Iceland Liechtenstein Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

UNIVERSITY OF BUCHAREST
VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of Science and Technology

Natural product chemistry

Introduction

Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022

Textbook: K. B. G. Torssell: Natural Product Chemistry, 2. utg., Apotekersocieteten/Taylor & Francis, 1997

Natural products

- All chemical compounds that are found in nature, produced by living cells.
- Ancient science:
  - Extracts from nature were used for preparation of food, coloring, fibers, toxins, medicine and stimulants
- Late 18th century:
  - Natural products were separated, purified and analyzed

Organic natural product chemistry

- Occurence of natural products
  - (sources and amounts)
- Structure and stereochemistry
- Physicochemical properties
- Function in the organism
- Biosynthesis
- Chemical synthesis (for structural evidence)

Why is natural product chemistry interesting today?

One reason: antibacterial and anticancer medicine

Natural products were precursors for 54% of new drugs on the market from 1981-2002

David J. Newman, Gordon M. Cragg and Kenneth M. Snader, *J. Nat. Prod*, 2003, *66*, 1022-1037

Primary and secondary metabolites

Metabolism	=	**anabolism** (synthesis of molecules needed by the organism)	+	**catabolism** (breakdown of molecules for energy)

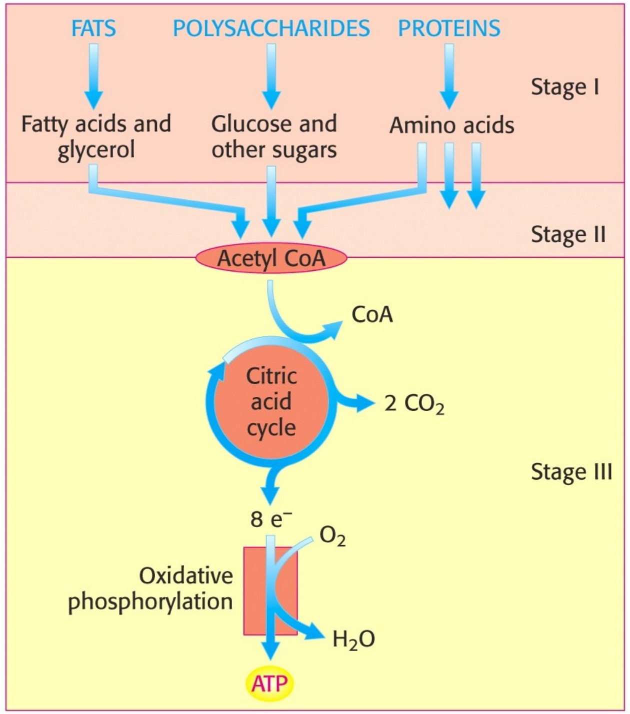
Primary metabolites

- Compounds that are essential for life
- Photosynthetic processes

$CO_2 + H_2O \rightarrow$ carbohydrates $+ O_2$

- Starting materials for secondary metabolites

- Low molecular weight carboxylic acids (Krebs cycle)
- α-amino acids
- Carbohydrates
- Fats
- Protein
- Nucleic acids

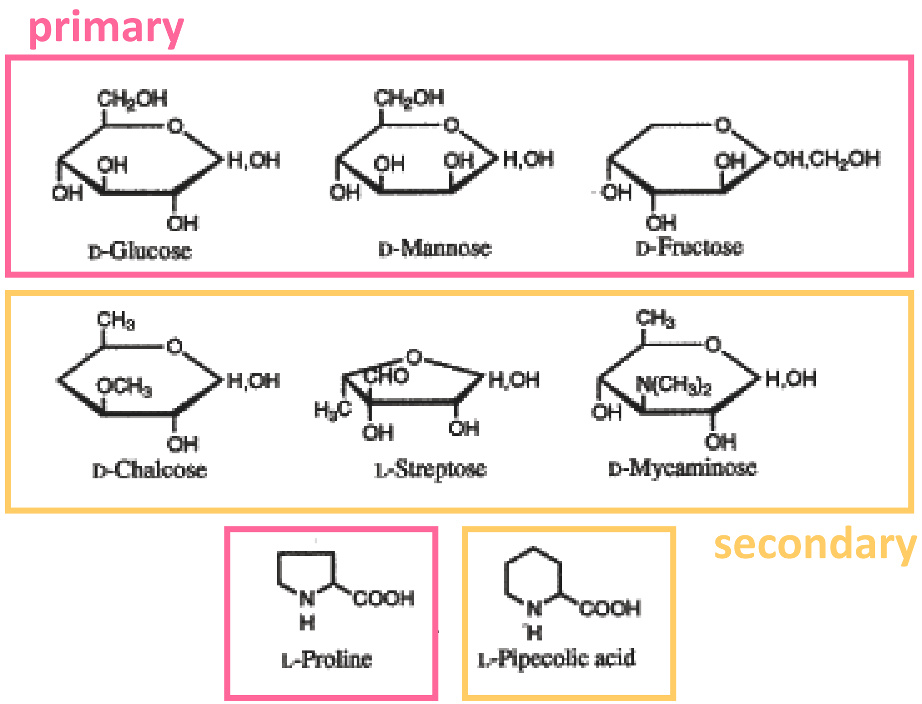


Primary and secondary metabolites

Metabolism = **anabolism** (synthesis of molecules needed by the organism) + **catabolism** (breakdown of molecules for energy)

Secondary metabolites

- Non-essential, «specialized» molecules
- Often characteristic for groups of organisms
- Natural product chemistry
- No sharp line between primary and secondary metabolites



Examples of secondary metabolite functions

Attract other individuals

Pheromones, pigments, aroma
Reproduction & propagation

Repel other individuals

Bad taste, smell, toxicity, phytoalexins
Defence & protection

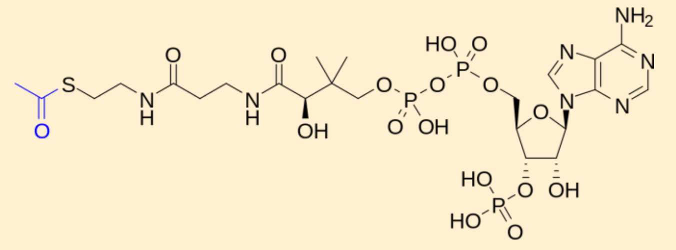
Modify membranes

Protecting the organism from the environment (temperature, etc.)

Aid in metabolism

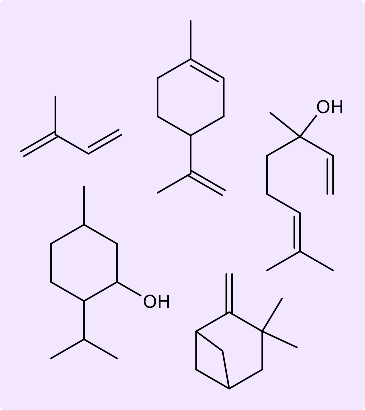
Prosthetic groups/coenzymes, vitamins, hormones, etc.

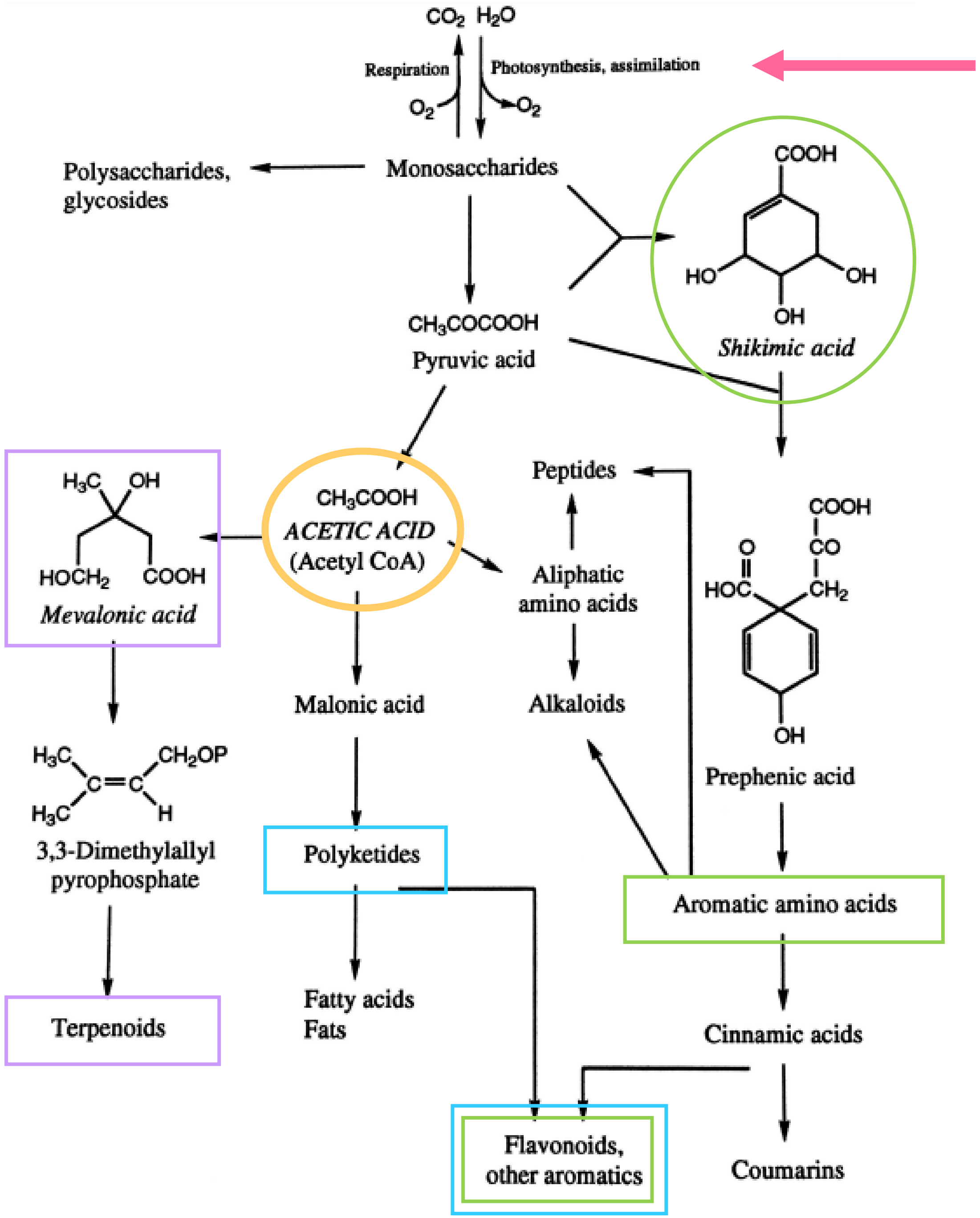
Main streams of secondary metabolism



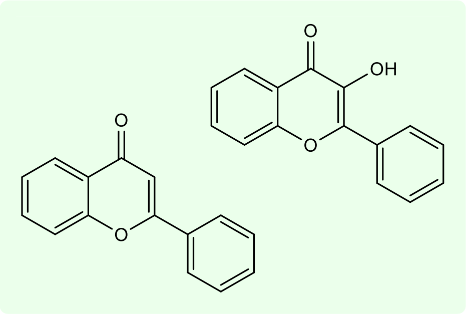
Acetyl CoA

Terpenes/terpenoids

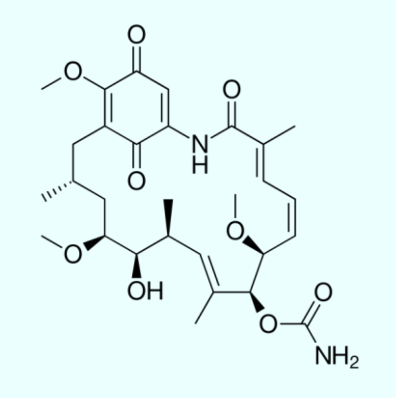




Primary metabolism



Flavones/flavonoids



Geldanamycin (a polyketide)

We will go through:

3: Carbohydrates and primary metabolites

4: The shikimic acid pathway

5: The polyketide pathway

6: The mevalonic acid pathway & the terpenes

7: Amino acids, peptides and proteins

8: The alkaloids

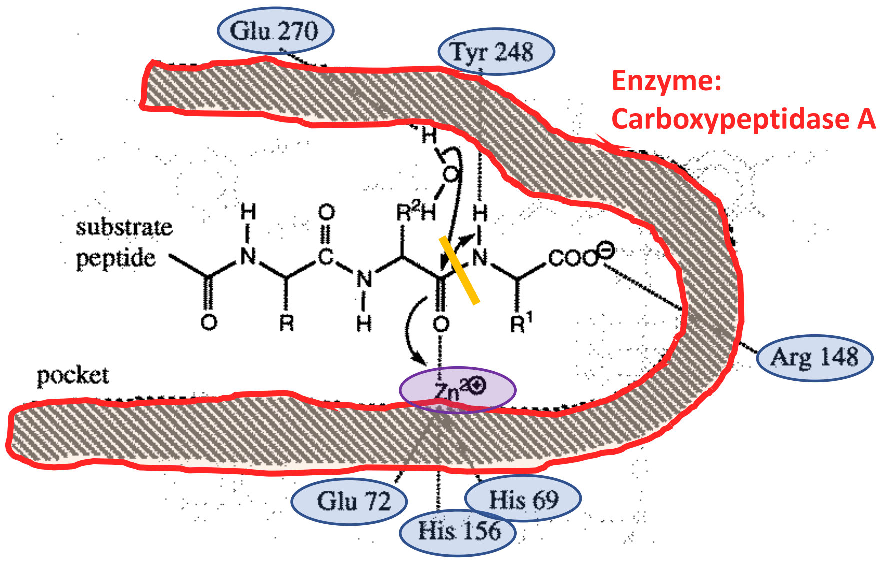
9: The N-heteroaromatics

Biochemical reactions (reminders)

- Enzymatic reactions: can easily form enantiopure compounds, because of the chiral structure of enzymes.

Example: Hydrolysis of a terminal amino acid of a peptide.

- This enzyme is a chain of 307 amino acids.
- Through hydrogen bonding and other types of bonds, the substrate is ideally placed for the reaction.
- The dipolar character of the carbonyl bond is enhanced thanks to Zn^{2+}.
- Protonation of the amino function lead the cleavage of the peptide bond.



Biochemical reactions (reminders)

- A lot of enzymatic reactions require coenzymes. They will act as carriers of some necessary groups.

TABLE 14.2 Some activated carriers in metabolism

Carrier molecule in activated form	Group carried	Vitamin precursor
ATP	Phosphoryl	
NADH and NADPH	Electrons	Nicotinate (niacin)
$FADH_2$	Electrons	Riboflavin (vitamin B_2)
$FMNH_2$	Electrons	Riboflavin (vitamin B_2)
Coenzyme A	Acyl	Pantothenate
Lipoamide	Acyl	
Thiamine pyrophosphate	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2	Biotin
Tetrahydrofolate	One-carbon units	Folate
S-Adenosylmethionine	Methyl	
Uridine diphosphate glucose	Glucose	
Cytidine diphosphate diacylglycerol	Phosphatidate	
Nucleoside triphosphates	Nucleotides	

Note: Many of the activated carriers are coenzymes that are derived from water-soluble vitamins (Section 8.6.1).

Biochemical reactions

- Several types of reactions can be distinguished:

Carbon-carbon coupling (Claisen and Michael)

In enzyme promoted acylation with thioesters for instance.

Electrophilic substitutions

In C-, N- and O-alkylations with S-adenosyl methionine and phospahtes

Eliminations

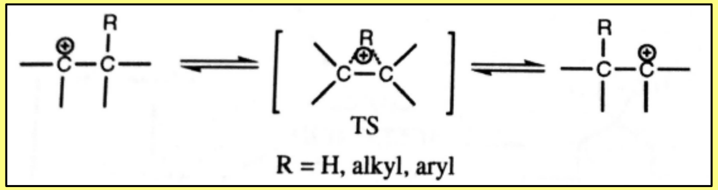
The nucleophile group of an enzyme (-OH,-NH_2,-SH) will lead to the elimination of, for instance, water or ammonia.

Oxidations/reductions

Cofactors like NAD^+ and FAD are used as electron carrier/receiver.

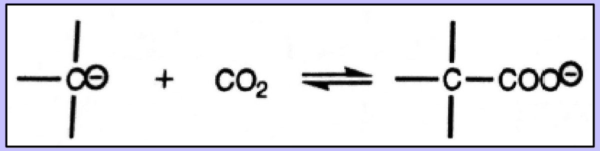
Carbonium anion rearrangements

Happens for instance in terpene biosynthesis



Carboxilation/decarboxylation

Happens for instance in fatty acid synthesis.



Elucidation of metabolic sequences

- Intermediate are only present in very small quantities in normal organisms.
- Use of defective organisms (obtained for ex. by UV or X-rays irradiation):
  - In mutant **1**, D will accumulate, and E will be needed for the mutant to grow
  - The filtrate of **1** will allow **2** to grow, but the opposite is false.
  - The order of the sequence can be proven this way.

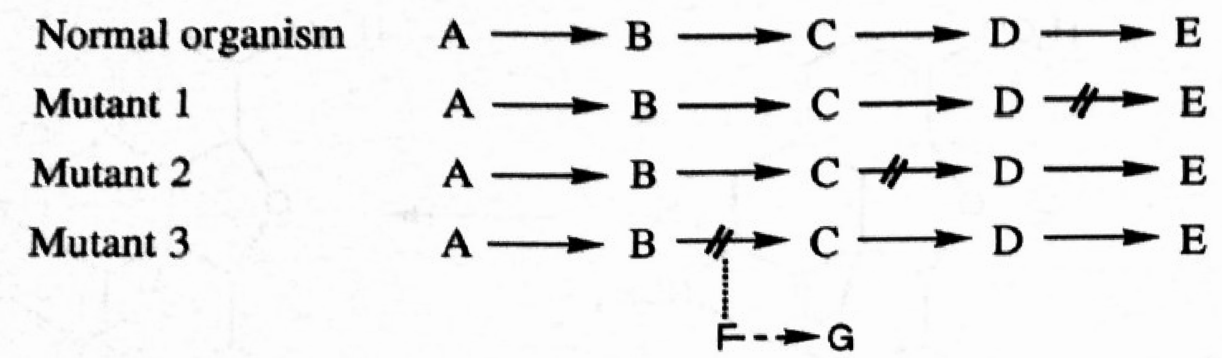
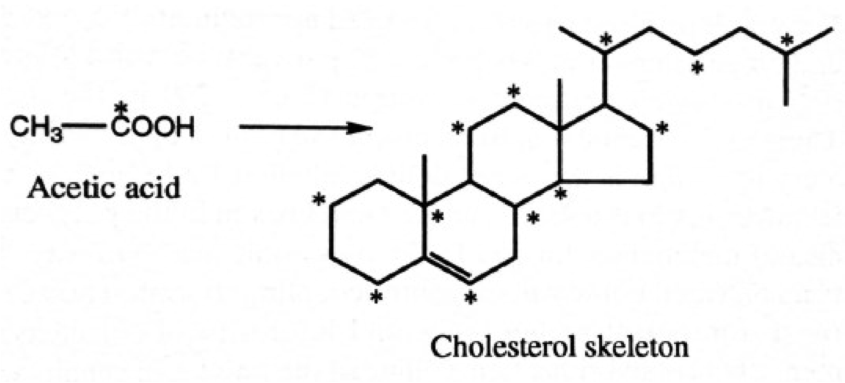


Fig. 9 Mutants with defective enzymes at different points along a metabolic chain

Elucidation of metabolic sequences

- Use of isotopes:
  - Radioactive substrates are given to the organism.
  - In a sequence A→E, radioactivity will first appear in A, then B,...
  - Allow us to know precisely where a certain carbon in a final product comes from (isotope labeling).
  - The pathway from acetic acid to cholesterol was found by degrading a labeled cholesterol molecule.
  - C^{13}-NMR and H-NMR are powerful tools to analyze labeled compounds



Prebiotic chemistry

<u>How were the first amino-acids created on earth?</u>

- An electrical discharge in a mixture of basic compounds (water, methane, ammonia and nitrogen) gives amino-acids in a low yield.

$$CH_4 + H_2O \xrightarrow{\text{Discharge}} CH_2{=}CHCHO \quad (15)$$

$$CH_4 + H_2S \xrightarrow{\text{Discharge}} CH_3SH \quad (16)$$

$$CH_3SH + CH_2{=}CHCHO \xrightarrow{\text{Addition}} CH_3SCH_2CH_2CHO \quad (17)$$

$$CH_3SCH_2CH_2CHO + HCN + NH_3 \xrightarrow{\text{Hydrolysis}} \underset{\text{Methionine}}{CH_3SCH_2CH_2CH(NH_2)COOH} \quad (18)$$

- A lot of amino-acids may have been formed from oligomerization of hydrocyanic acid.
- The preference for the L form of chiral compounds in living beings doesn’t have any good explanation.

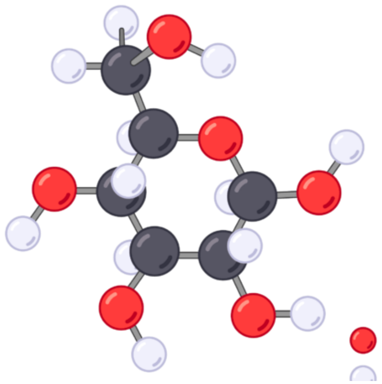
Iceland Liechtenstein Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

University of Bucharest
VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of Science and Technology

Chapter 3
Carbohydrates and primary metabolites

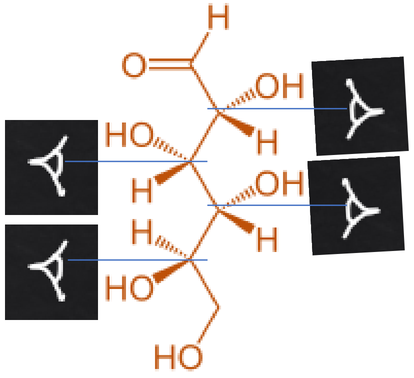


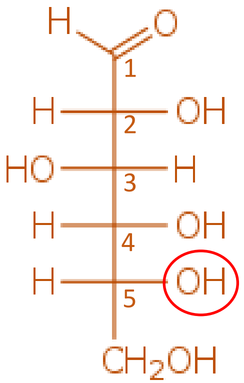
Elisabeth Jacobsen and Lucas Boquin, NTNU

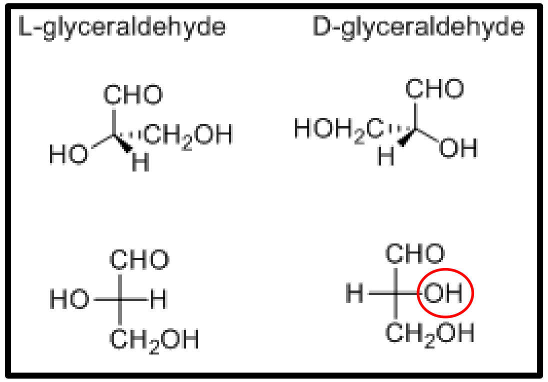
Spring 2022

Classification of carbohydrates

- Number of carbon atoms in one unit (most common: hexoses)
- Aldehyde or keto function
- Number of units (monosaccharides, disaccharides,...)
- Fischer representation:



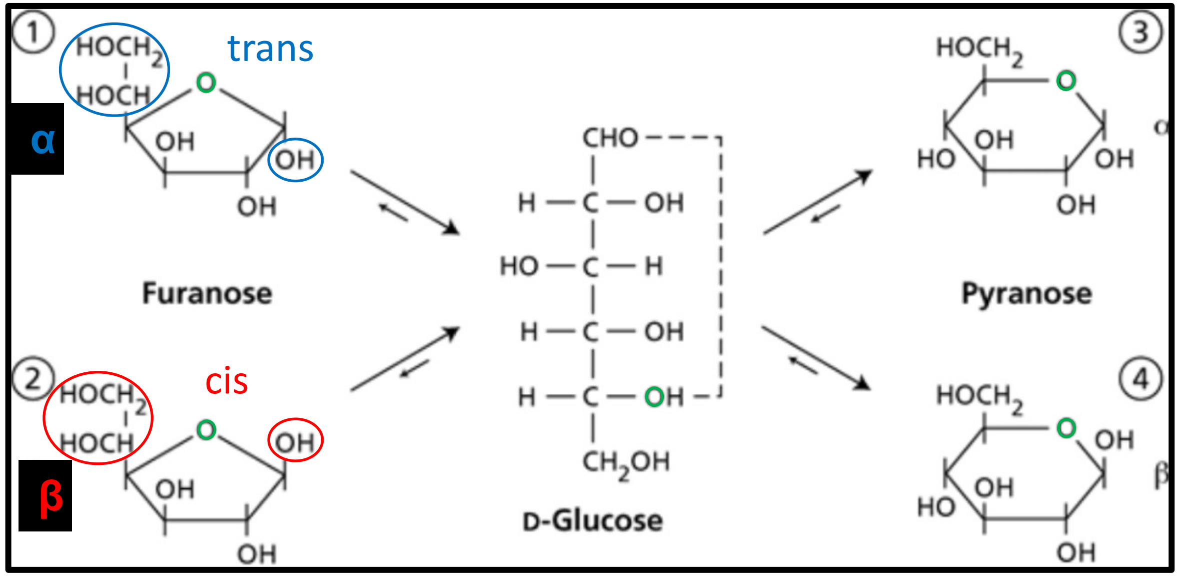




Conformations and stereoisomerism

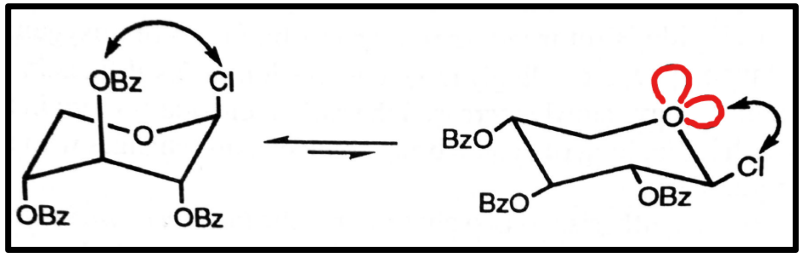
- The open form of hexoses are in equilibrium with their hemiacetal/hemiketal form.

α / β : Different physical properties



6-membered rings:
In chair and boat form

- The preferred isomer isn't always the one with bulky groups in equatorial position.



Carbohydrate biosynthesis in plants: Photosynthesis

- 1- Light reaction

energy rich

$$H_2O + NADP^+ + P + ADP \xrightarrow{h\nu} \tfrac{1}{2}O_2 + NADPH + ATP + H^+$$

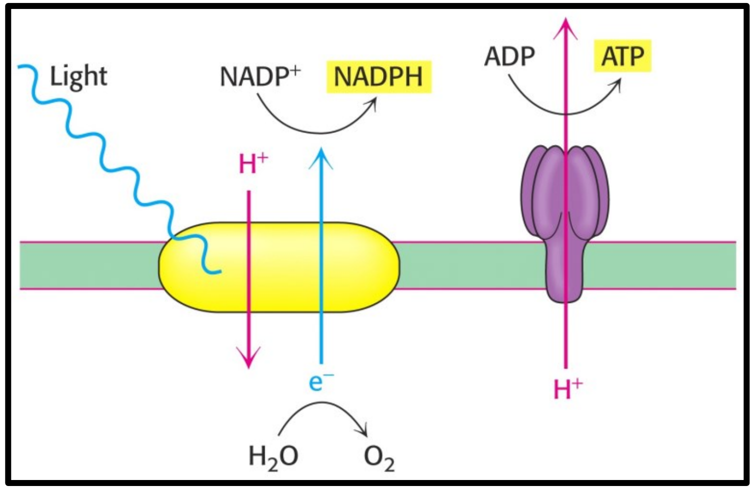
reducing agent

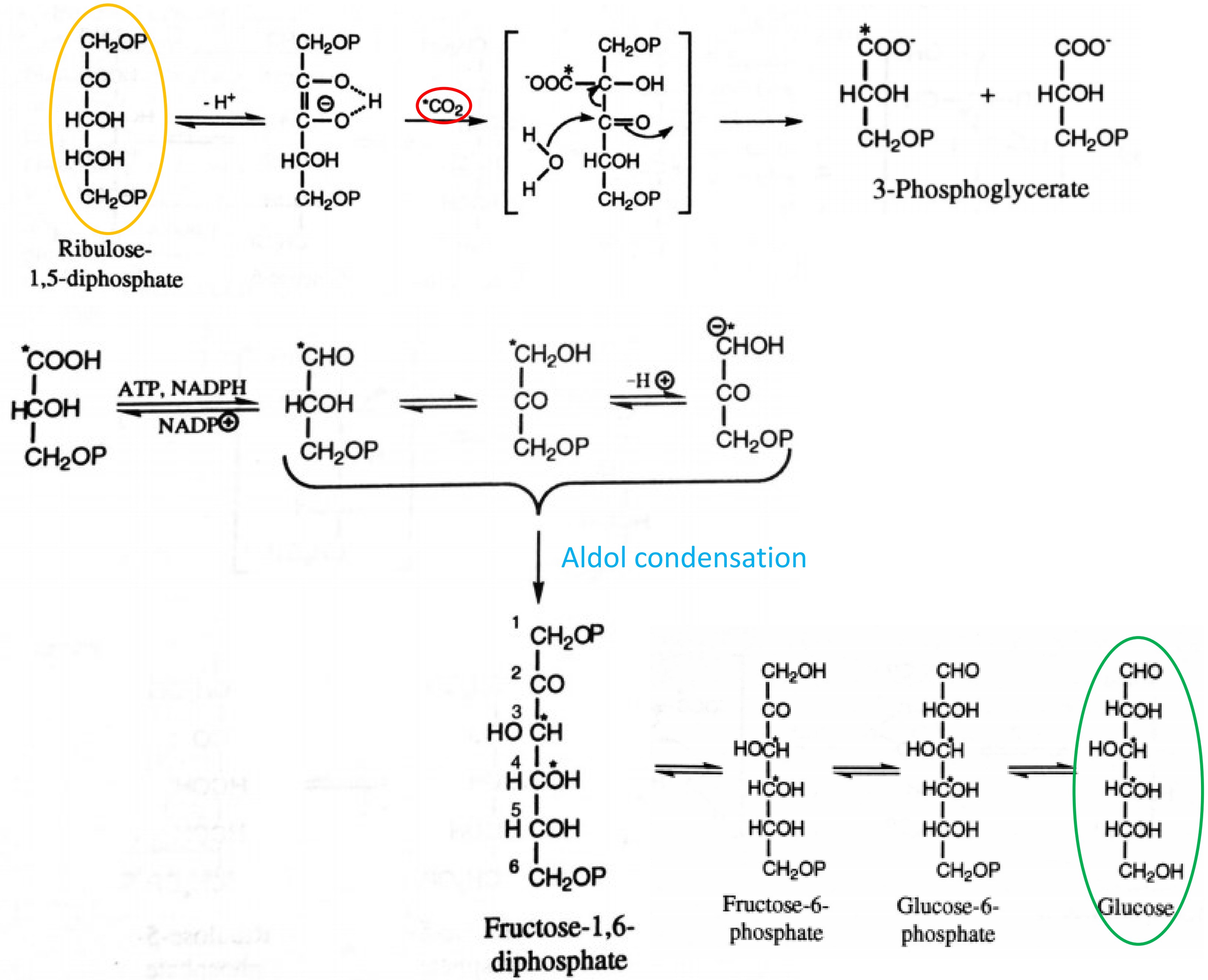
ATP will be used to phosphorylate some groups, making them more reactive.

The oxygen here comes from water, and not CO_2.

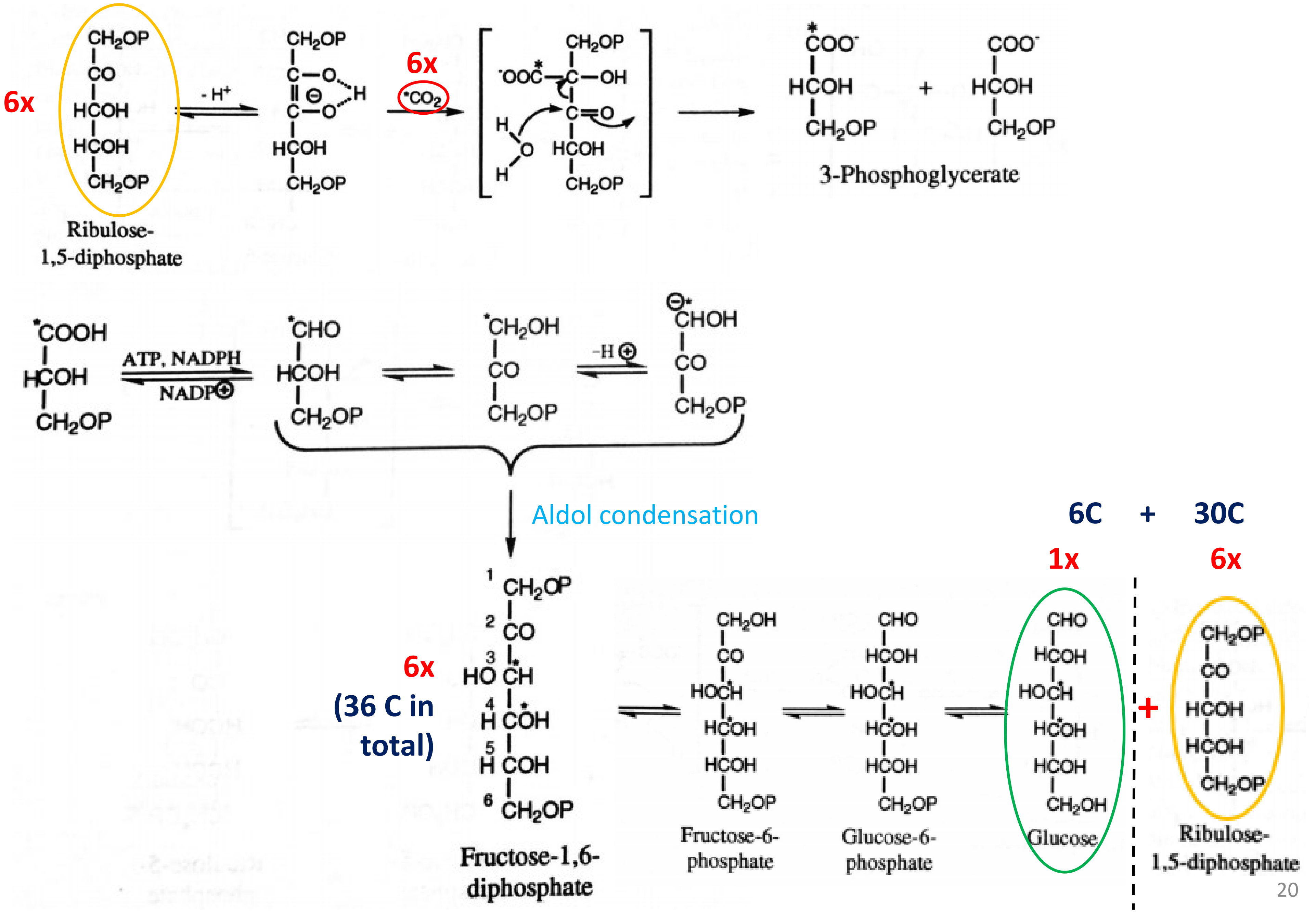
$$2H_2O \xrightarrow{h\nu} 4\,H^+ + 4e^- + O_2$$

- 2- Dark reaction: CO_2 is reduced with the help of NADPH and ATP.
  - Very complex mechanism





- H+
*CO2
Ribulose-
1,5-diphosphate
3-Phosphoglycerate
ATP, NADPH
NADP⊕
-H⊕
Aldol condensation
Fructose-1,6-
diphosphate
Fructose-6-
phosphate
Glucose-6-
phosphate
Glucose



6x
Ribulose-1,5-diphosphate
- H+
6x
*CO2
3-Phosphoglycerate
ATP, NADPH
NADP⊕
-H⊕
Aldol condensation
6C + 30C
1x
6x
6x
(36 C in total)
Fructose-1,6-diphosphate
Fructose-6-phosphate
Glucose-6-phosphate
Glucose
Ribulose-1,5-diphosphate

Carbohydrate biosynthesis in plants: Photosynthesis

- 1- Light reaction

energy rich

$$H_2O + NADP^+ + P + ADP \xrightarrow{h\nu} \tfrac{1}{2}O_2 + NADPH + ATP + H^+$$

reducing agent

ATP will be used to phosphorylate some groups, making them more reactive.

The oxygen he

$$2H_2O \xrightarrow{h\nu} 4\,H^+ + 4e^- + O_2$$

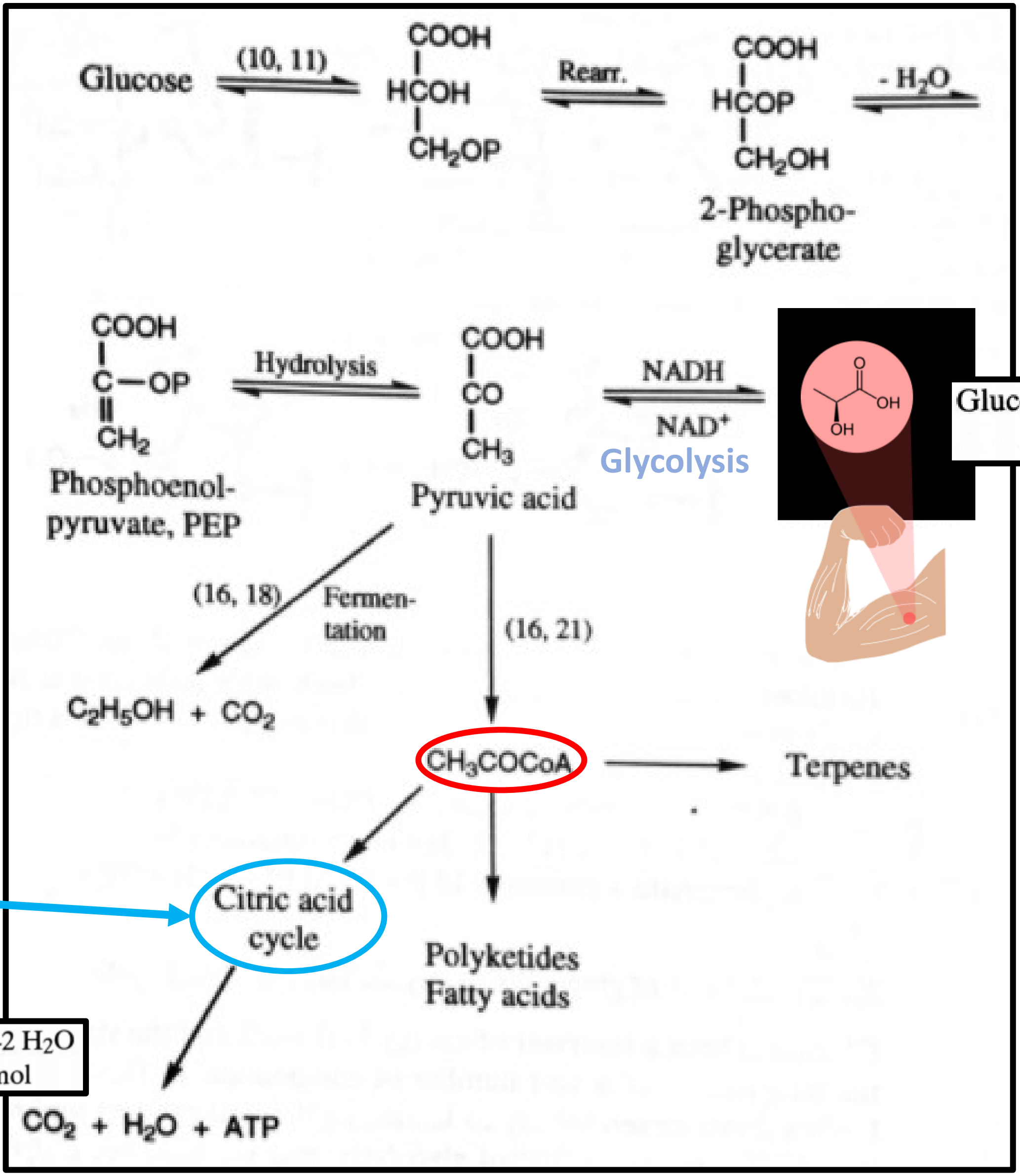
r, and not $CO_{2.}$

- 2- Dark reactio

$$6 \text{ Ribulose-1,5-diphosphate} + 6\,CO_2 + 18\,ATP + 12\,NADPH + 12\,H^{\oplus} + 12\,H_2O \rightarrow 6 \text{ ribulose-1,5-diphosphate} + \text{glucose} + 18\,P + 18\,ADP + 12\,NADP^{\oplus}$$

DPH and ATP.

Breakdown of glucose



Glucose + 2 P + 2 ADP → 2 lactate + 2 ATP + 2 H_2O

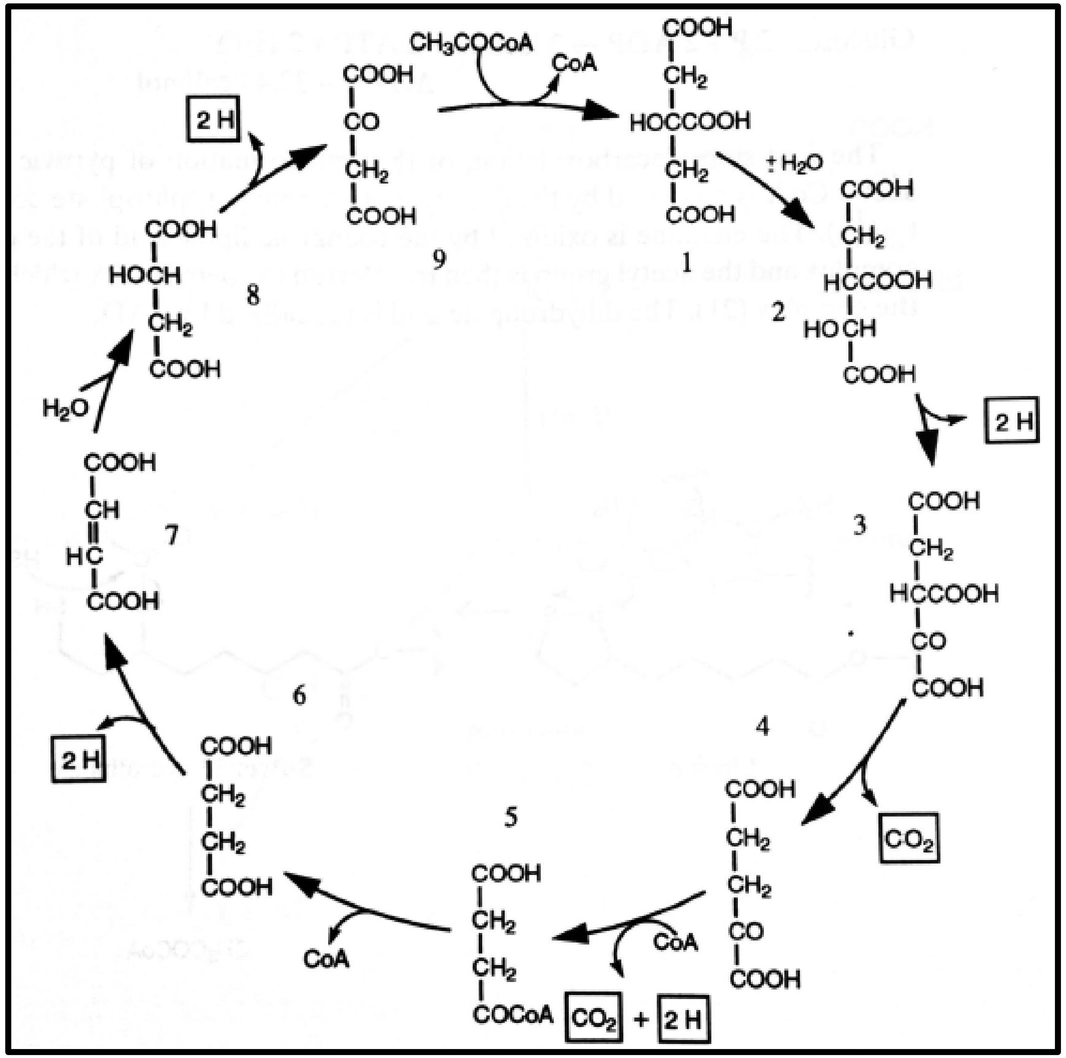
$\Delta G^{O'} = -32.4$ kcal/mol

Coupled with oxidative phosphorylation, the citric acid cycle is the essence of respiration

Glucose + 6 O_2 + 36 P + 36 ADP → 6 CO_2 + 36 ATP + 42 H_2O

$\Delta G^{O'} = -423$ kcal/mol

The citric acid cycle

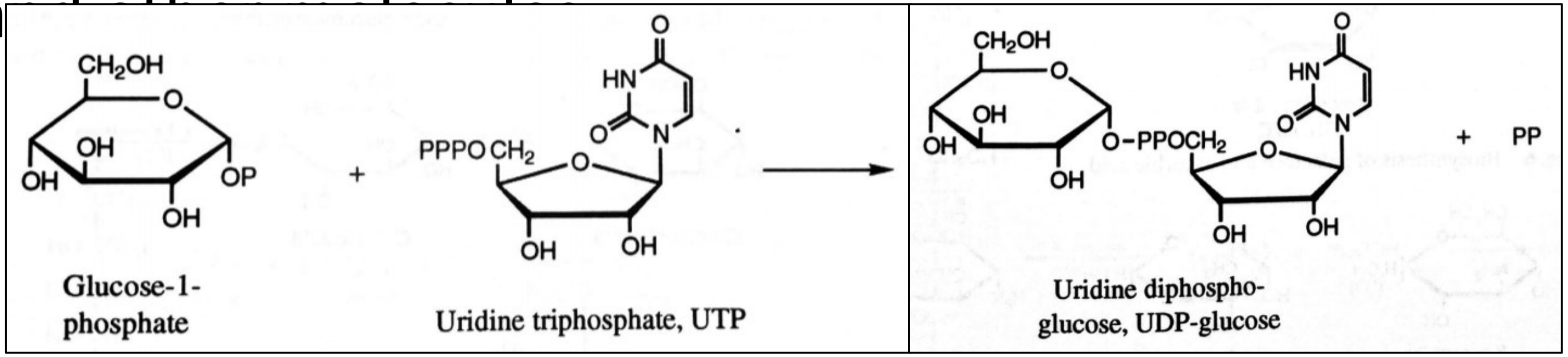


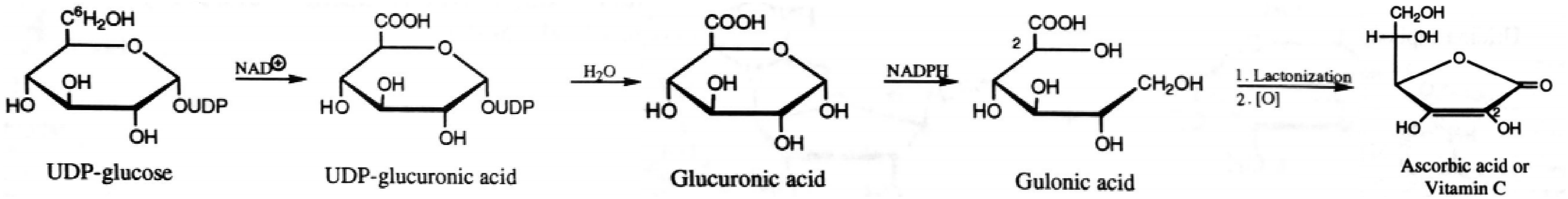
- 1 molecule of acetyl CoA is consumed.
- 8 H and 2 CO_2 are released.
- The 8 H are oxidized to water with production of free energy and ATP

- The citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant.
- It removes electrons from acetyl CoA and uses these electrons to form NADH and $FADH_2$, which will be reoxidized in oxidative phosphorylation.

Formation of monosaccharides

- Glucose is the precursor of other hexoses, disaccharides, polysaccharides, glycosides a





Example: Biosynthesis of vitamin C

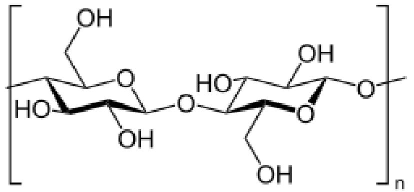
Disaccharides and polysaccharides

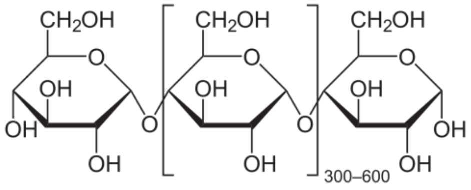
- Disaccharides:
  - 2 monosaccharides joined by an acetal or ketal link
  - Classified according to their reducing power
    - Non-reducing: The carbonyl function is blocked as acetal (for instance sucrose)
    - Reducing: For instance lactose

sucrose

lactose

- Polysaccharides:
  - Long chain of linear or branched monosaccharides, used as storage (starch,





Iceland Liechtenstein Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

University of Bucharest

NTNU
Norwegian University of Science and Technology

Chapter 4-1
The Shikimic Acid Pathway

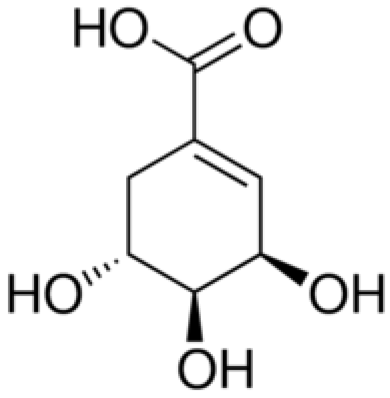
Elisabeth Jacobsen and Susanne Hansen Troøyen, NTNU

Spring 2022

Shikimic acid

Shikimic acid could replace the aromatic amino acids in *E.coli* mutants

- It has to be an intermediate in their biosynthesis



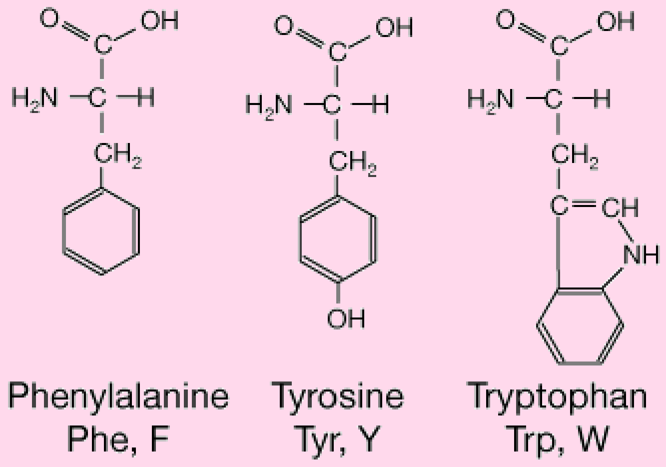
Shikimic acid

Illicium anisatum

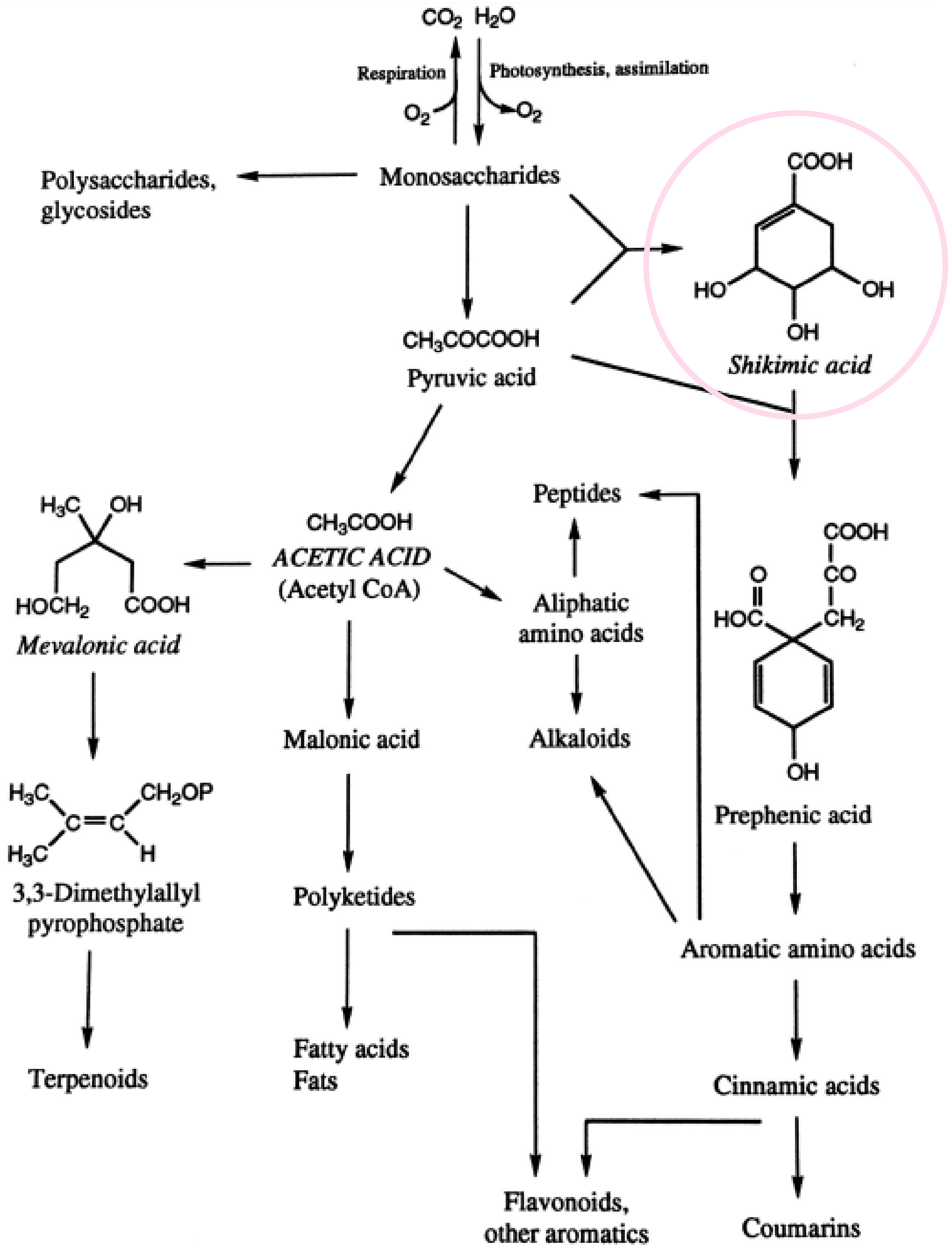
Shikimic acid was first isolated from japanese star anise, which is where it got its name.

Shikimic acid pathway

Biosynthesis of aromatic amino acids



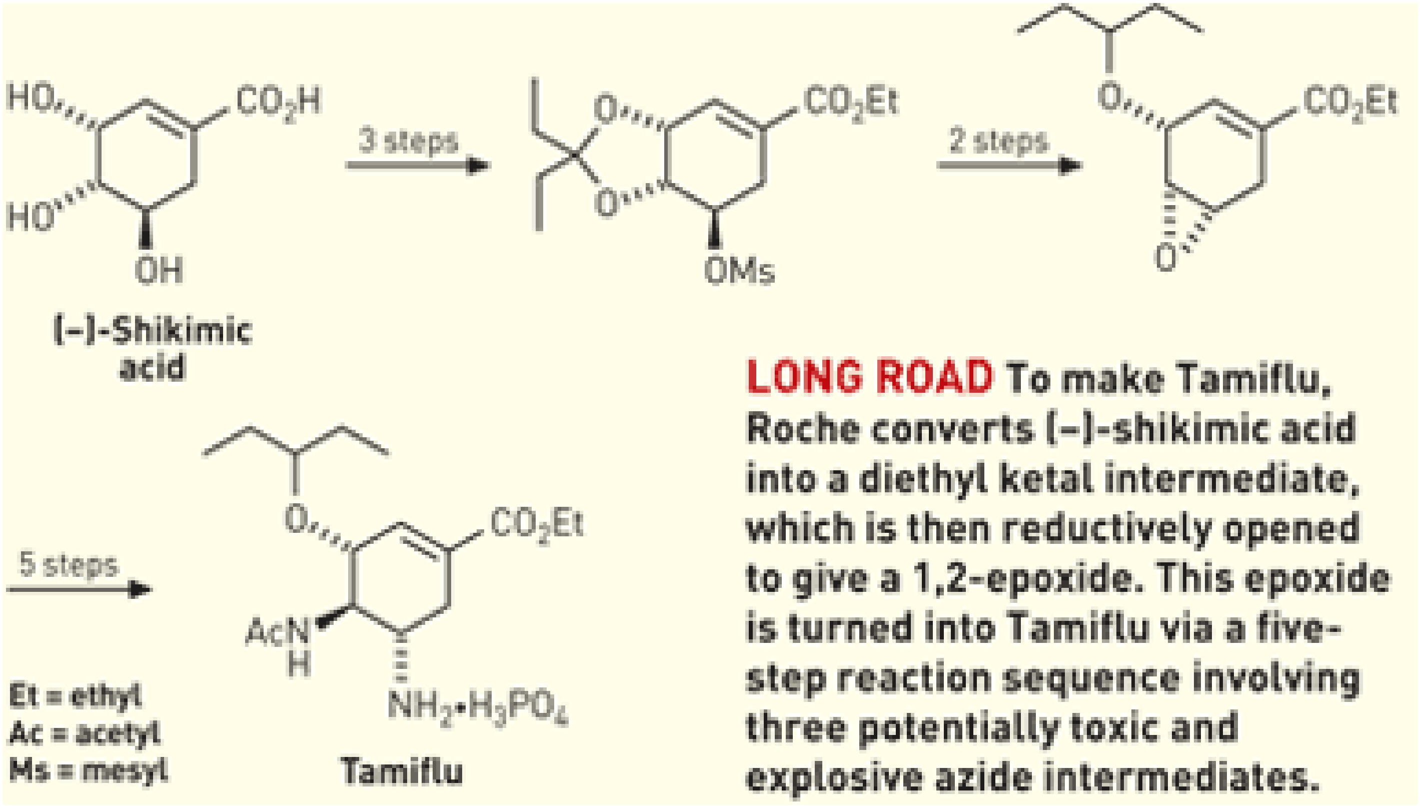
This biosynthetic pathway is present in bacteria, funghi and higher plants – but not in mammals.



Tamiflu

Tamiflu = oseltamivir phosphate

Important drug for bird flu (and other influenza viruses).

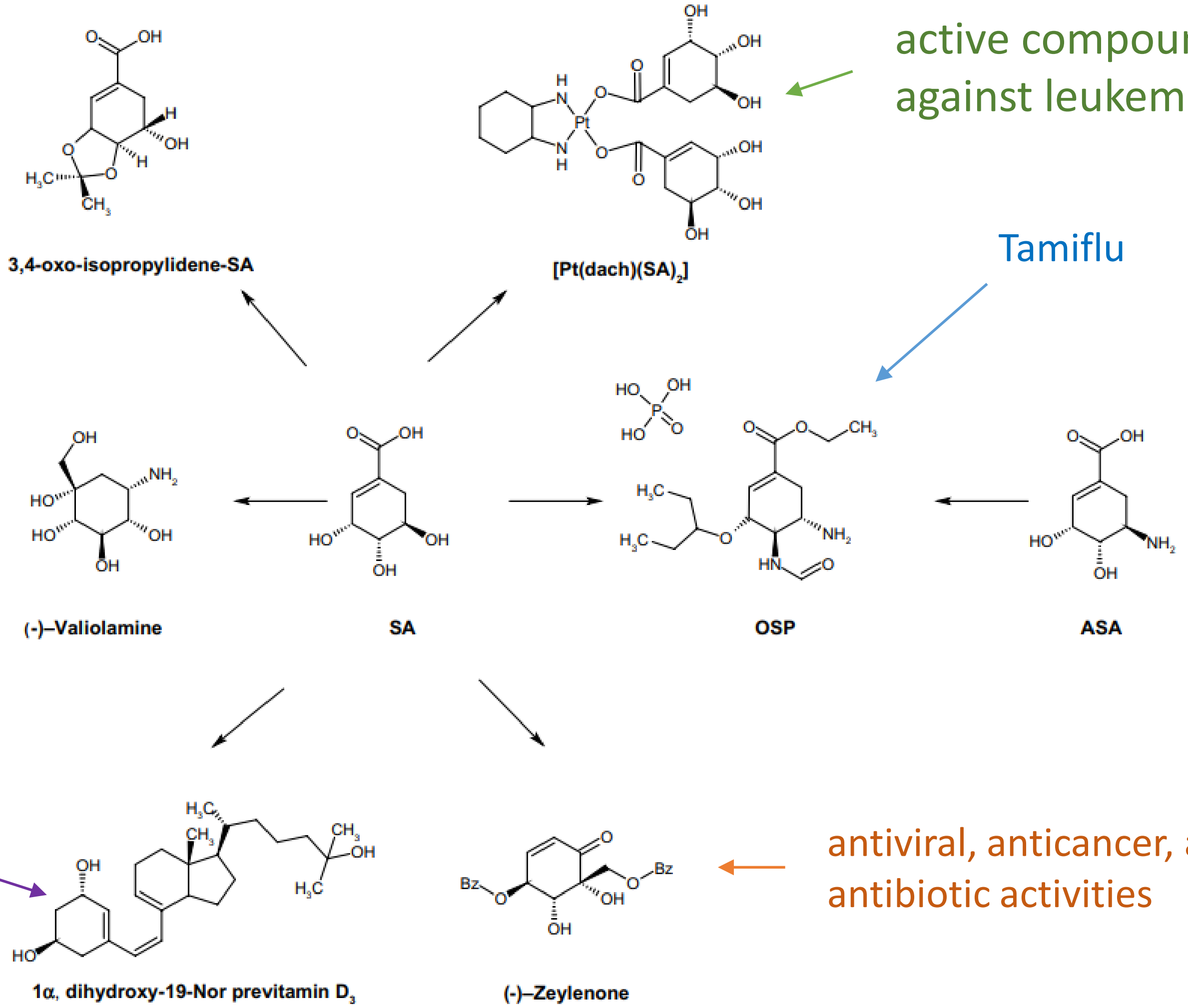


Chem. & Eng. News Aug. 29, 2005, Vol 28, No 35, p 22

Pharmaceutical products derived from SA

active compound against leukemia

Tamiflu

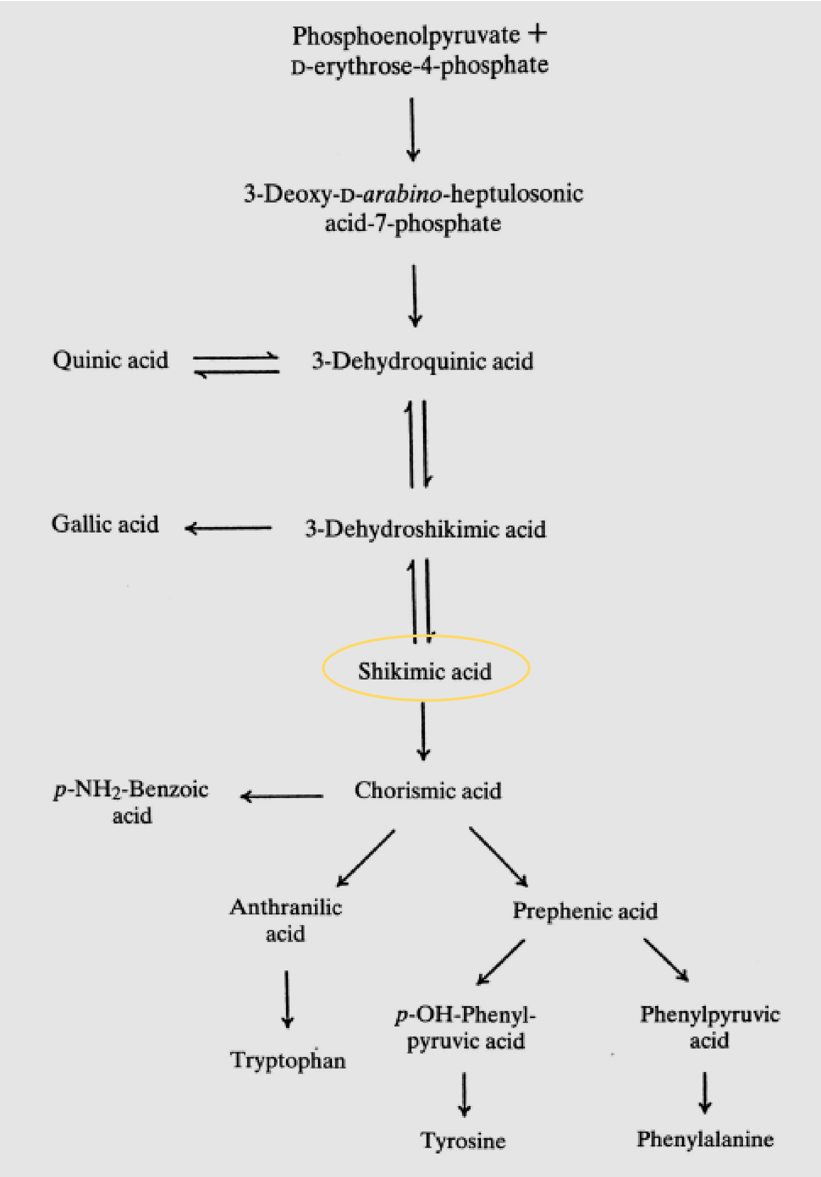


promise for the treatment of osteoporosis

antiviral, anticancer, and antibiotic activities

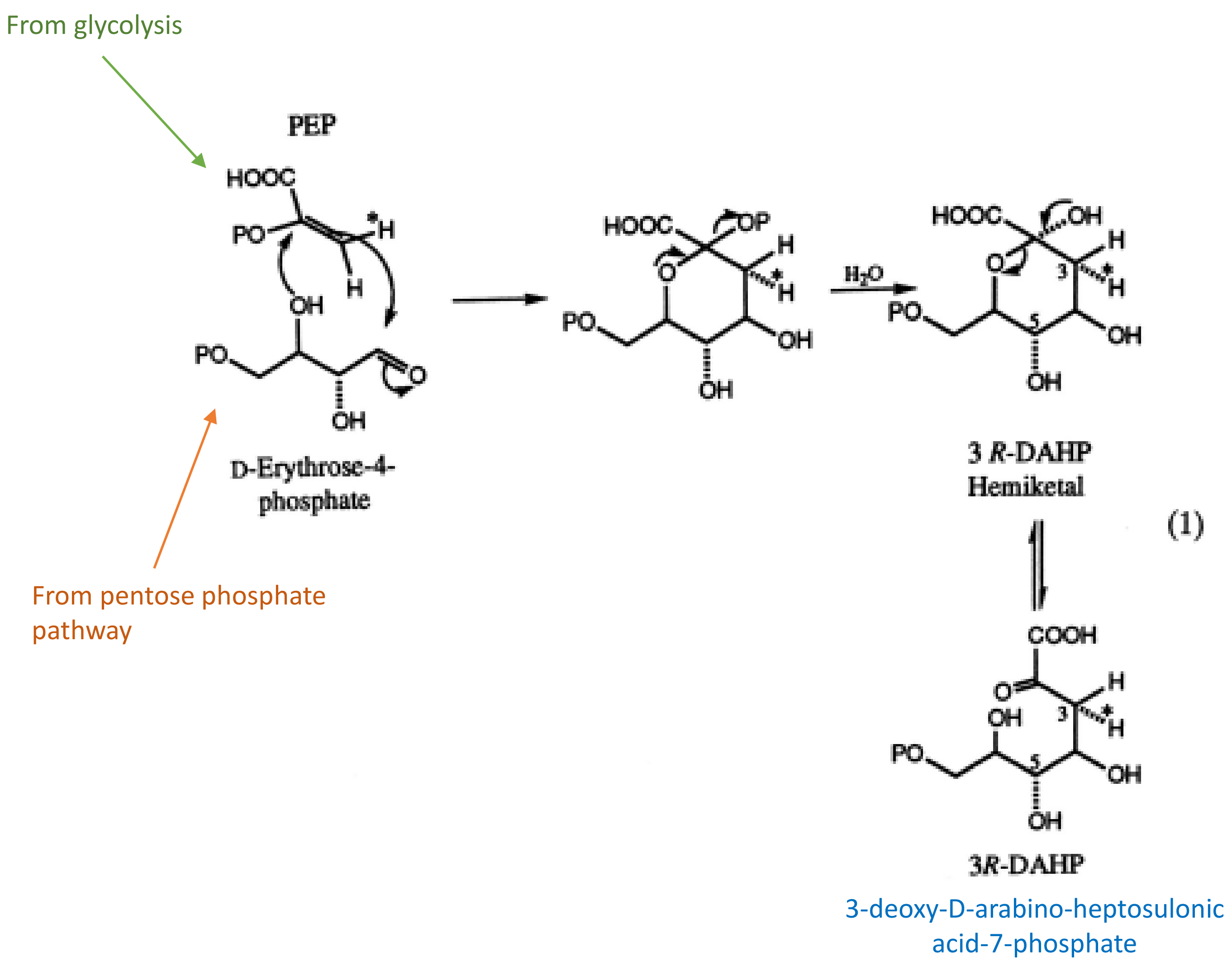
Díaz Quiroz *et al.* Annu Rep Med Chem 2014; 4:35-46

Shikimic acid pathway



Step 1:
Condensation of D-Erythrose-4-phosphate with phosphoenol pyruvate (PEP)

- Catalyzed by DAHP synthases
- Stereospecific reaction
  - Si face of PEP adds to Re face of erythrose-4-phosphate.
- Gives 3-deoxy-D-arabino-heptosulonic acid-7-phosphate (DAHP)



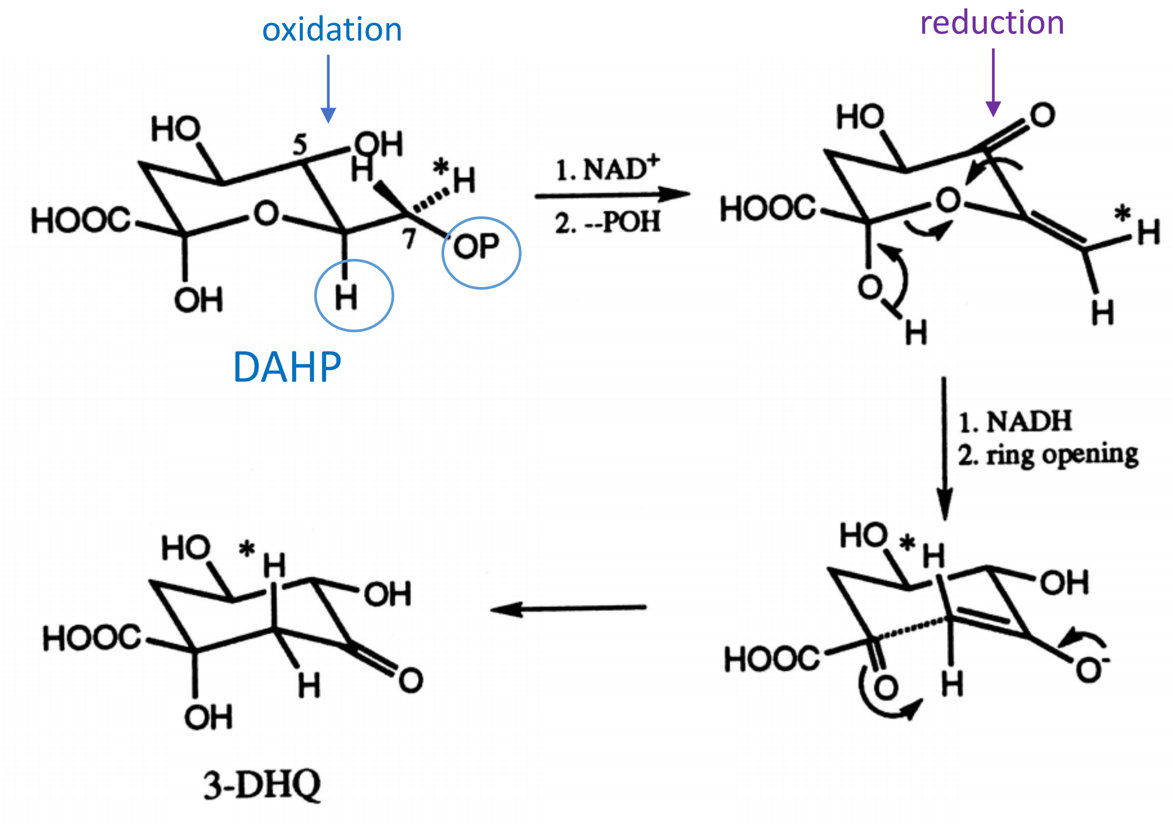
Step 2:
Formation of 3-dehydroquinic acid (DHQ)

- Catalyzed by DHQ synthase
- NAD+/NADH

α-β-elimination of phosphate group (cis elimination)
Oxidation at C5

Reduction at C5
Ring opening

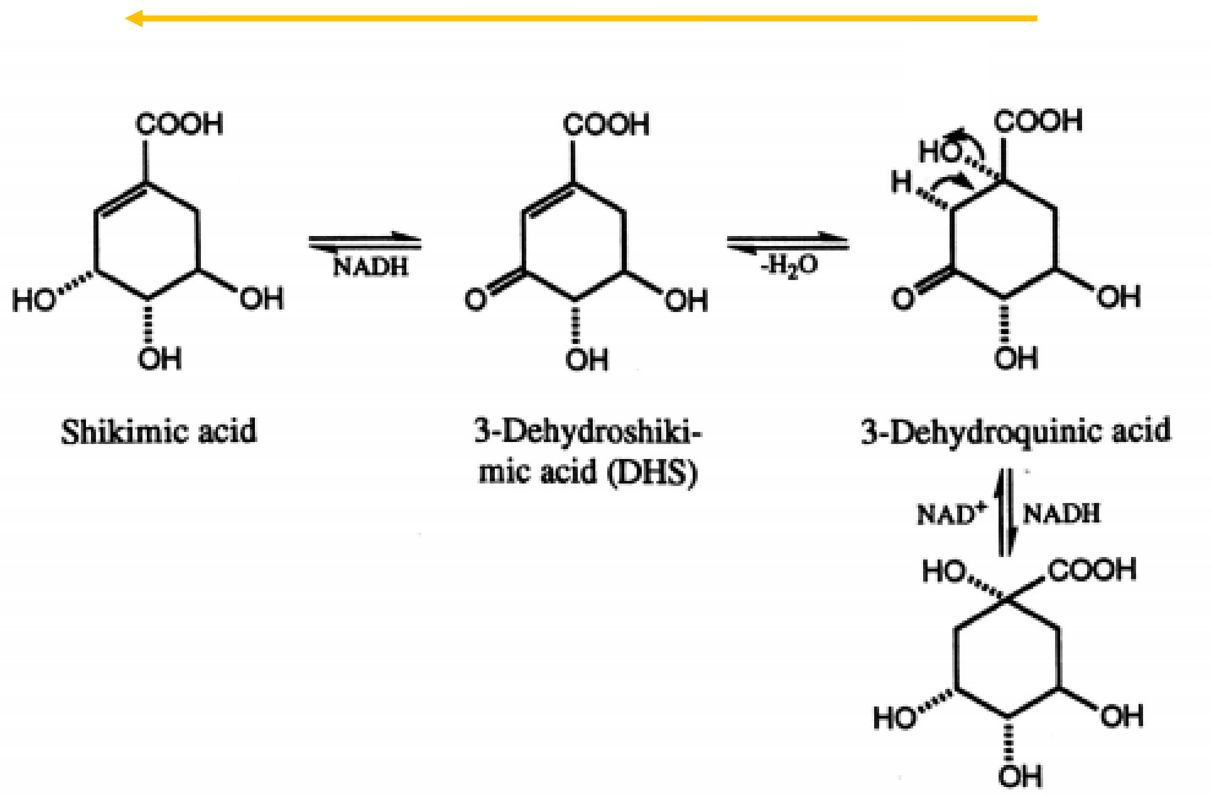
Ring closing forms 3-dehydroquinic acid.



Step 3:
Dehydration and reduction

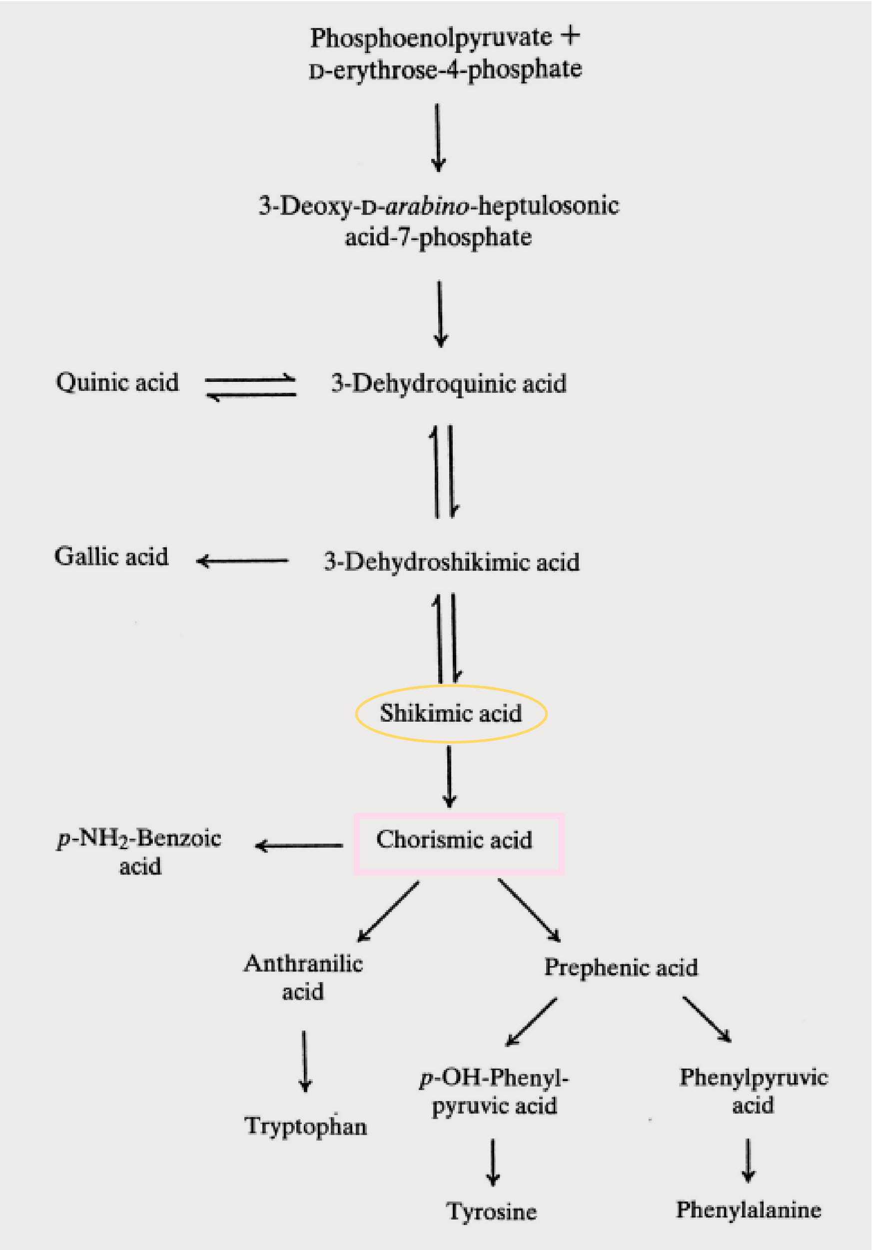
- Catalyzed by DHQ dehydratase and shikimic acid dehydrogenase

Quinic acid
Abundant in nature
Once it is formed it is not so easily metabolized
Except many microorganisms that can convert it back to DHQ



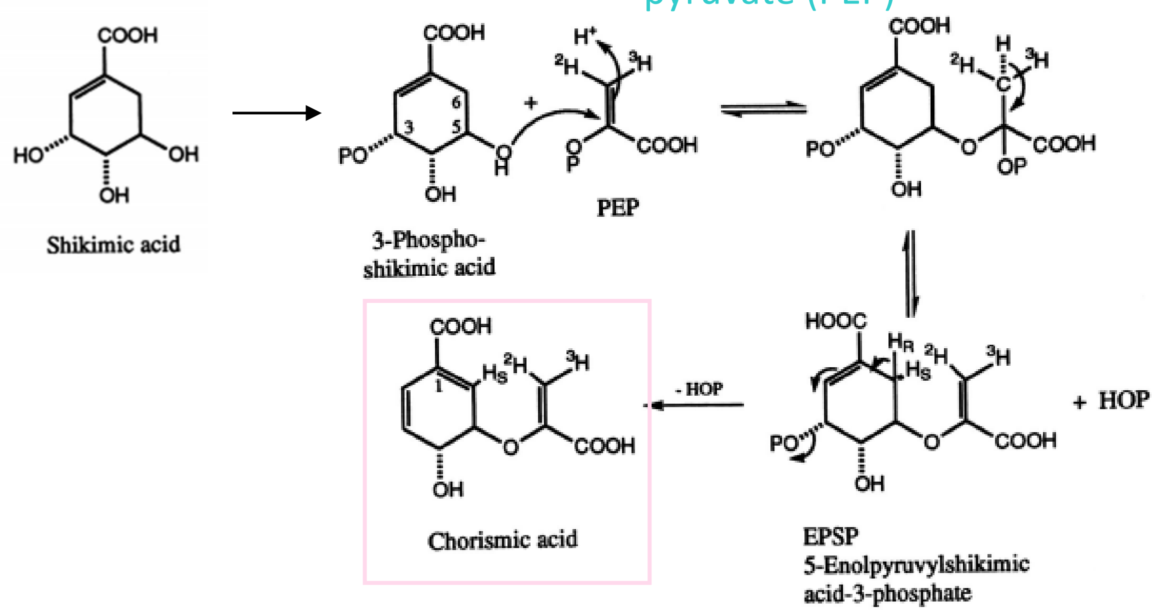
Quinic acid

Conversion of shikimic acid to chorismic acid



Phosphorylation
(creates a better LG for elimination that follows)

Addition of phosphoenol pyruvate (PEP)



Eliminations of phosphoric acid (HOP)

Biosynthesis of aromatic amino acids

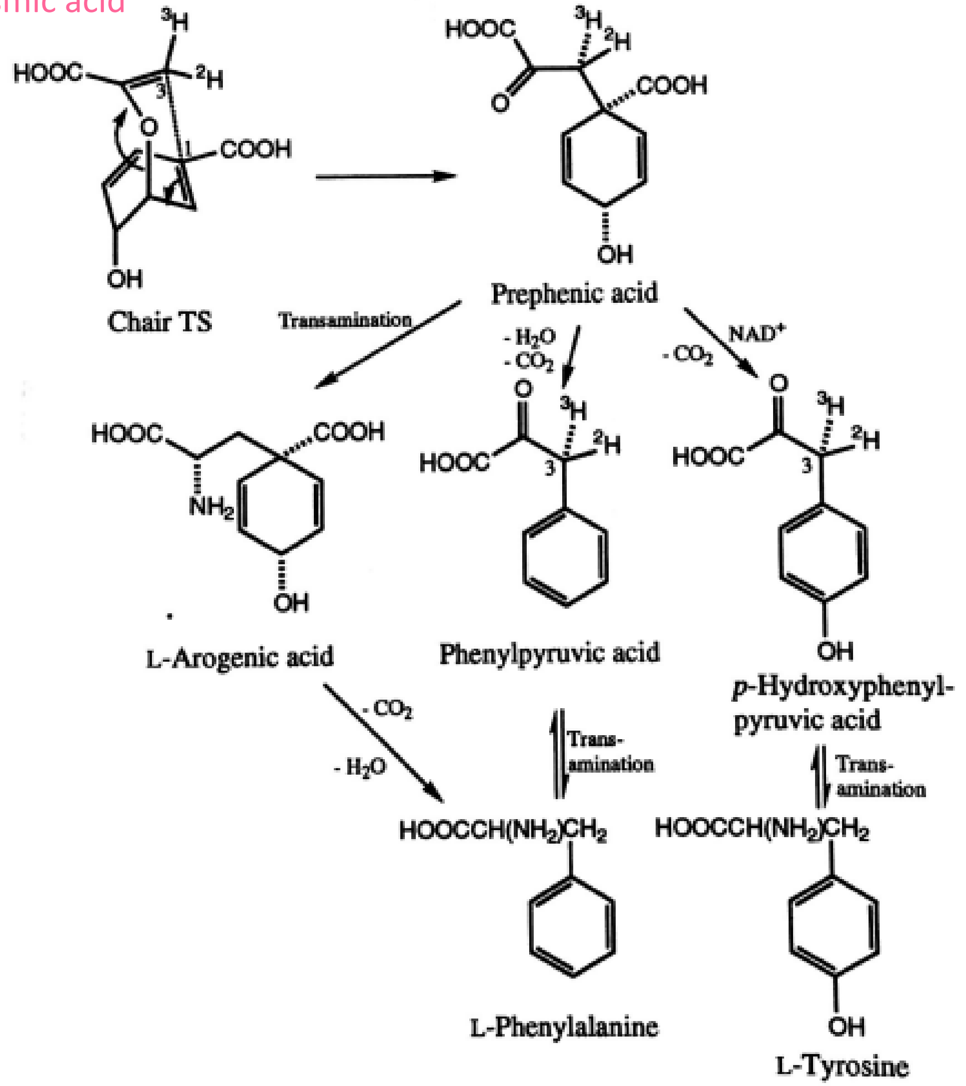
Claisen rearrangement to prephenic acid

L-phenylalanine
Rapid *in vitro* conversion to phenylpyruvic acid
Transamination to phenylalanine

Some organisms can do transamination directly from prephenic acid

L-tyrosine
Conversion to *p*-hydroxyphenylpyruvic acid
Transamination to tyrosine

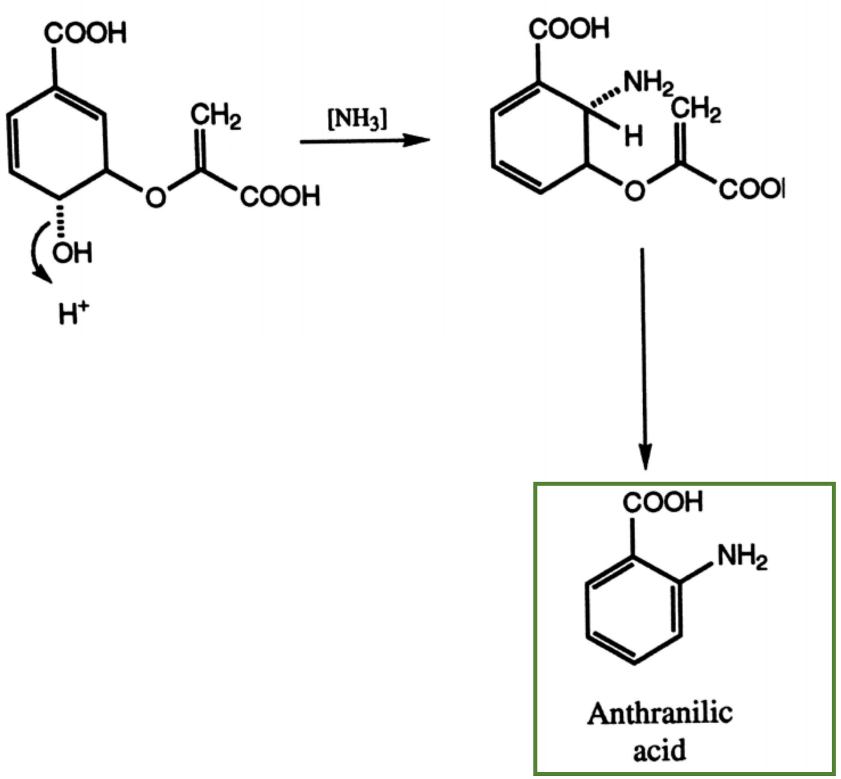
Chorismic acid



Biosynthesis of aromatic amino acids

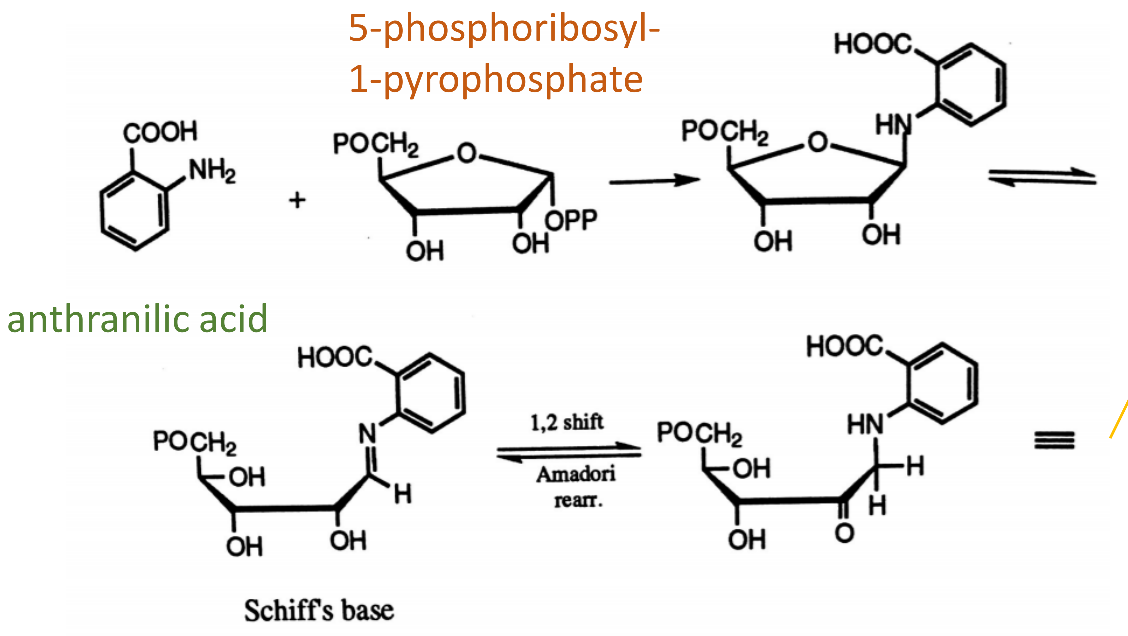
Conversion of chorismic acid to anthranilic acid:

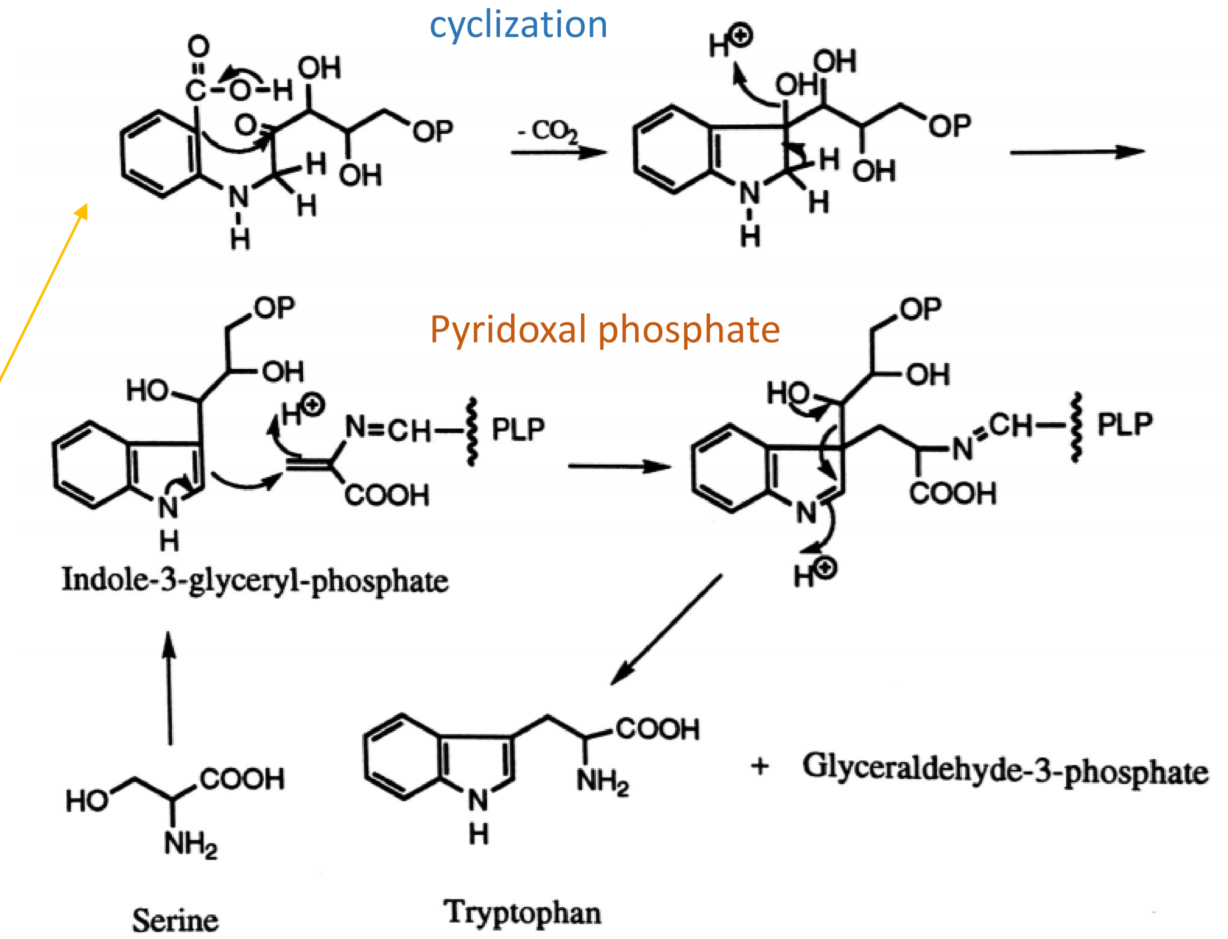
L-tryptophan biosynthesis

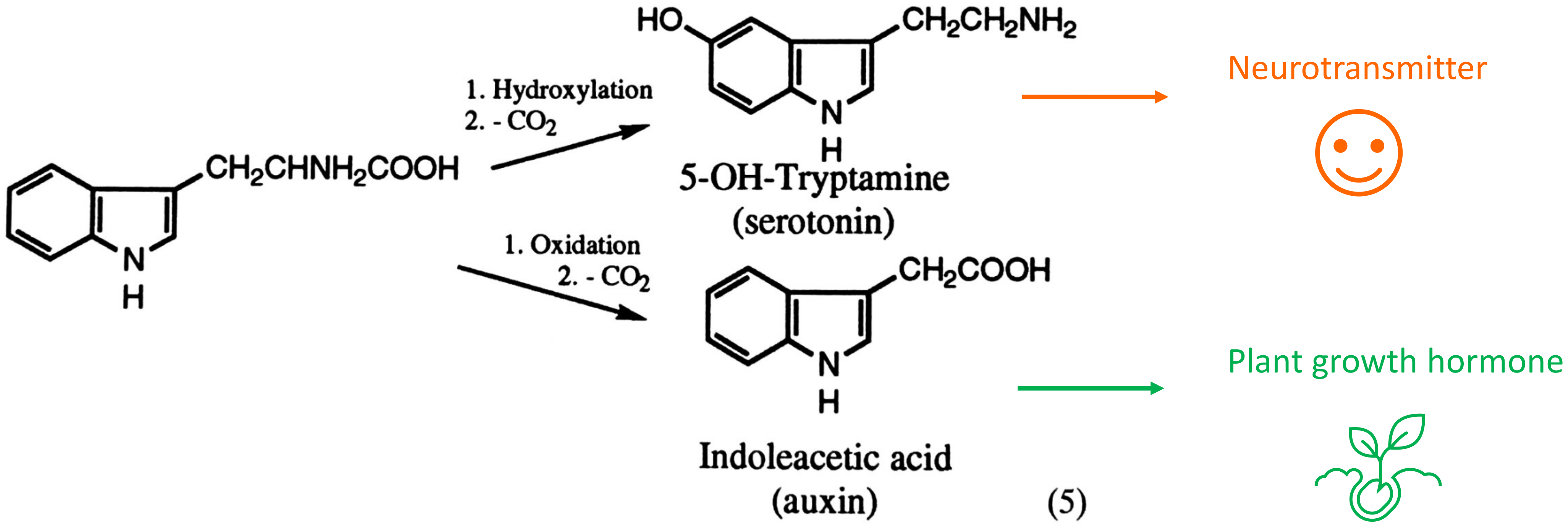


Biosynthesis of aromatic amino acids

L-tryptophan biosynthesis






CH2CHNH2COOH
N
H
1. Hydroxylation
2. - CO2
1. Oxidation
2. - CO2
HO
CH2CH2NH2
5-OH-Tryptamine
(serotonin)
CH2COOH
Indoleacetic acid
(auxin)
Neurotransmitter
Plant growth hormone

(5)

Biological hydroxylation – redox reactions

- Oxidation and reduction of carbonyls

$$-CH_2OH \rightleftharpoons -CHO \rightleftharpoons -COOH$$

- Oxygen insertion into unactivated C-H bonds

Catalyzed by:

- Dehydrogenases (without molecular O_2)

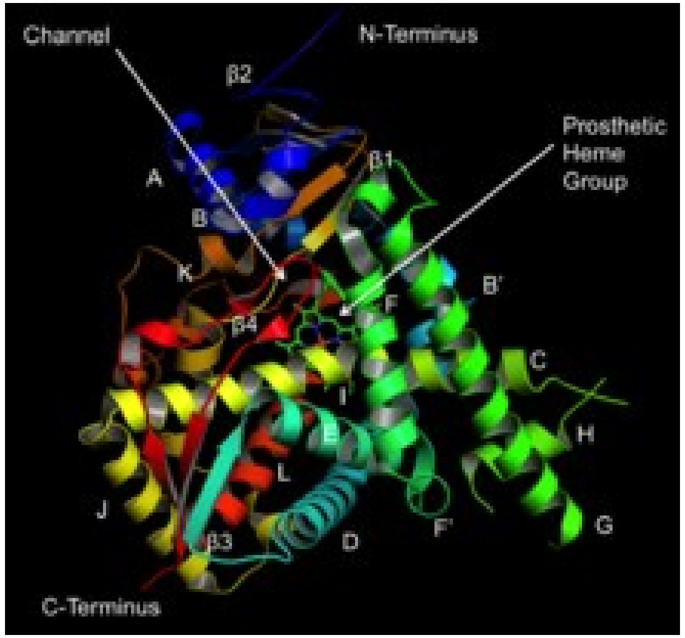
$$RH_2 + NAD^+ \leftrightarrow R + NADH + H^+$$

- Oxidases (electron transfer to O_2)

$$RH_2 + O_2 \leftrightarrow R + H_2O_2$$

- Oxygenases (incorporating molecular oxygen)

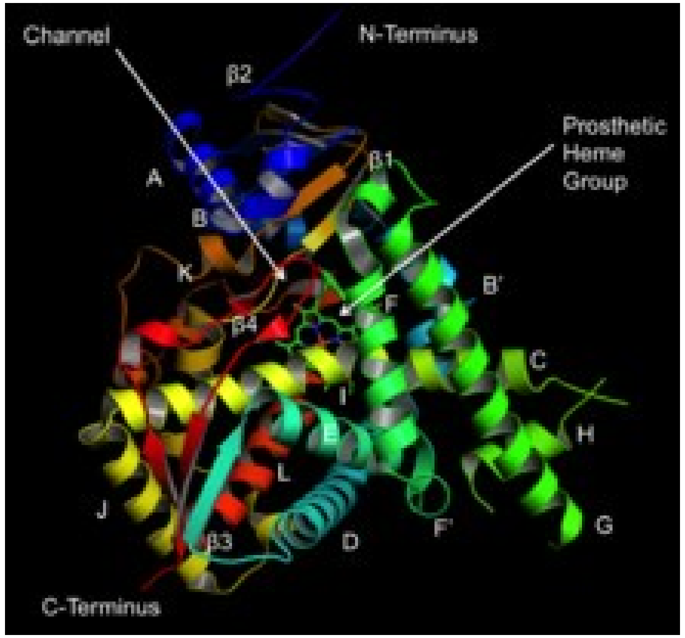
$$RH_2 + O_2 + NADPH + H^+ \rightarrow RHOH + H_2O + NADP^+$$



Cytochrome P450s are monooxygenases

Biological hydroxylation

- Monooxygenases are especially important
  - Can oxidize non-activated hydrocarbons
    - Steroids
    - Fatty acid α-oxidation
    - Hydroxylation of aromatics
      - i.e. Conversion of phenylalanine to tyrosine
    - Oxidation of amino groups to nitro groups
    - Dealkylation of amines, ethers, thioesters
    - etc.



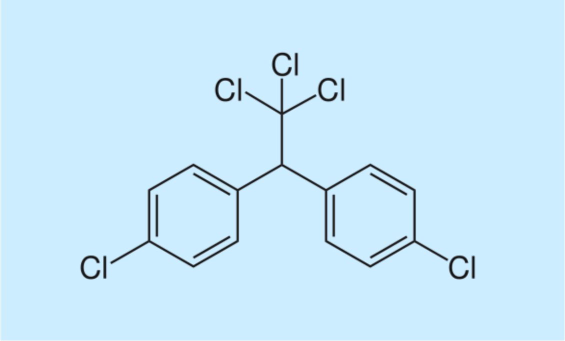
Cytochrome P450s are monooxygenases

Biological hydroxylation

- Drug metabolism
  - Oxidizing foreign substances make them more polar, easier to excrete

Example: DDT is hard to oxidize

- Accumulated in fat cells – dangerous!
- DDT is a pesticide – banned in the 70s



Dichlorodiphenyl-
trichloroethane (DDT)

Spraying DDT in Oregon, USA, 1955

ENVIRONMENT

Malaria Control

Resurging use of the banned pesticide DDT to prevent malaria poses dilemma for health, environment

by **Bette Hileman**

JULY 24, 2006 | APPEARED IN **VOLUME 84, ISSUE 30**

Late last year, the U.S. Agency for International Development (**USAID**), after a long hiatus, announced that it would fund DDT spraying on the inside walls of houses to prevent malaria. For many years, USAID had supported the DDT spraying, but after the pesticide was banned in developed countries, USAID stopped funding its use.

Spraying DDT on interior walls presents a dilemma. It may be the cheapest, most effective way to reduce malaria deaths in some parts of Africa. But the DDT intended for interior spraying may end up on crops, endangering wildlife and beneficial insects. Also, new evidence indicates that prenatal exposure to DDT may retard child development and lead to preterm birth.

Beginning in 1945, DDT was used extensively to eradicate malaria-carrying mosquitoes from all of southern Europe and the southern U.S. It was also widely employed for malaria control in Asia, Latin America, and Africa. By 1966, according to the **U.S. National Academy of Sciences**, DDT had saved 500 million lives.

DDT was banned in the developed world in the early 1970s because of its environmental effects. Thereafter, DDT spraying for mosquito control ceased in most of Africa, though its use was continued relatively unnoticed in South Africa, Botswana, Indonesia, and India. Declining foreign aid budgets also contributed to dwindling efforts to control mosquitoes with other insecticides or with alternative methods in less developed countries.

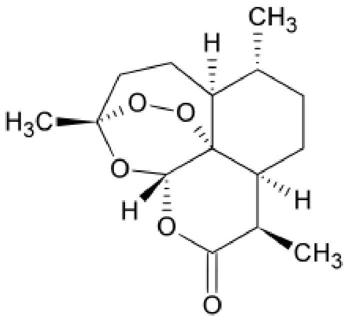
LINE OF DEFENSE

[+]Enlarge

Credit: USAID

Lambda-cyhalothrin insecticide is being sprayed on the walls of a house in Angola to prevent malaria. Spraying in Angola started last December.

"Malaria parasites have become resistant to the commonly used, inexpensive drugs chloroquine and sulfadoxine-pyrimethamine. The newer, more effective artemisinin drugs are prohibitively expensive for poor African patients."



Artemisinin

Chem & Eng News, July 24, 2006, Volume 84, Number 30, pp. 30-31