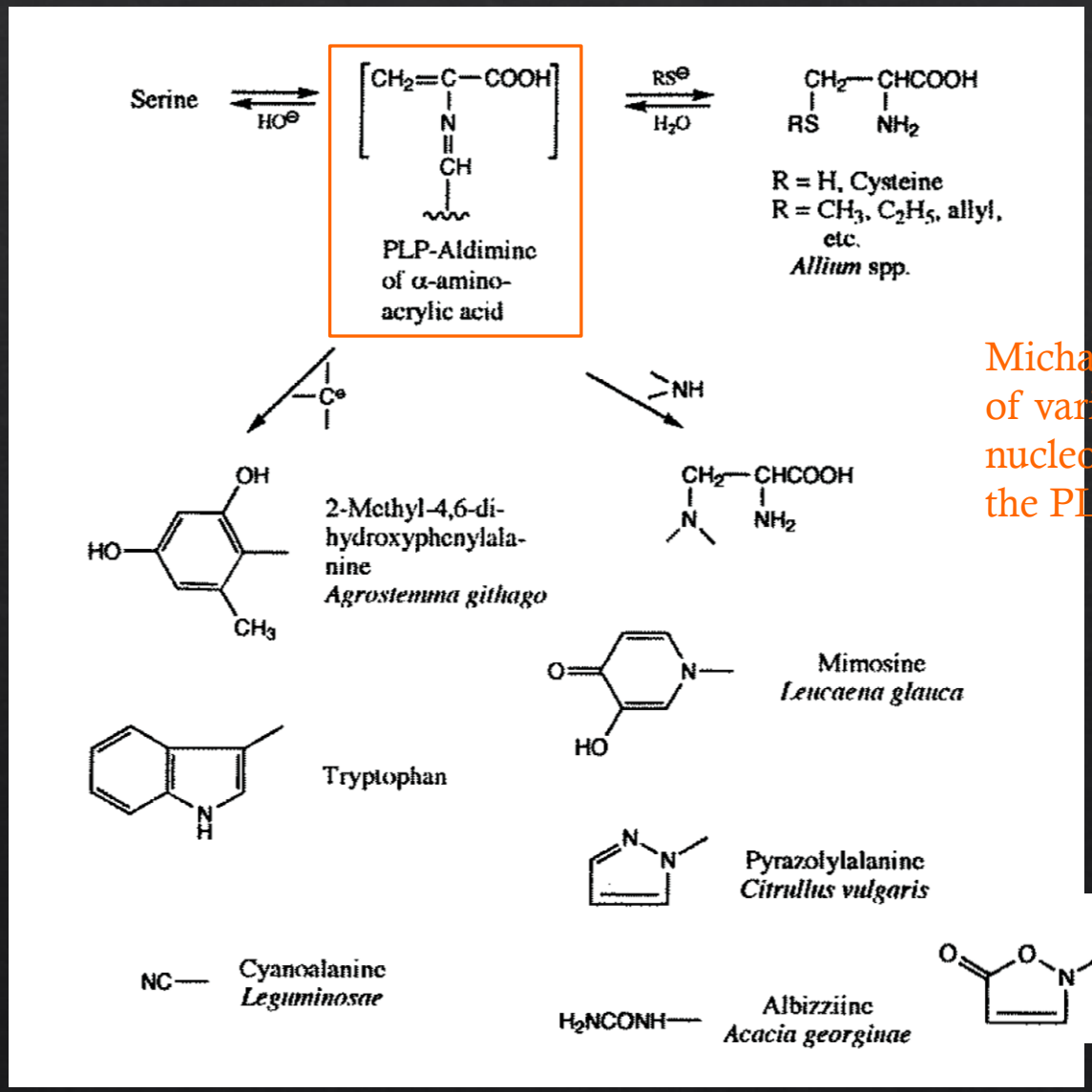
The urea cycle

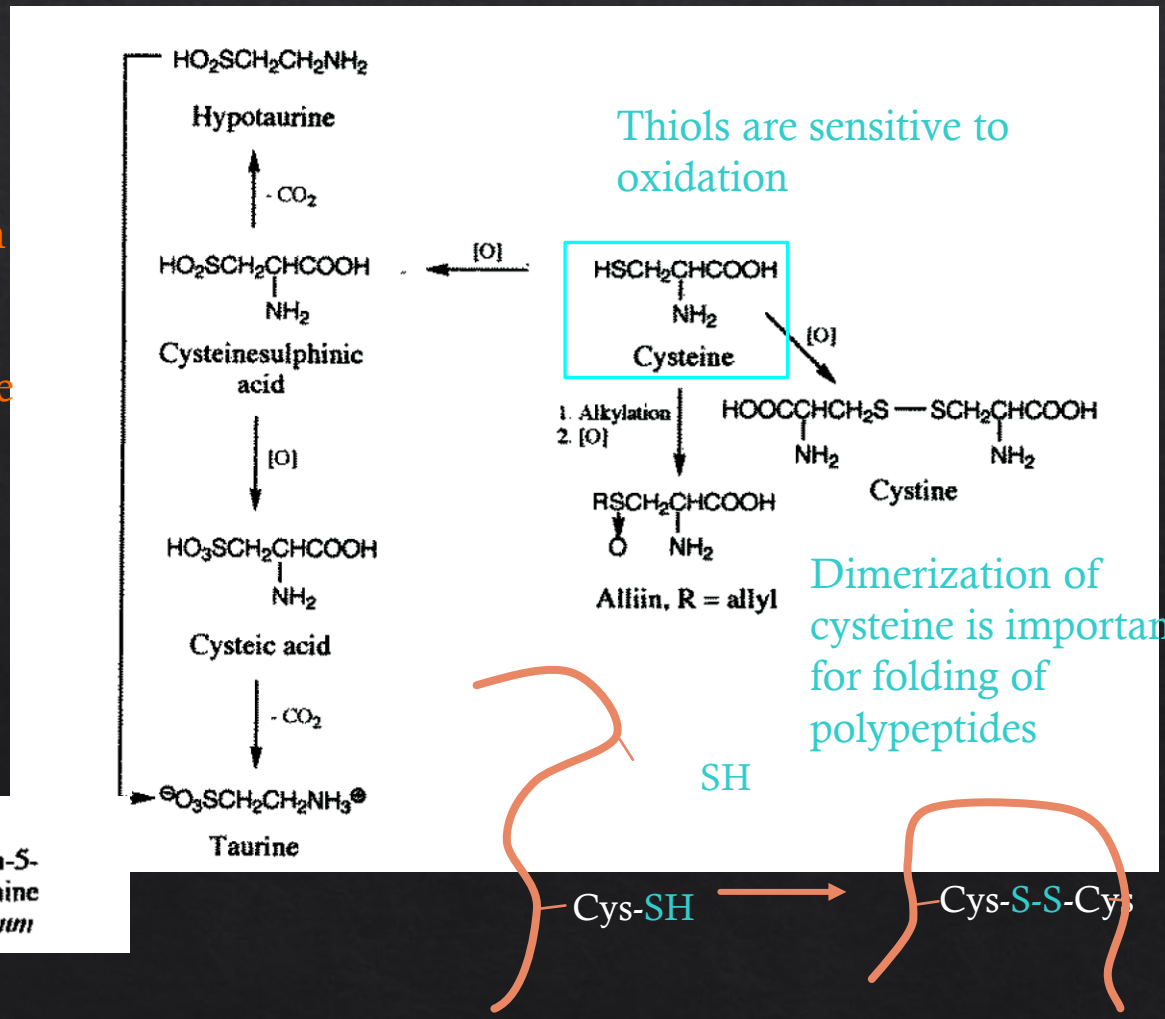
1. Formation of citrulline from carbamoyl phosphate and ornithine
2. Aspartic acid enters to form argininosuccinic acid from citrulline – through a citrulline-AMP intermediate (requires ATP)
3. Release of fumarate gives arginine
4. Hydrolysis of arginine releases urea



Secondary products of serine and cysteine



Michael addition of various nucleophiles to the PLP-aldimine



Thiols are sensitive to oxidation

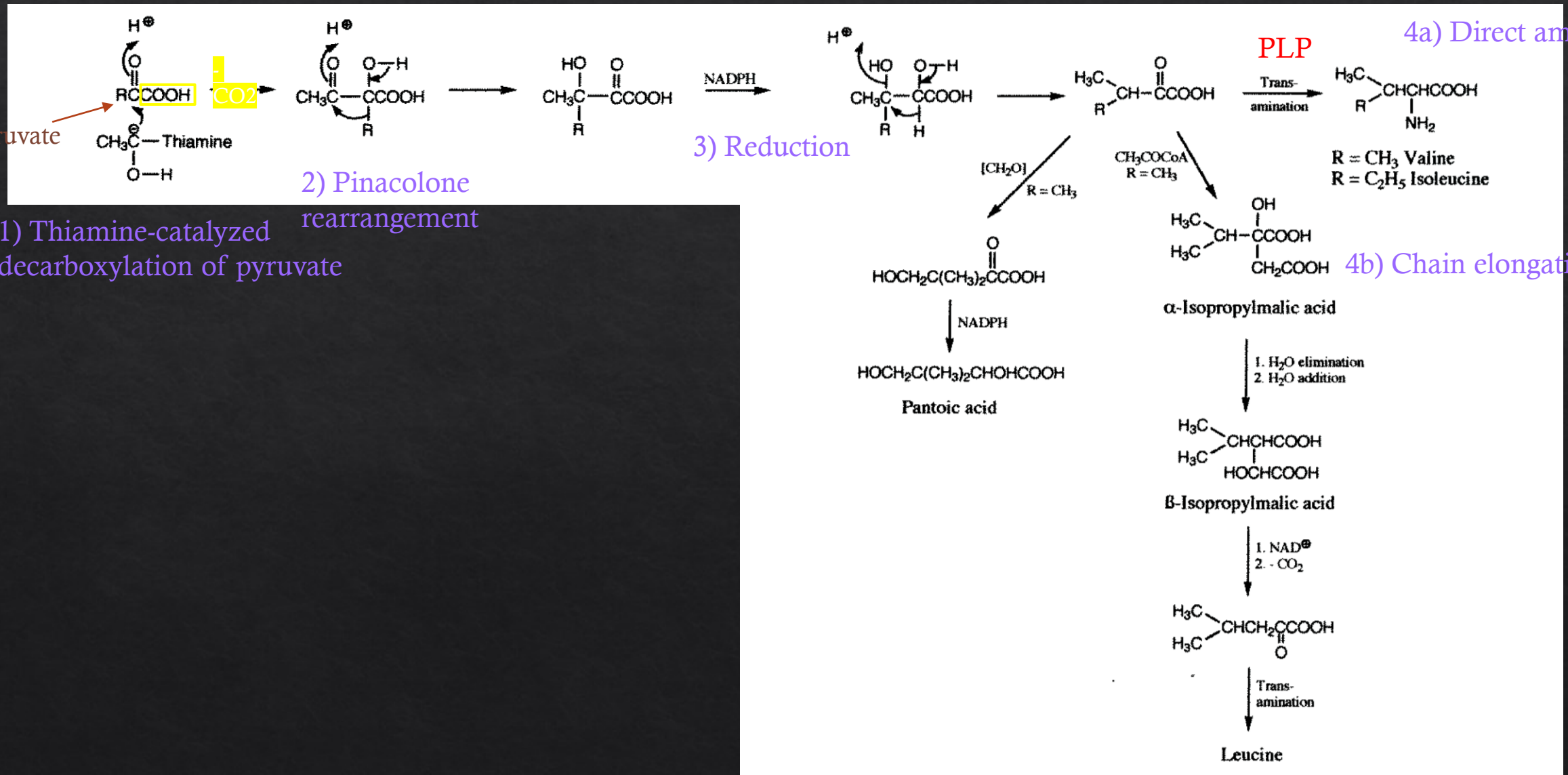
Dimerization of cysteine is important for folding of polypeptides

Serine

Glycine

Cysteine

Biosynthesis of leucine, valine and isoleucine



1) Thiamine-catalyzed decarboxylation of pyruvate

2) Pinacolone rearrangement

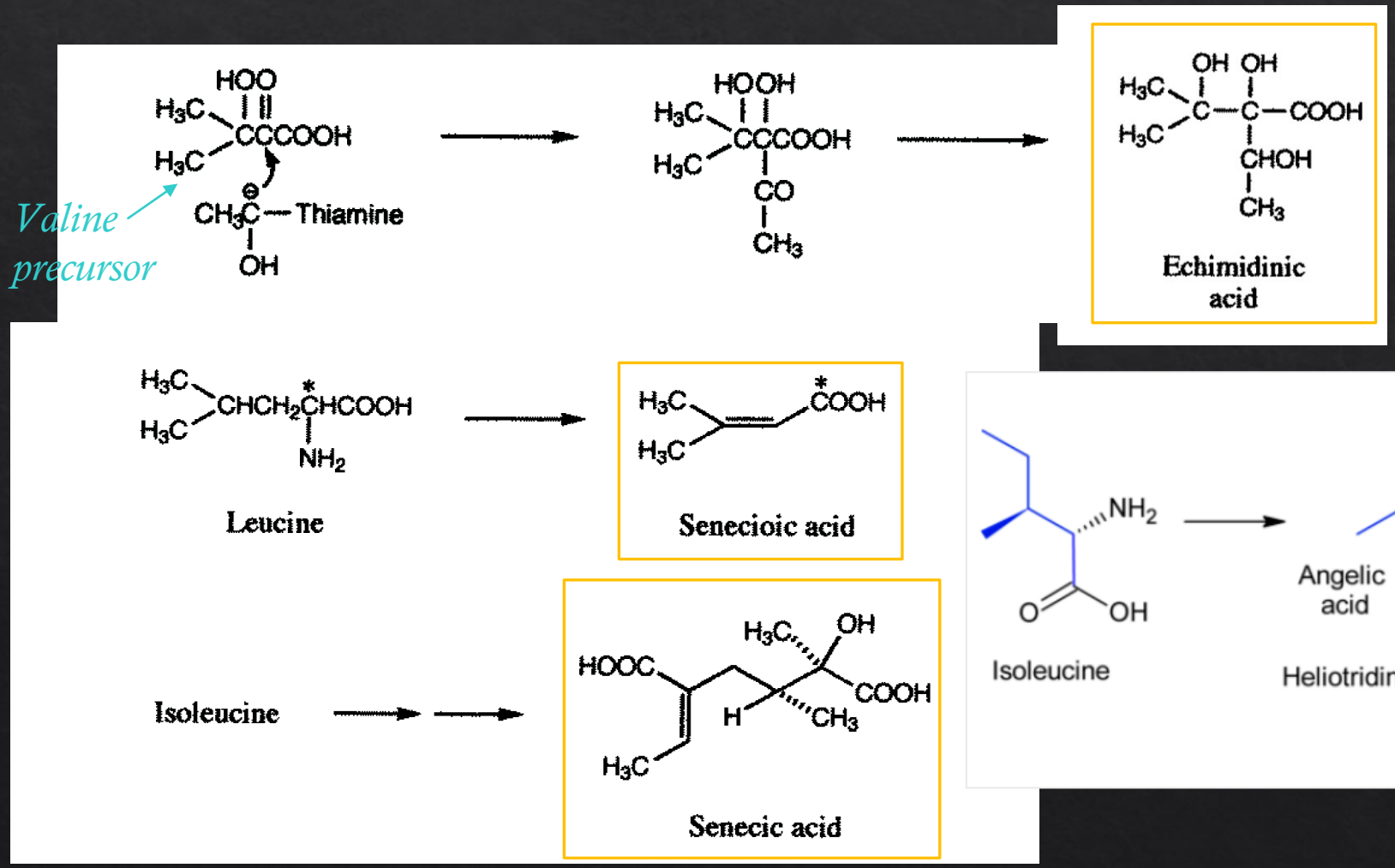
3) Reduction

4a) Direct amination

4b) Chain elongation

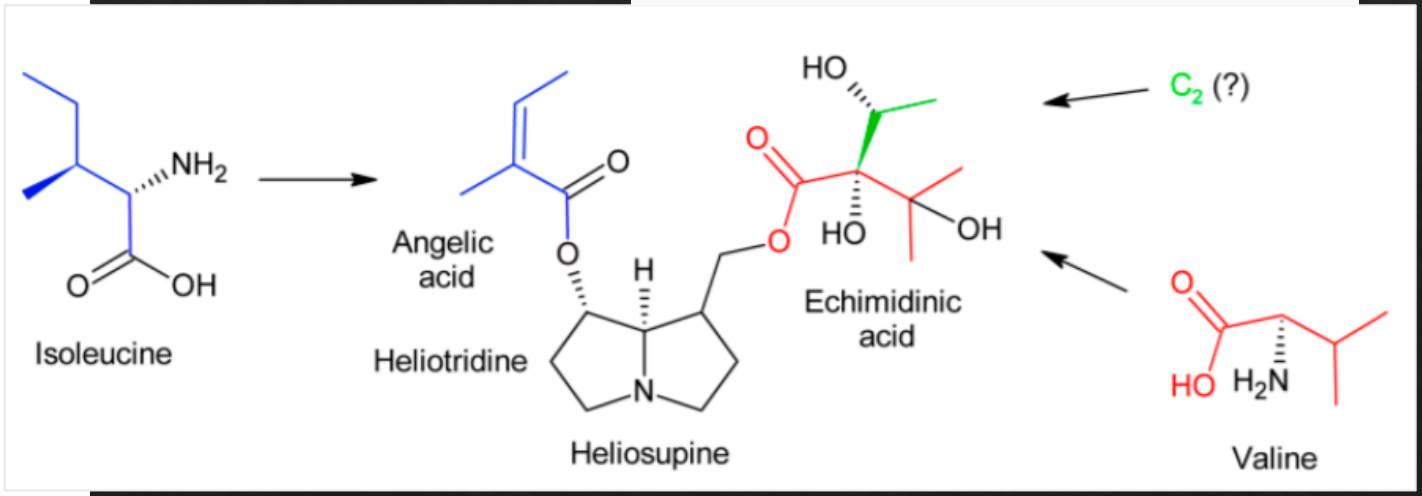
Secondary products of valine, isoleucine and leucine

Necic acids are often derived from these amino acids (not from acetate, as originally suspected):



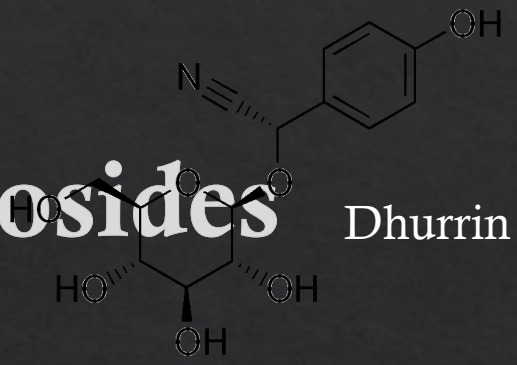
Necic acids are important building blocks of pyrrolizidine alkaloids such as heliosupine

(Plant defence chemicals, highly toxic compounds)



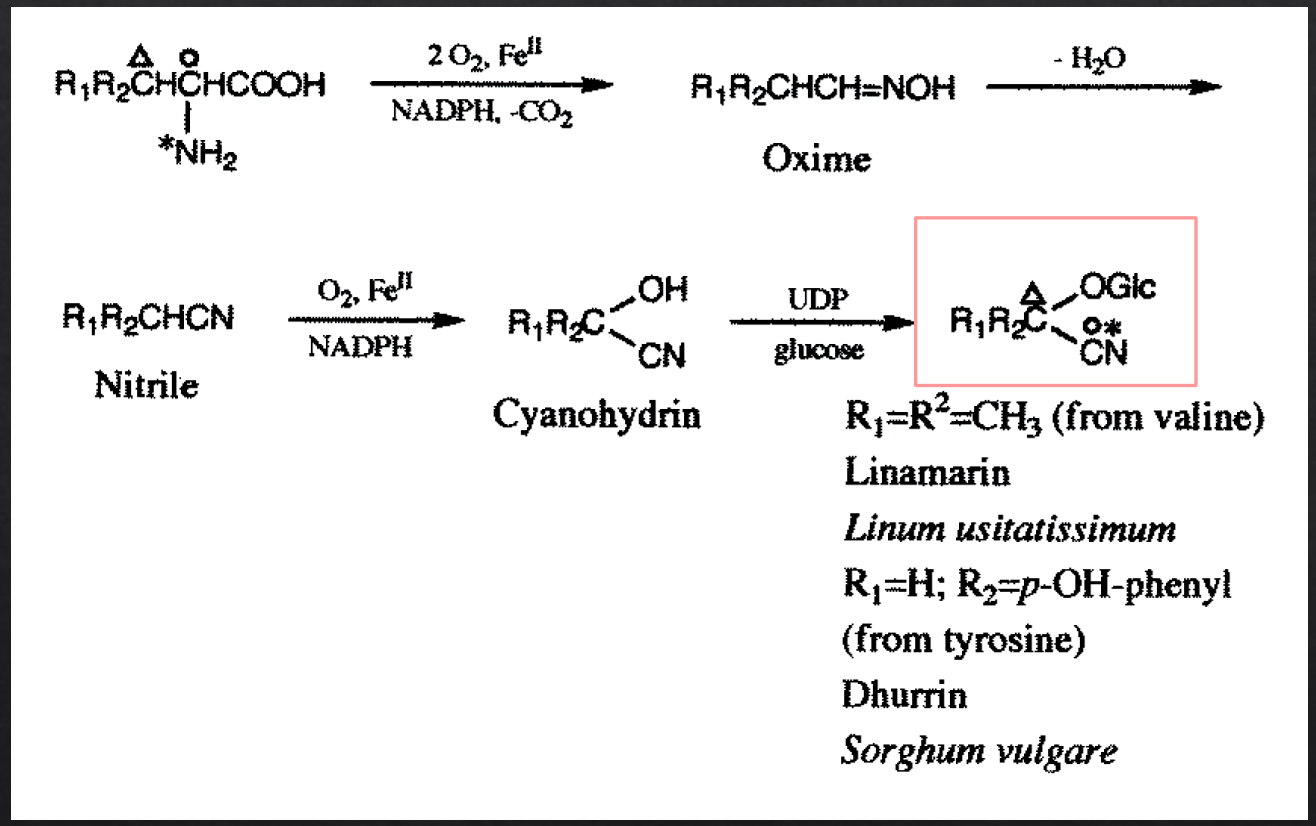
Schramm *et al. (2019)* Molecules, 24, 498

Cyanogenic glycosides



Dhurrin

Sorghum vulgare



Amino acids → Cyanogenic glycosides → HCN

Cyanogenic glycosides:

Precursors for HCN in some plants

Decomposition gives sugar, ketone/aldehyde and HCN

Defence mecanism:

Tissue damage in plant releases enzymes that catalyze decomposition => HCN released

Vetter J. (2017) Plant Cyanogenic Glycosides. Plant Toxins. Toxinology. Springer, Dordrecht.