UNIT-I

Acetate Pathways

Presented By

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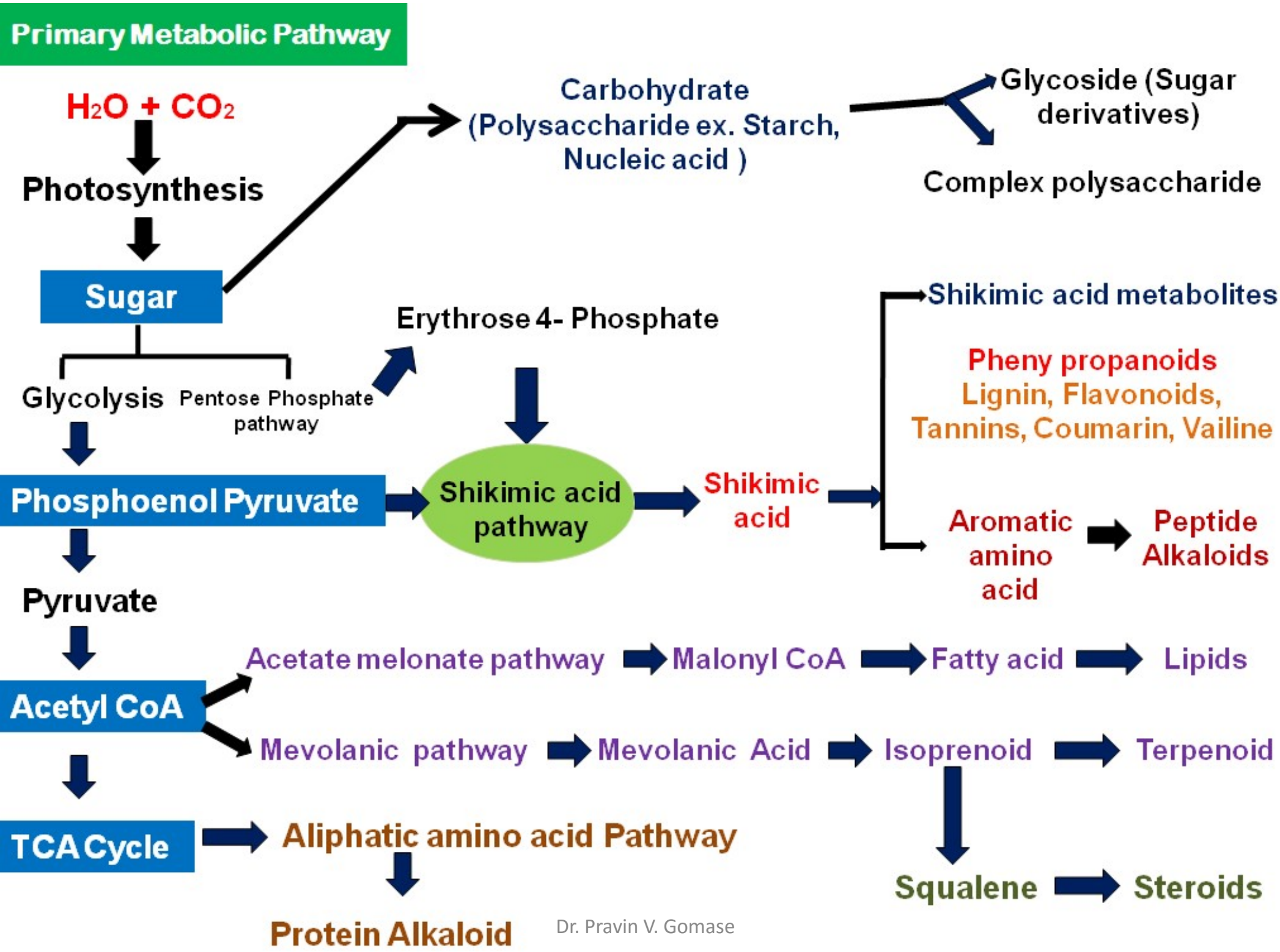
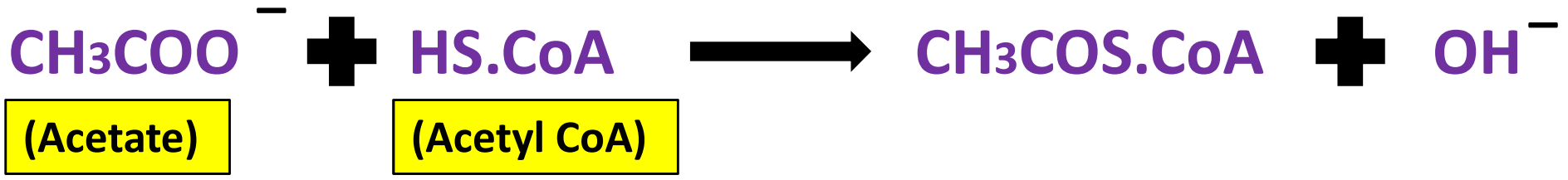
What is Biosynthesis ?

- Living plants are solar-powered biochemical and biosynthetic laboratory which manufactures both **primary and secondary metabolites** from air, water, minerals and sunlight
- Metabolites are organic compound **synthesized by organisms using enzyme-mediated chemical** reaction called **metabolic pathways. Or**
- The reaction sequence occurring with in the organisms in an orderly and regulate way known as **metabolic pathways**.


Primary Metabolic Pathway
H2O + CO2
Photosynthesis
Sugar
Carbohydrate
(Polysaccharide ex. Starch,
Nucleic acid)
Glycoside (Sugar
derivatives)
Complex polysaccharide
Glycolysis
Pentose Phosphate
pathway
Erythrose 4- Phosphate
Phosphoenol Pyruvate
Shikimic acid
pathway
Shikimic
acid
Shikimic acid metabolites
Pheny propanoids
Lignin, Flavonoids,
Tannins, Coumarin, Vailine
Aromatic
amino
acid
Peptide
Alkaloids
Pyruvate
Acetate melonate pathway
Malonyl CoA
Fatty acid
Lipids
Acetyl CoA
Mevolanic pathway
Mevolanic Acid
Isoprenoid
Terpenoid
TCA Cycle
Aliphatic amino acid Pathway
Squalene
Steroids
Protein Alkaloid
Dr. Pravin V. Gomase

Acetate Pathway

- It is one of the most important pathway for the production of **secondary metabolites**.
- In this pathway acetate is used in most biochemical reaction is **acetyl CoA.**
- Acetate mainly formed from carbohydrates via pyruvic acid (oxidative decarboxylation)
- Acetate occupies central position in relation to general metabolism.



Acetate Pathway

- **It consist of two main pathway**

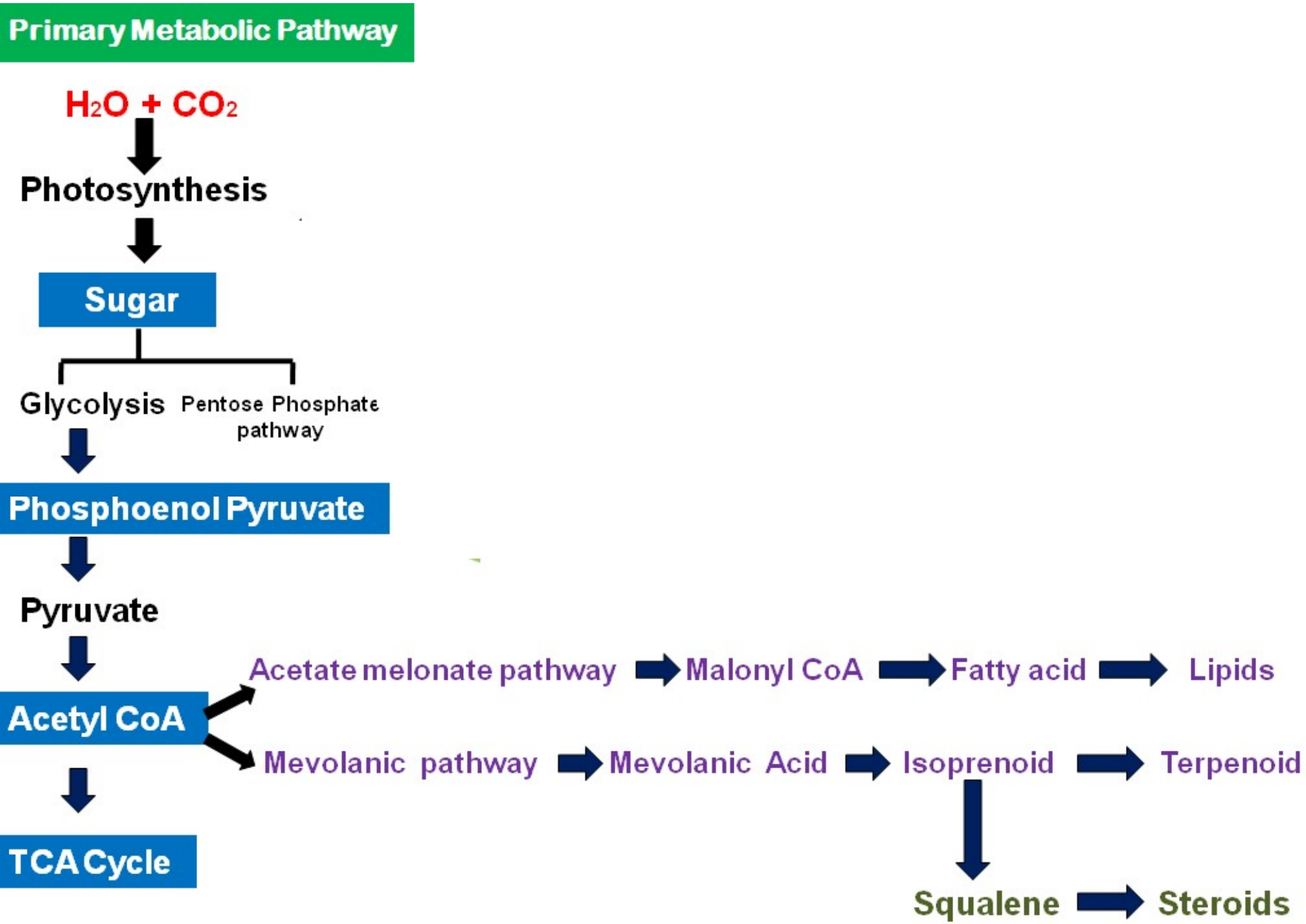
1. **Acetate Mevalonate Pathway/ Isoprenoid Pathway**
2. **Acetate Melonate Pathway/ Fatty acid & Polyketides.**

- Acetate Pathway is also known as **Mevalonate pathway** or **HMG-CoA reductase pathway**.

- It is an essential metabolite pathway present in Eukaryotes, archaea and some bacteria.

- This produced two 5 Carbon building blocks called **Isopentenyl Pyrophosphate (IPP)** and **Dimethyle alkyl-pyrophosphate (DMAPP).**

- The Mavalonate pathway starts with **Acetyl CoA** and ends with production of **IPP and DMAPP.**


Primary Metabolic Pathway
H2O + CO2
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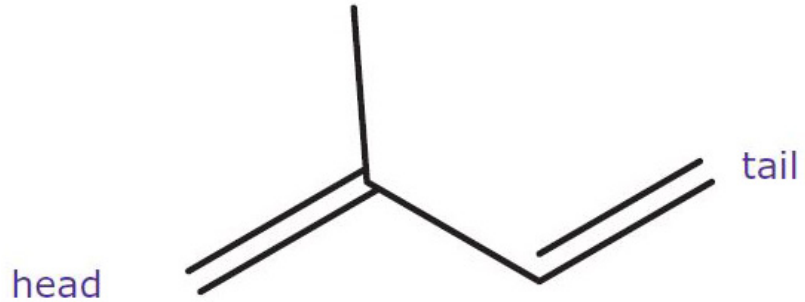
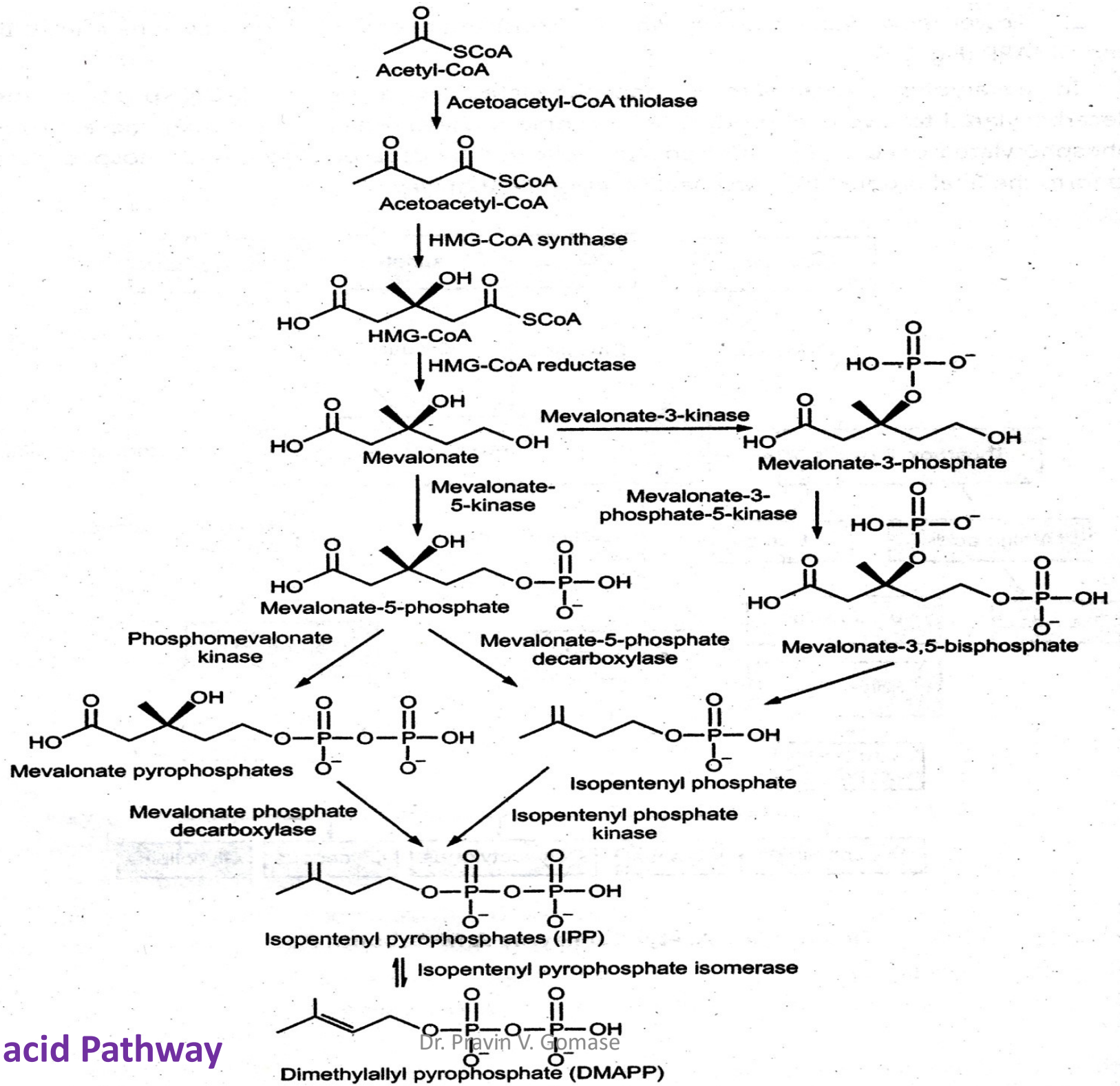
Phosphoenol Pyruvate
Pyruvate
Acetyl CoA
TCA Cycle
Acetate melonate pathway
Malonyl CoA
Fatty acid
Lipids
Mevolanic pathway
Mevolanic Acid
Isoprenoid
Terpenoid
Squalene
Steroids

Terpenoid:

- The terpenoids, sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene, and the isoprene polymers called terpenes. Like C10 (monoterpenes), C15 (Sesquiterpenes) and C20 (diterpenes).
- Isoprene rule: Terpenoids are derived from "isoprene units" **(C_5H_8)**



Isoprene (2-methyl-1,3-butadiene)



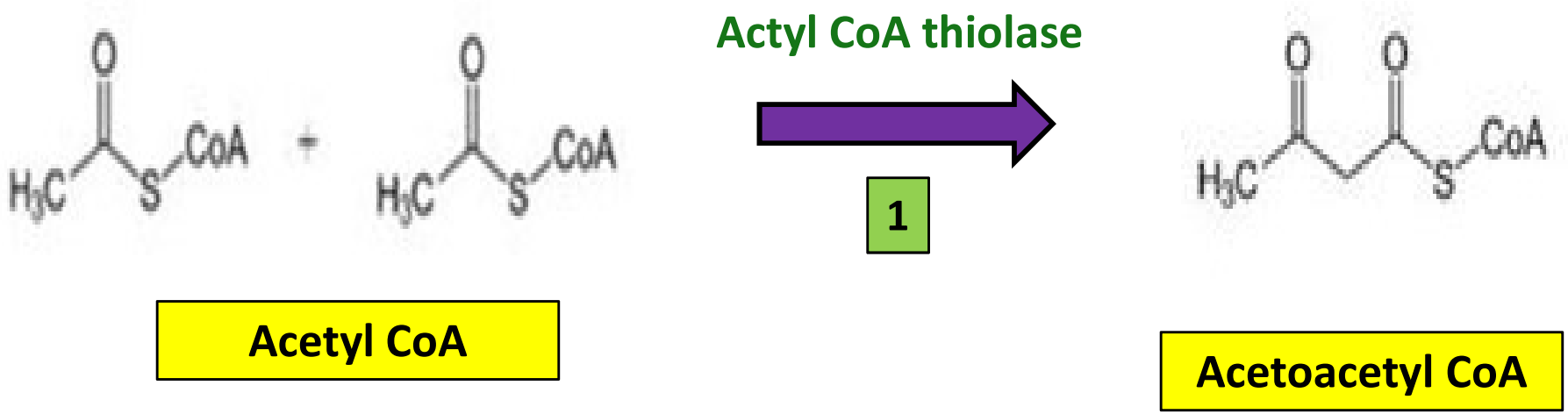
Mevalonic acid Pathway

Mevalonic acid Pathway

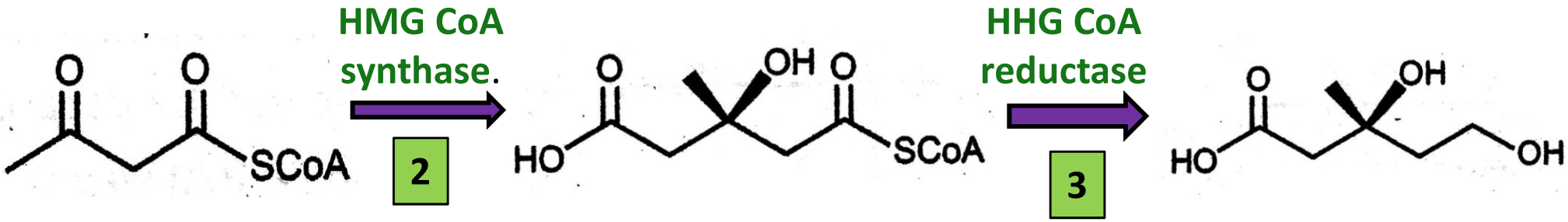
- Mavalonic acid pathway is the biosynthesis precursure to the actual **C5 "isoprene units"** which are isopentyl diphosphate (IPP, tail) and dimethylallyl diphosphate (DMAPP, head).

- Mevalonate pathway starts with Acetyl CoA(which is active form of Acetate) comes from the Glycolysis pathway and finally it converts to Pyruvate.

- **Step 1:** Two molecules of **Acetyl CoA** combines with each other and converts into the **Acetoacetyl CoA** by the enzyme **Actyl CoA thiolase.**



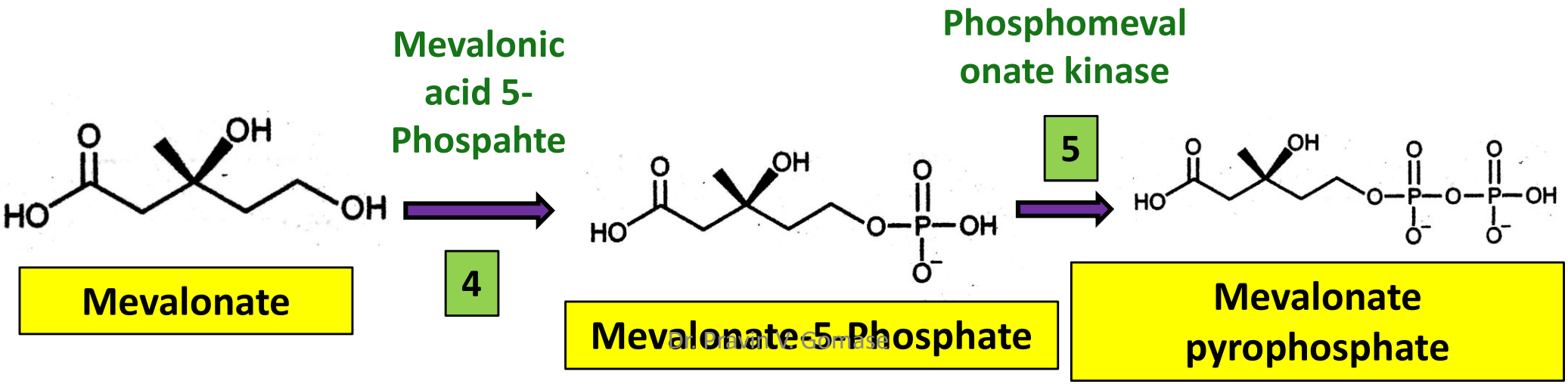
- **Step 2:** The **acetoacetyl CoA** combines with another **Acetyl CoA** to form **3-Hydroxy-3-methyl glutary CoA (HMG CoA)** with the help of enzymes **HMG CoA synthase**.
- **Step 3: HMG CoA** undergoes reduction to form **Mevalonate (Mevalonic acid)** with help of enzymes **HHG CoA reductase** and **NADPH and 2H +**



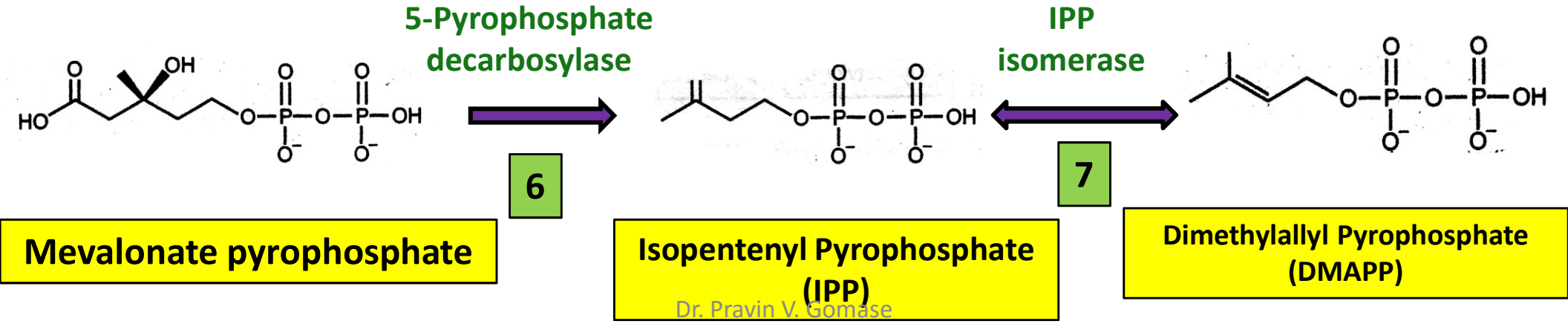
Acetoacetyl CoA | **3-Hydroxy-3-methyl glutary CoA (HMG CoA)** | **Mevalonate**

Conversion of Mevalonic acid IPP and DMAPP

- **Step 4: Mevalonate** undergoes **phosphorylation** in presence of ATP to from **Mevalonate-5-Phosphate** at C-5, enzyme involves is **Mevalonic acid 5-Phospahte**.
- **Step 5: Mevalonate-5-Phosphate** again undergoes **phosphorylation** to form **Mevalonate pyrophosphate** with the help of ATP in presence of enzyme **Phosphomevalonate kinase.**



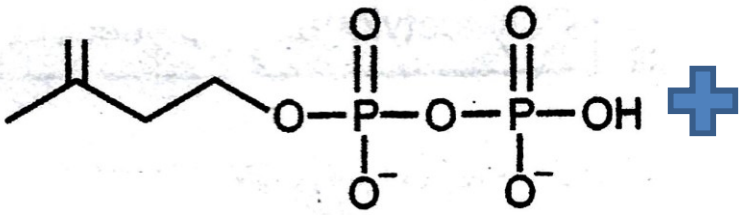
- **Step 6: Mevalonate pyrophosphate** is then converted in to two important precursors i.e. **Isopentenyl Pyrophosphate (IPP) (C_5)** and **Dimethylallyl Pyrophosphate (DMAPP)** which is responsible for the synthesis of different types of **Isoprenoids** with the help of enzymes **5-Pyrophosphate decarbosylase**.
- **Step 7: Isopentenyl Pyrophosphate (IPP)** undergoes **Isomerisation** to form **Dimethyl allyl Pyrophosphate** with the help of enzyme **IPP isomerase**.



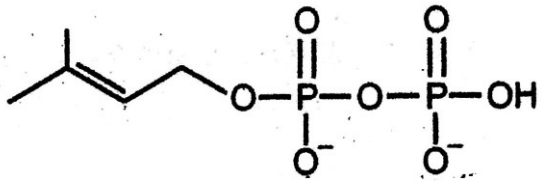
Carbon-Carbon bond formation in Terpene biosynthesis

Conversion of IPP and DMAPP to Geraniol-PP (C10unit) and Farnesyl-PP (C15unit) .

- **Step 8:** **Isopentenyl Pyrophosphate (IPP) (C5unit)** and **Dimethyl allyl Pyrophosphate** undergoes condensation to form **Geranyl Pyrophosphate (C10unit) .**

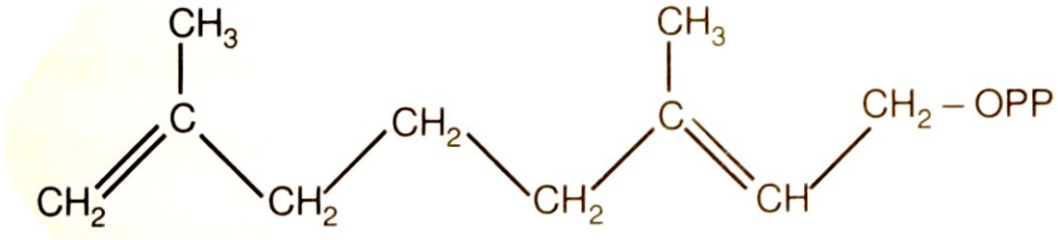


Isopentenyl Pyrophosphate (IPP)



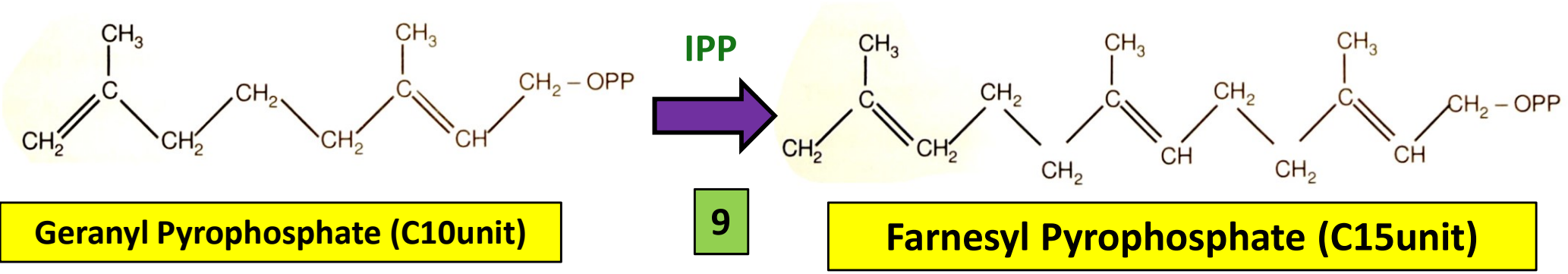
Dimethylallyl Pyrophosphate (DMAPP)

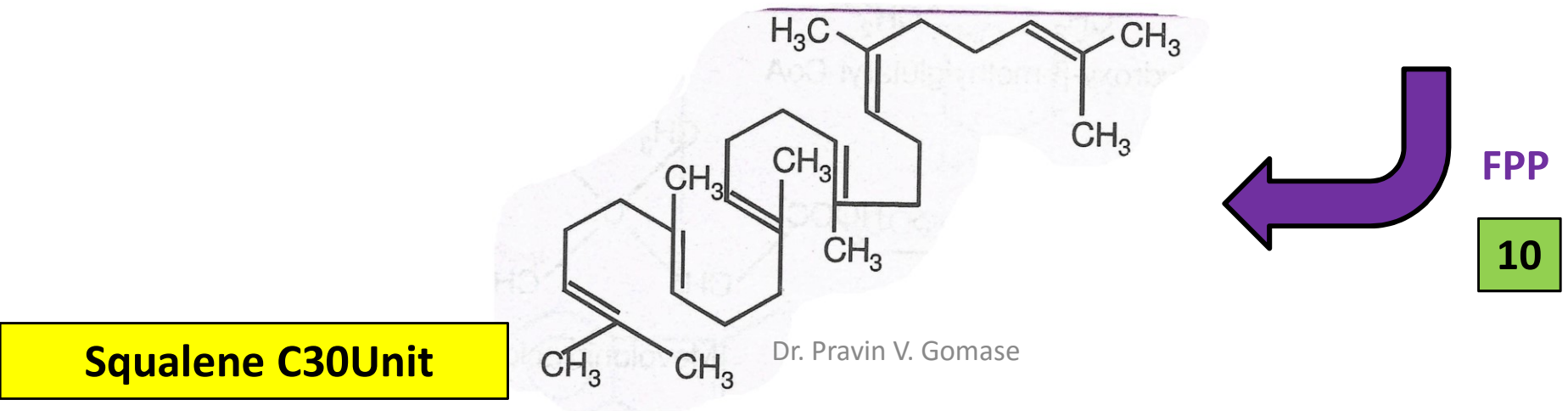
8

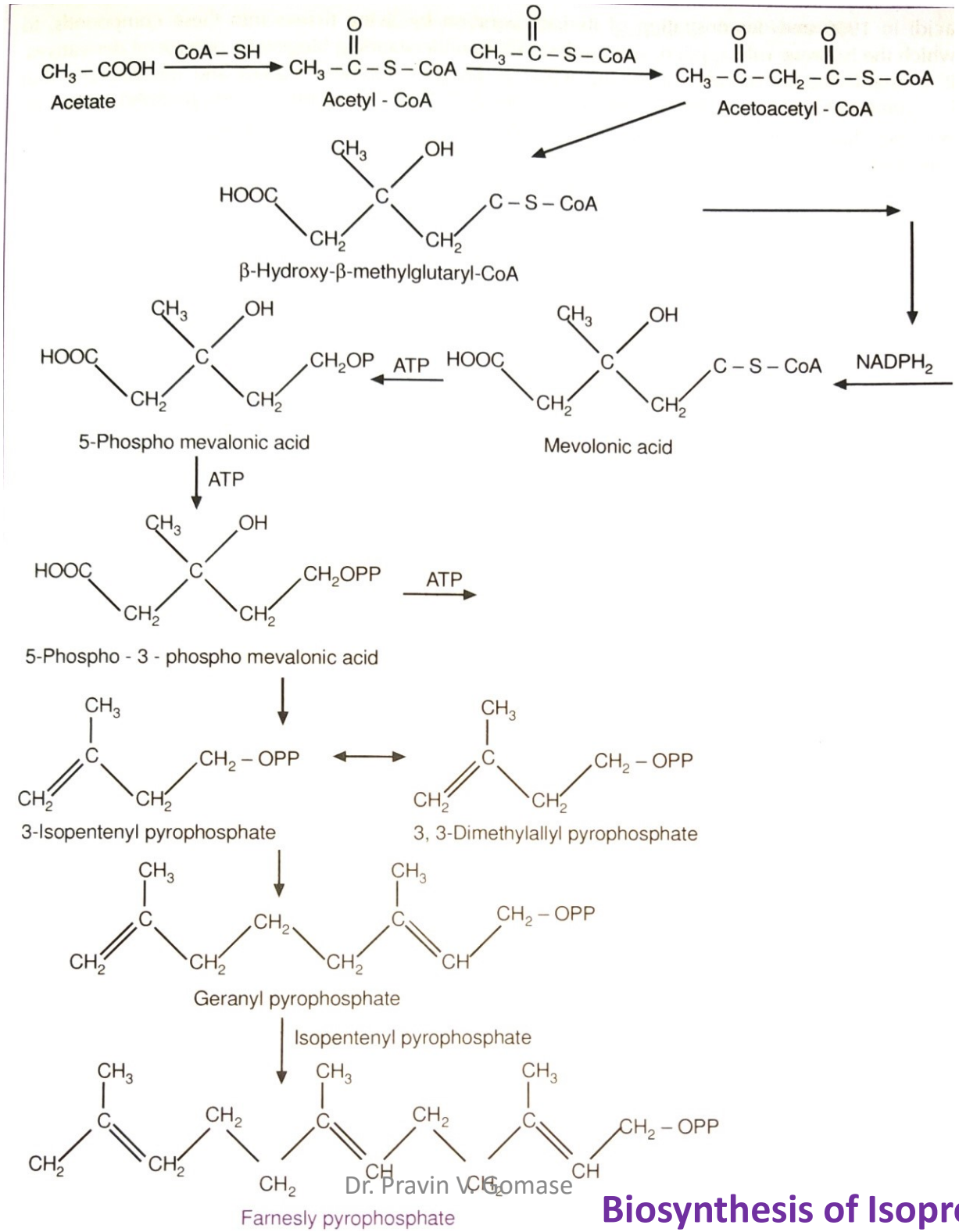


Geranyl Pyrophosphate (C10unit)

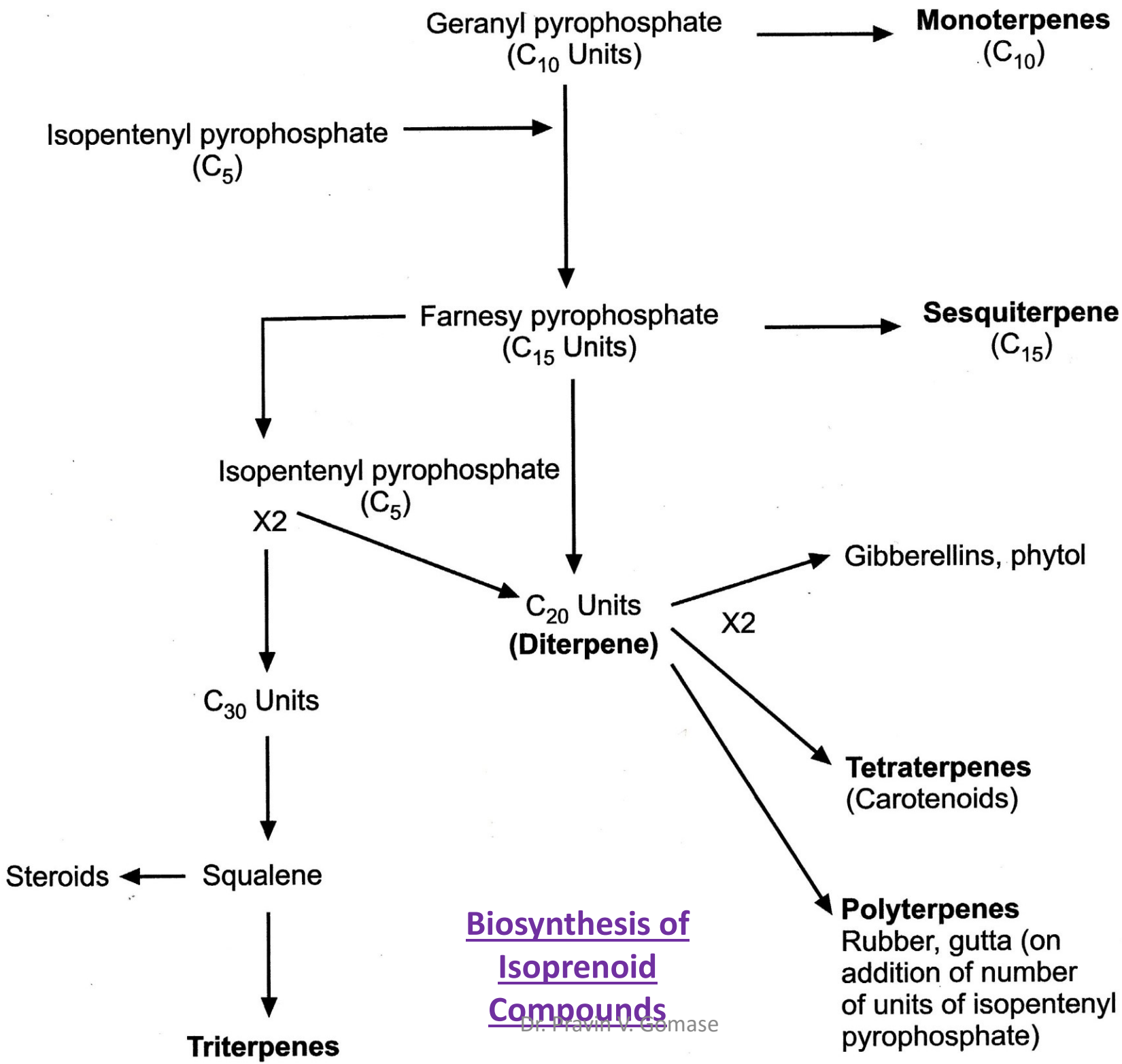
- **Step9:** **Geranyl Pyrophosphate (C10unit)** is further converted to **Farnesyl Pyrophosphate (C15unit)** due to **addition of IPP.**
- **Step 10:** To the **Farnesyl Pyrophosphate**, add one **FPP** to form **Squalene**.



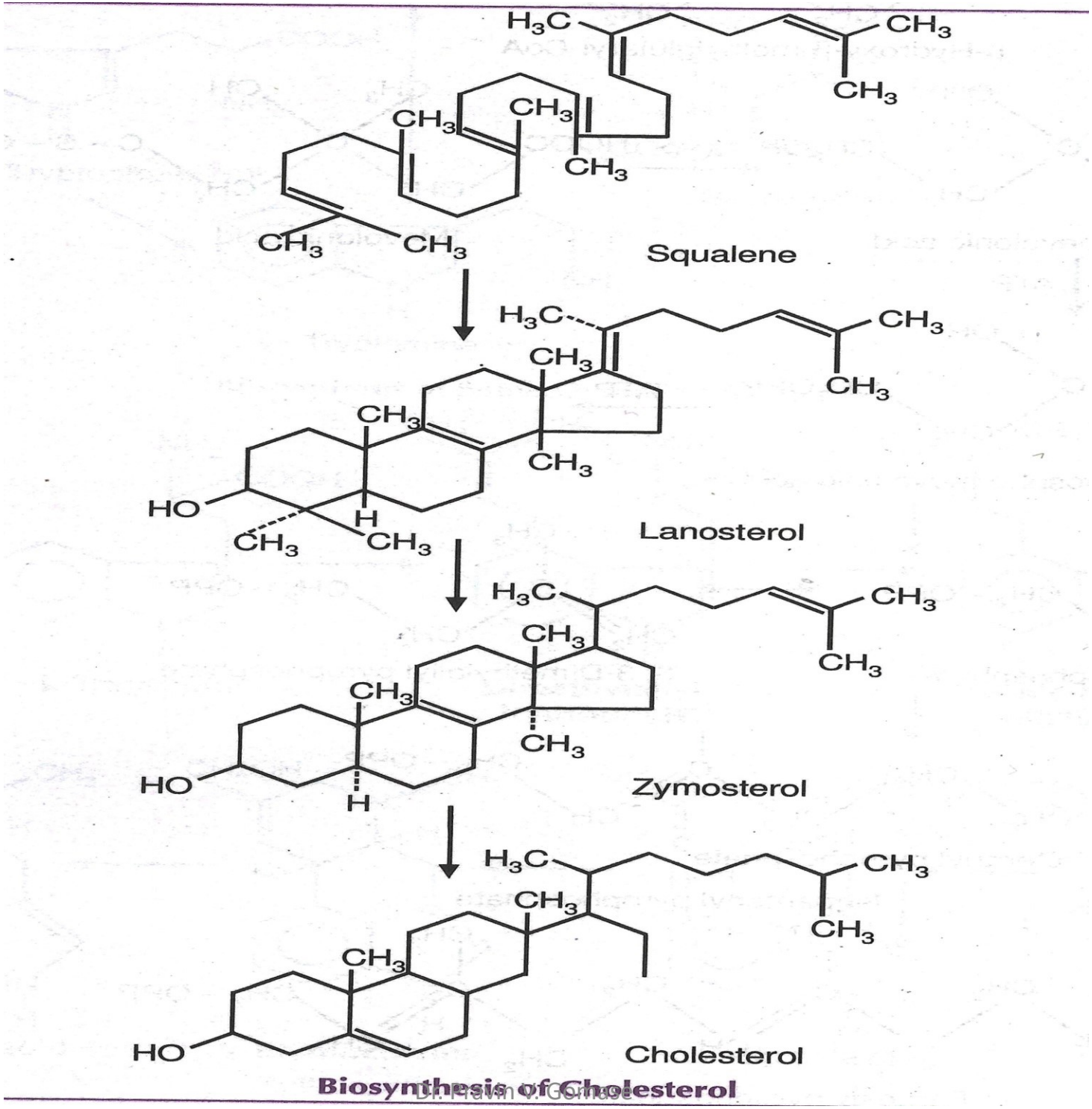




Biosynthesis of Isoprenoid Compounds



Biosynthesis of Isoprenoid Compounds



Biosynthesis of Cholesterol

Types of Mevalonate Pathway

- **Upper mevalonate pathway**
- The mevalonate pathway of eukaryotes, archaea, and eubacteria all begin the same way.

- The sole carbon feed stock of the pathway is acetyl-CoA. The first step condenses two acetyl-CoA molecules to yield acetoacetyl-CoA. This is followed by a second condensation to form HMG-CoA (3-hydroxy-3- methyl-glutaryl-CoA).

- Reduction of HMG-CoA yields (R)-mevalonate. These first 3 enzymatic steps are called the **upper mevalonate pathway.**

Lower mevalonate pathway

- The lower mevalonate pathway which converts (R)-mevalonate into **IPP and DMAPP** has 3 variants. In eukaryotes, mevalonate is phosphorylated twice in the 5-OH position, then decarboxylated to yield IPP.

- In some archaea such as Haloferax volcanii, mevalonate is phosphorylated once in the 5-OH position, decarboxylated to yield isopentenyl phosphate (IP), and finally phosphorylated again to yield IPP.

- A third mevalonate pathway variant found in Thermoplasma acidophilum, phosphorylates mevalonate at the 3-OH position followed by phosphorylation at the 5-OH position.

- The resulting metabolite, mevalonate-3,5-bisphosphate, is decarboxylated to IP, and finally phosphorylated to yield IPP

Thank You

UNIT-I

Amino acid pathway

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Introduction

- Amino acid synthesis is the set of **biochemical processes (metabolic pathways)** by which the **amino acids are produced.**
- The substrates for these processes are various compounds in the organism's diet or growth media.
- Not all organisms are able to synthesize all amino acids.
- Amino acids are organic compounds containing amine **(-NH_2)** and carboxyl **(-COOH)** functional groups, along with a side chain(R group) specific to each amino acid.

- Many amino acids contain only carbon, hydrogen, oxygen and nitrogen, but other atoms may be present (e.g. sulphur in cystine, and iodine in thyroxin).
- As already mentioned, more than one amino group may be present (e.g. Lysine, diaminocaproic acid) and more than one carboxylic acid group (e.g. aspartic or amino succinic acid).

CLASSIFICATION

Nonpolar/hydrophobic aminoacids

- Glycine, alanine, valine, leucine, methionine, phenyalalanine, proline.

Polar/hydrophilic amino acids

- Serine, cysteine, tyrosine, glutamic acid, asparaticacid, lysine, arginine

Sulphur containing amino acids

- Cystine, methionine, cysteine

List of essential and non essential amino acids

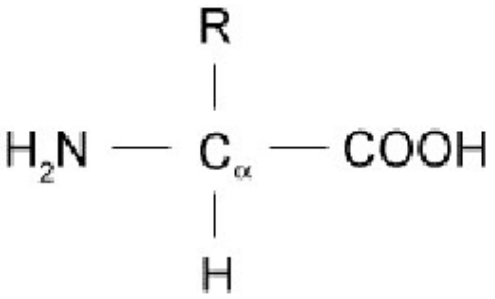
Nutritionally essential amino acids	Nutritionally non-essential amino acids
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic acid
Leucine	Cysteine
Lysine	Glutamic acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

- **Properties**
- Amino acids are generally soluble in water but only slightly soluble in alcohol. A general test is to warm with ninhydrin, when, with the exception of **proline**, which gives a yellow, they give a **pink, blue or violet colour**.
- Amino acids do not respond to the biuret test (compare polypeptides and proteins).
- Certain amino acids are detected by more specific tests (e.g. histidine gives colour reactions with diazonium salts).

Amino acid pathway

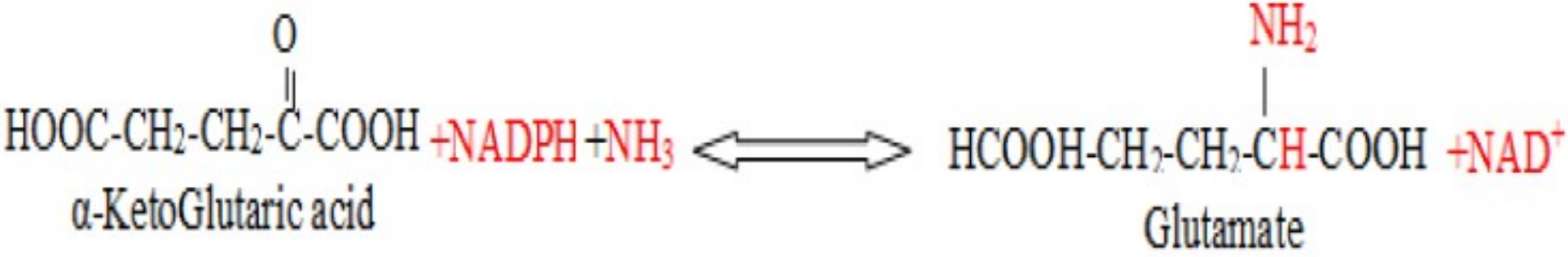
- Amino acid synthesis is the set of biochemical processes (Metabolic pathway) by which the amino acids are produced.
- All amino acids are derived from the intermediates in glycolysis, the Citric Acid Cycle, or Pentose Phosphate Pathway.
- Nitrogen enters the pathway by way of Glutamate and Glutamine.
- Organisms vary greatly in their ability to synthesize the 20 common amino acids.

- Whereas most bacteria and plants can synthesize all **20**, mammals can synthesize about half of them- generally those with simple pathways. The non-essential amino acids not needed in the diet.
- The remaining, the essential amino acids, must be obtained from food.
- Amino acids are the precursors of some secondary metabolite (Eg. Alkaloids).
- Most amino acids are found in nature – α amino acids.

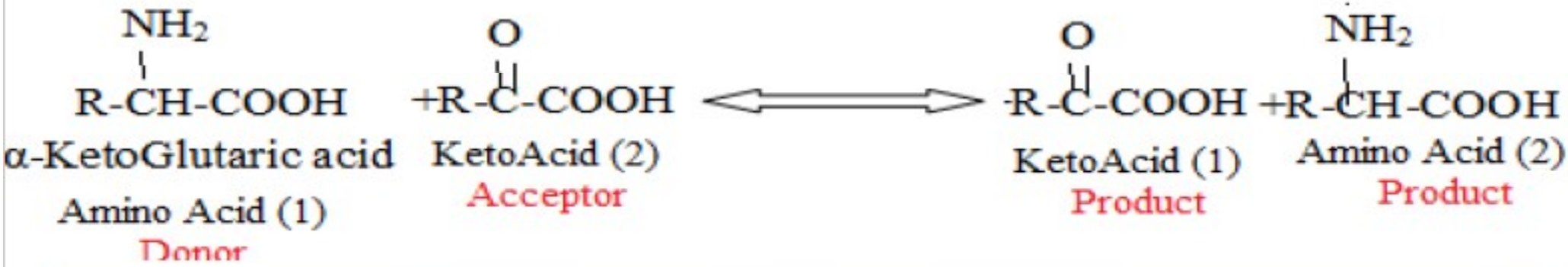


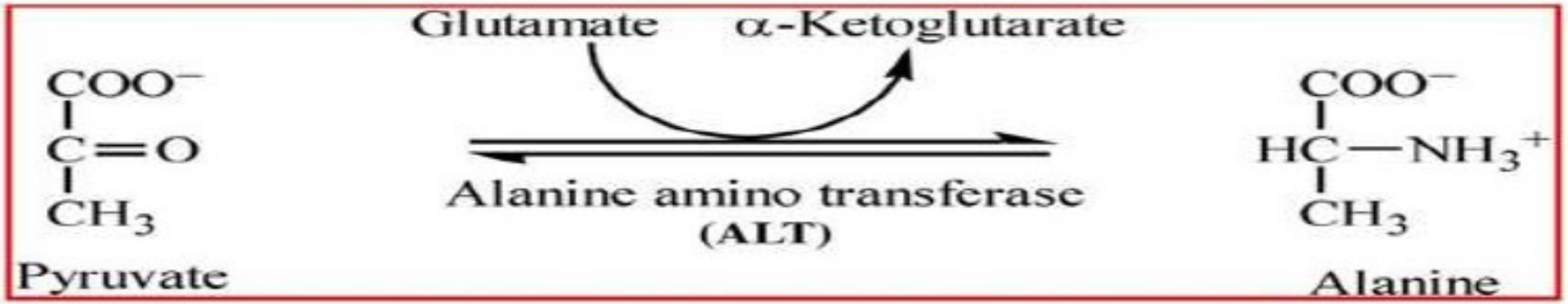
- Amino acid pathway starts from Glycolysis, Kreb's cycle (TCA Cycle) – Branched from its intermediates.
- Plant synthesize all 20 amino acids (Aliphatic, Aromatic Heterocyclic).
- Nitrogen enters metabolic reaction by Reductive amination.

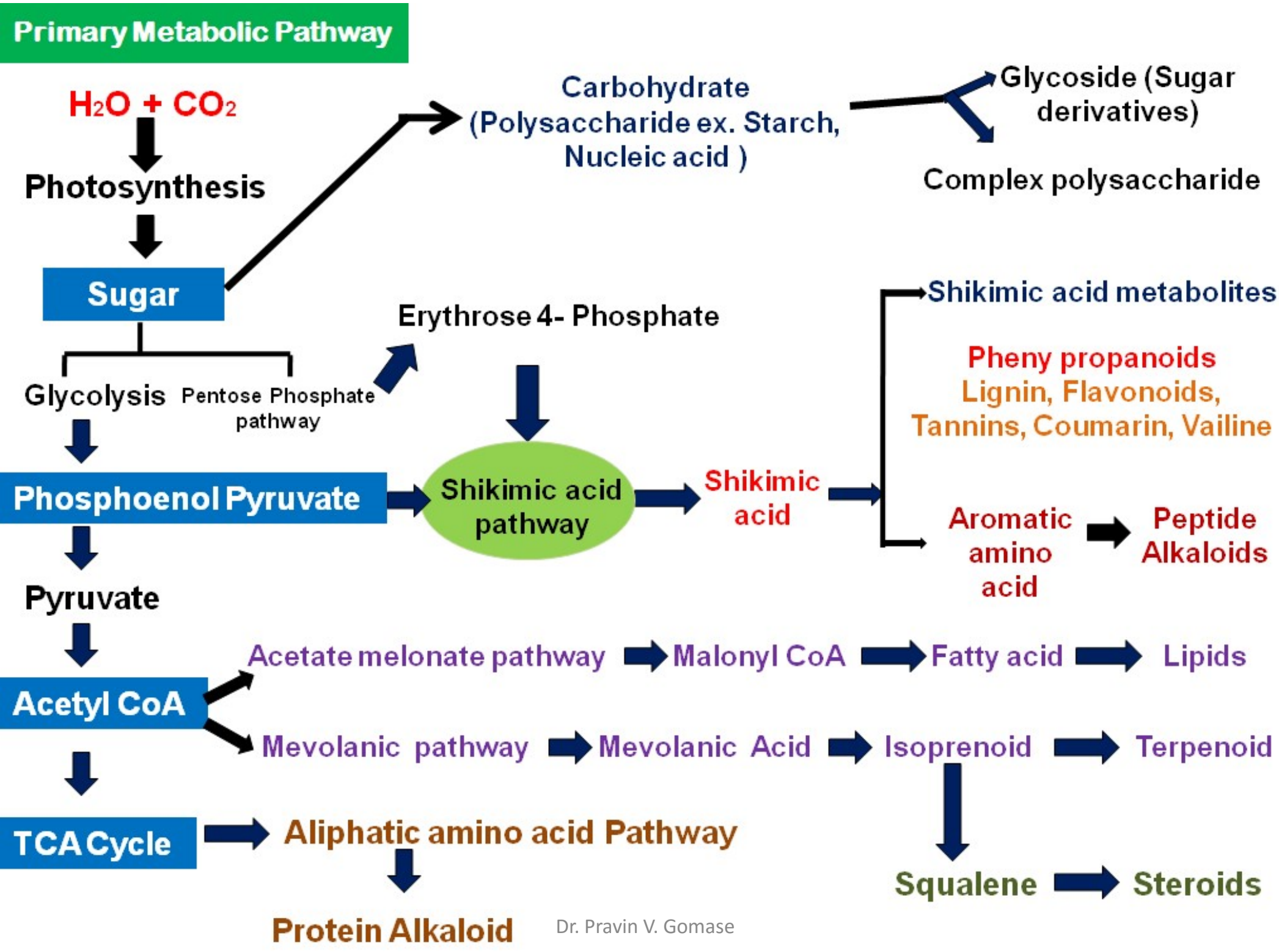
α-Keto acid $\xleftrightarrow{NH_3}$ Aliphatic Amino acid (serve as α amine donor for Transamination reaction)



Transamination Reaction



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Shikimic acid metabolites
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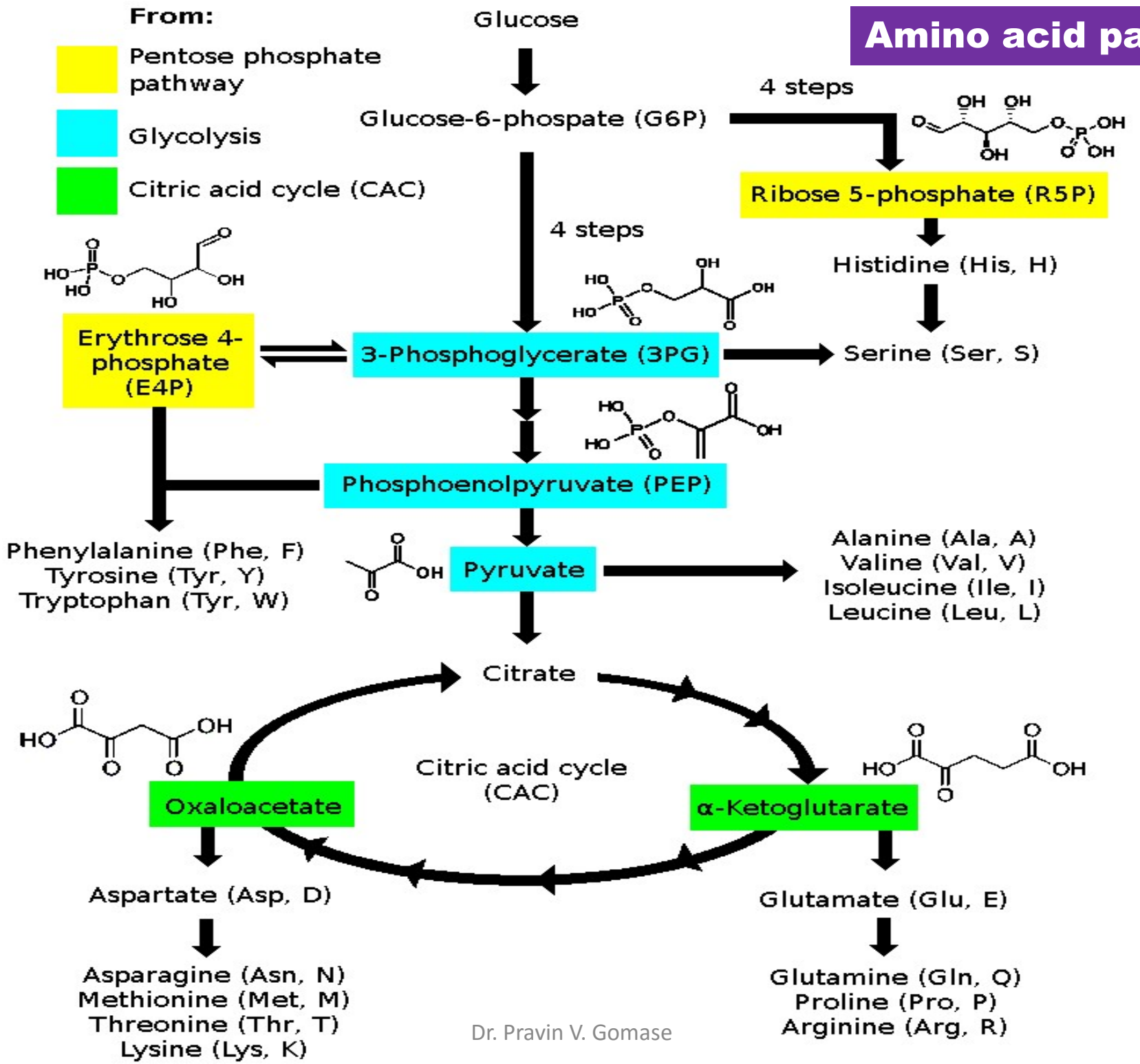
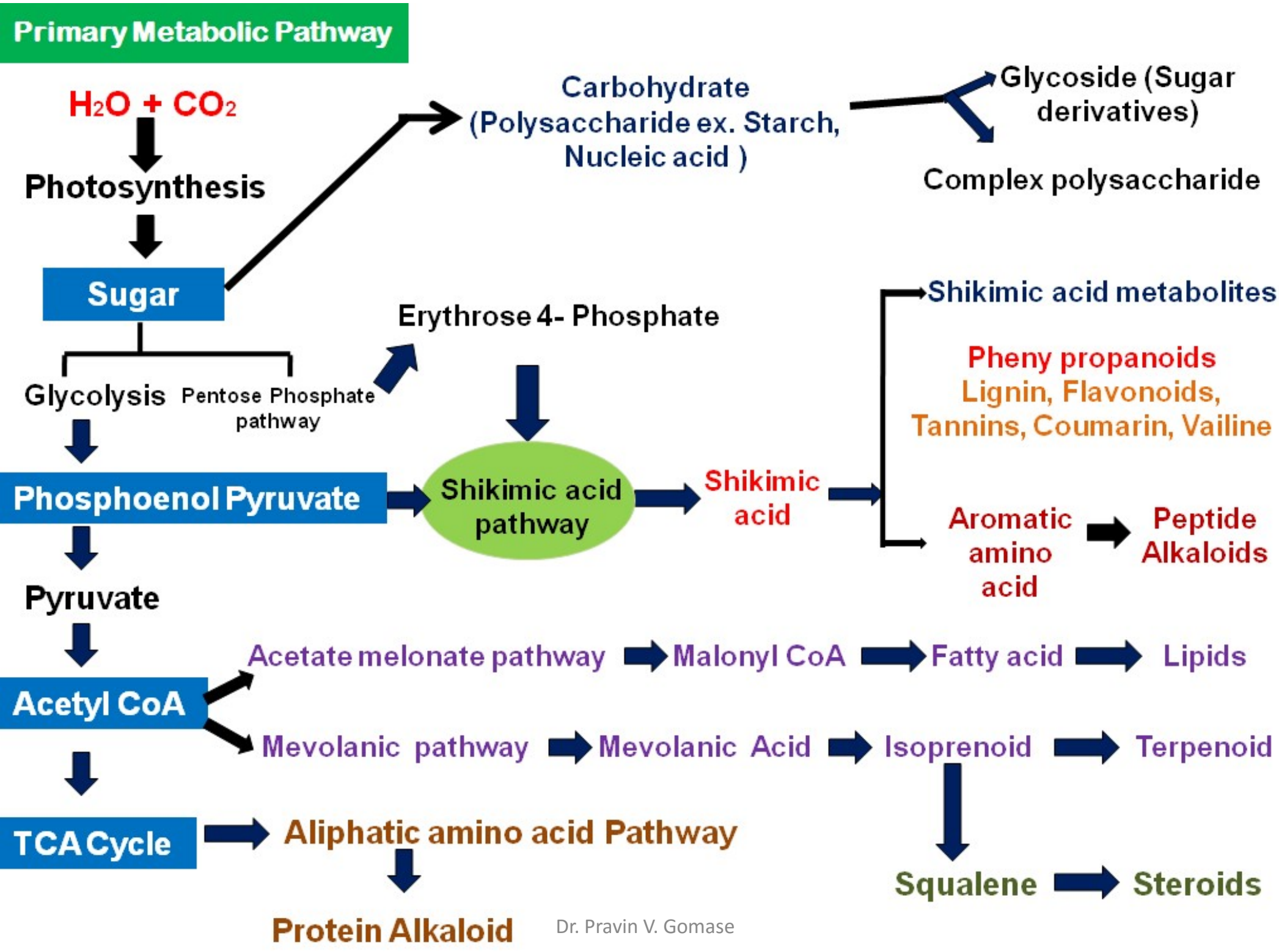
Amino acid pathway

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pathway
Shikimic
acid
Shikimic acid metabolites
Pheny propanoids
Lignin, Flavonoids,
Tannins, Coumarin, Vailine
Aromatic
amino
acid
Peptide
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Thank You

UNIT-I

Metabolic pathways in higher plants and their determination

Primary and Secondary Metabolites derived from Carbon metabolism

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What is Biosynthesis ?

- Biosynthesis is a process of forming **larger organic compounds** from **small subunits** within a living organism.

- Biosynthesis is mainly done by **enzymes.**

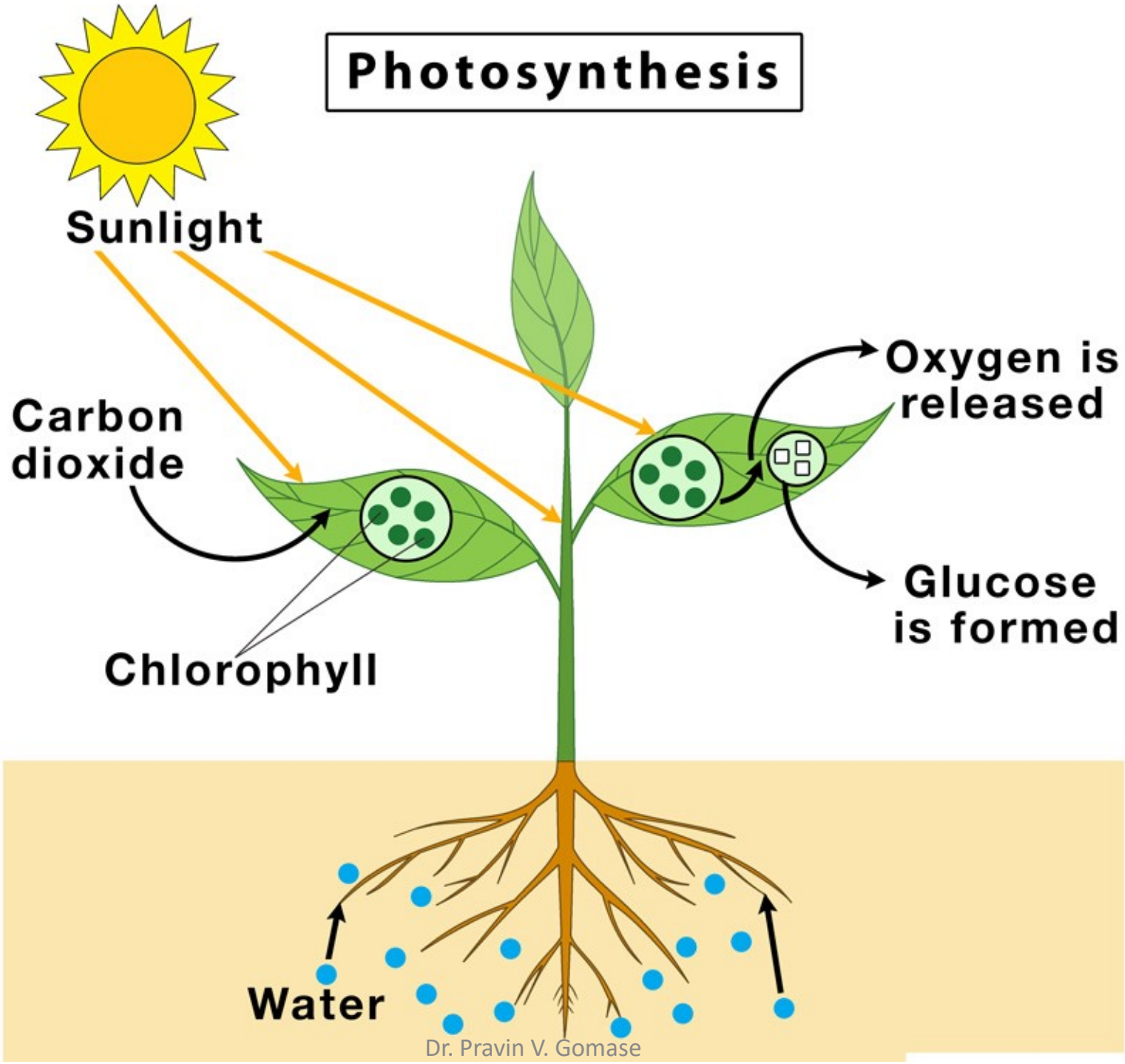
- Biosynthesis is also known as **anabolism** since simple compounds are joined together to form macromolecules by enzymes.

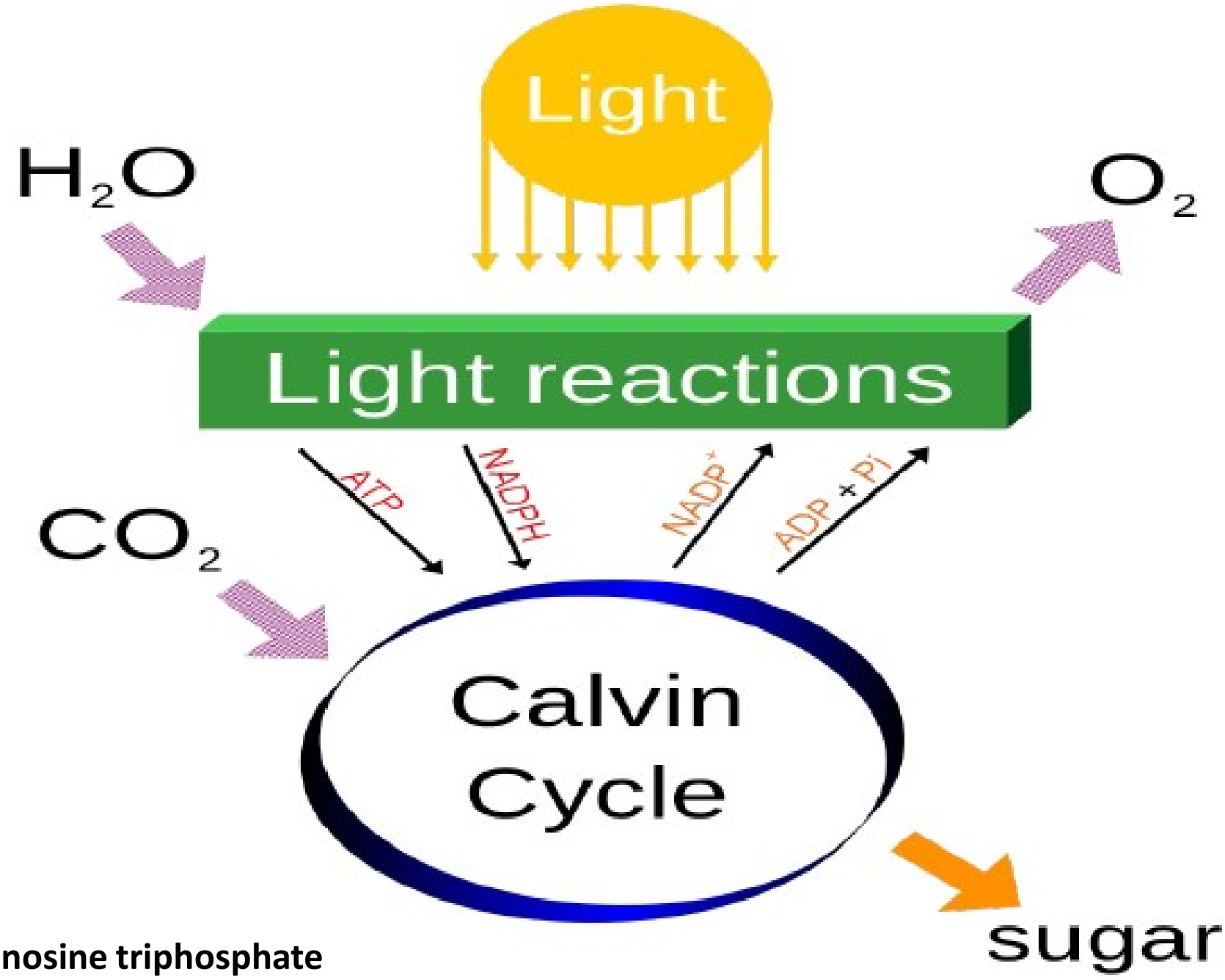
the synthesis of complex molecules in living organisms from simpler ones together with the storage of energy; constructive metabolism

- As an example, photosynthesis occurs inside the **chloroplast**.

- The **light energy is converted into chemical energy** during photosynthesis.

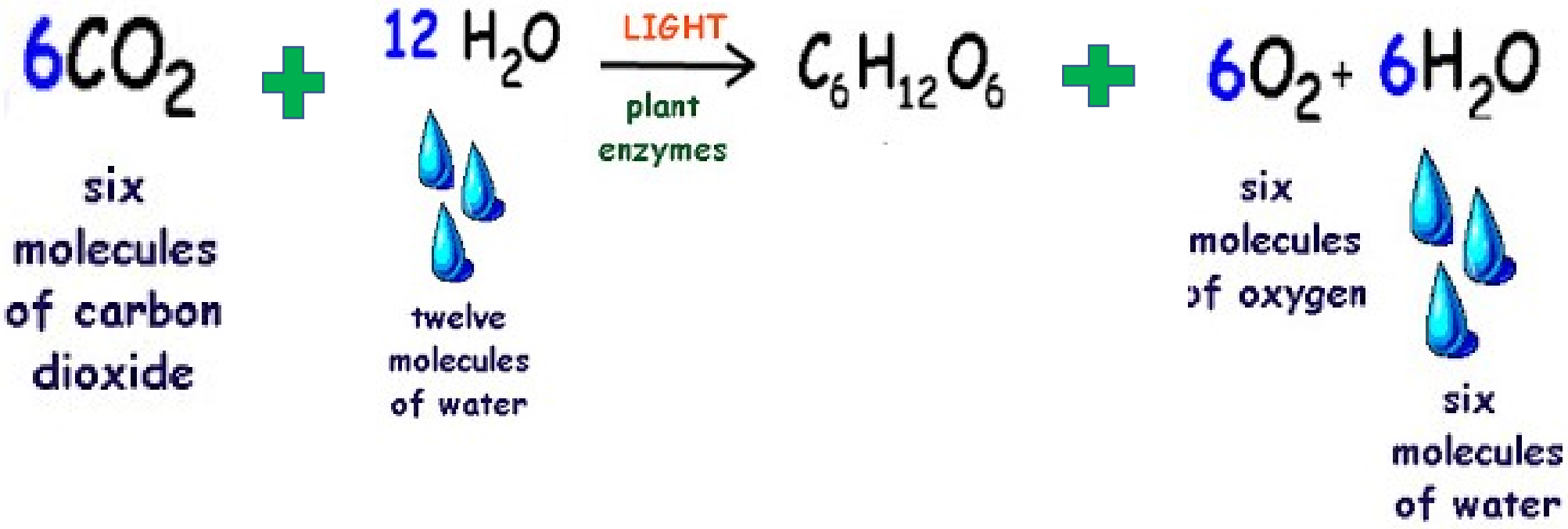
- The larger molecule glucose is biosynthesized from **water and carbon dioxide** by photosynthetic organisms**.(ATP, Enzyme, Cofactors)**


Photosynthesis
Sunlight
Carbon dioxide
Chlorophyll
Oxygen is released
Glucose is formed
Water



ATP- Adenosine triphosphate

NADPH- Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

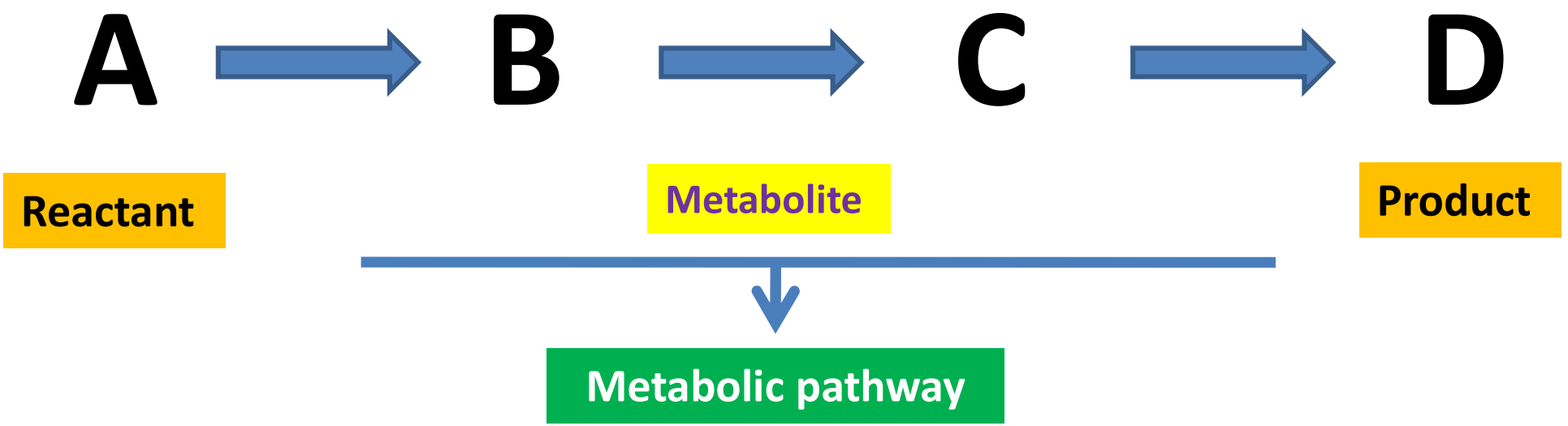

6CO2
six molecules of carbon dioxide
12 H2O
twelve molecules of water
LIGHT
plant enzymes
C6H12O6
6O2 + 6H2O
six molecules of oxygen
six molecules of water

Photosynthesis

- **H_2O + light + ADP + P ---> O_2 + ATP + e-**
- After the above steps occur in photosystem II, the electron is finally sent to photosystem I, where the following happens.
- **e- + NADP+ + H ---> NADPH**
- Now there are two high energy molecules, fully charged and ready to be used. Plants make more energy that it needs immediately, so the NADPH and ATP are used to make glucose as follows.
- **CO2 + ATP + NADPH ---> C6H12O6**

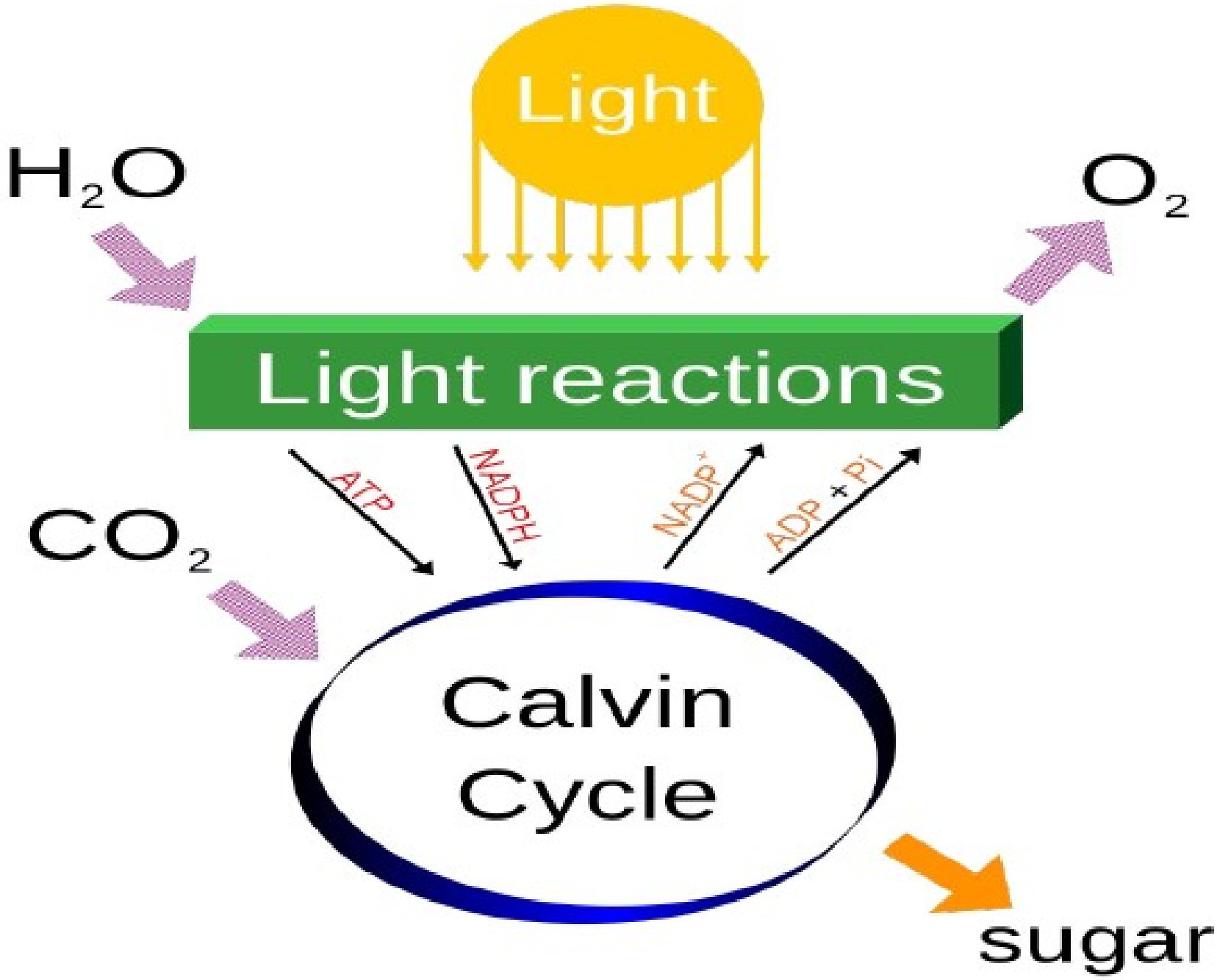
Biosynthesis of Primary Metabolites

- Living plants are solar-powered biochemical and biosynthetic laboratory which manufactures both **primary and secondary metabolites** from air, water, minerals and sunlight
- Metabolites are organic compound **synthesized by organisms using enzyme-mediated chemical** reaction called **metabolic pathways.** **Or**
- The reaction sequence occurring with in the organisms in an orderly and regulate way known as **metabolic pathways**.


A
B
C
D
Reactant
Metabolite
Product
Metabolic pathway

Types of Metabolites

- **Primary metabolites**
- The primary metabolites like **sugars, amino acids & fatty acids** that are needed **for general growth & physiological development** of plant which distributed in nature & also utilized as food by man.
- **Secondary metabolites**
- The secondary metabolites such as **alkaloids, glycosides, Flavonoids, volatile oils etc** are biosynthetically derived from primary metabolites.


Light
H_2O
O_2
Light reactions
ATP
NADPH
NADP+
ADP + Pi
CO_2
Calvin Cycle
sugar

Metabolism & Metabolic Pathways

- **Cell Metabolism:** Process by which living cell process nutrient molecule & living state.
- **Metabolic Pathway:** A complete set of chemical reactions that occur in living cells, allowing cells to grow and reproduce, maintain their structures, and respond to their environments.
- Living cell require energy for biosynthesis, transport of nutrient, motility and maintenance.
- Energy is obtained from the catabolism of carbon compounds (carbohydrate)
- Carbohydrates are synthesized from CO_2 and H_2O in the present of light by photosynthesis.

Types of Metabolism

- **Catabolism:**
- Metabolic reaction in the cell that degrade a substrate into smaller / simpler products
- Produce energy to the cell (**Glucose ⟹ CO_2**)

- **Anabolism:**
- Metabolic reaction that result into the synthesis of larger / more complex molecules.
- Required energy
- **Glucose to glycogen**

Metabolites

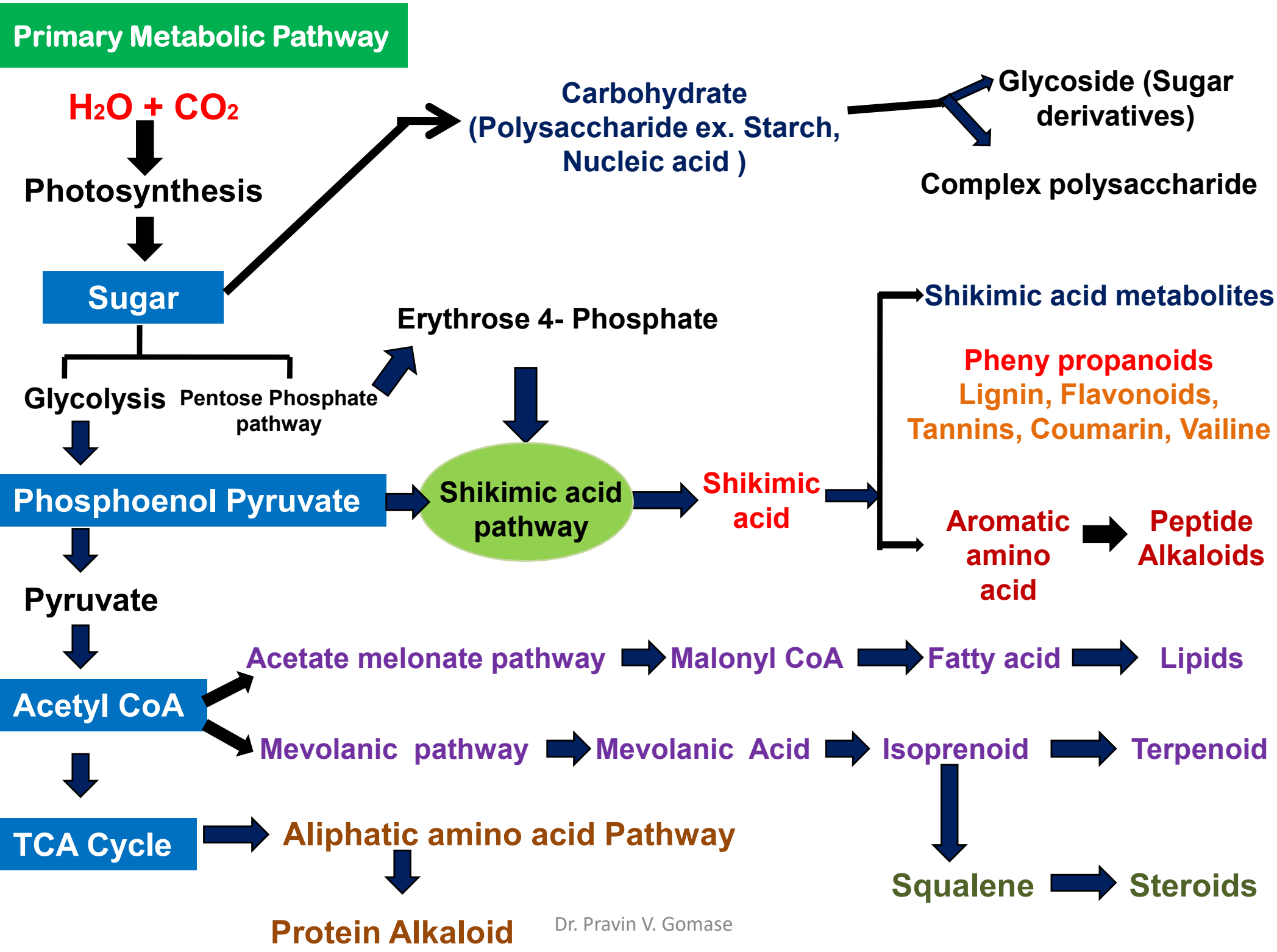
- Metabolites are the **intermediates & products** of metabolism.
- The term metabolite is usually restricted to **small molecules**.
- A primary metabolite is directly involved in the normal growth, development, and reproduction.
- A secondary metabolite is not directly involved in those processes, but usually has important ecological function.

Importance of photosynthesis information of primary metabolites

- Photosynthesis is the process where plants convert sunlight into energy, then store it as carbohydrates, sugars, such as glucose.
- Photosynthesis may be the most important process in ecosystems, both brings in energy needed within the ecosystem, and produce oxygen (O_2) needed for cellular respiration, and the production of more ATP.
- **Photosynthesis has three basic steps:**

1. Energy is captured from the sunlight.
2. Light energy is converted into chemical energy in the form of ATP and NADPH.
3. Chemical energy is used to power the synthesis of organic molecules (e.g. carbohydrates) from carbon dioxide (CO_2).

Primary and Secondary Metabolites derived from Carbon metabolism.


Primary Metabolic Pathway
H2O + CO2
Photosynthesis
Sugar
Carbohydrate
(Polysaccharide ex. Starch,
Nucleic acid)
Glycoside (Sugar
derivatives)
Complex polysaccharide
Glycolysis
Pentose Phosphate
pathway
Erythrose 4- Phosphate
Phosphoenol Pyruvate
Shikimic acid
pathway
Shikimic
acid
Shikimic acid metabolites
Pheny propanoids
Lignin, Flavonoids,
Tannins, Coumarin, Vailine
Aromatic
amino
acid
Peptide
Alkaloids
Pyruvate
Acetate melonate pathway
Malonyl CoA
Fatty acid
Lipids
Acetyl CoA
Mevolanic pathway
Mevolanic Acid
Isoprenoid
Terpenoid
TCA Cycle
Aliphatic amino acid Pathway
Squalene
Steroids
Protein Alkaloid
Dr. Pravin V. Gomase

Difference between Primary and Secondary metabolites

Primary Metabolites	Secondary metabolites
1. They are involved in normal growth development and reproduction	1. They are not directly involved in the normal growth development and reproduction.
2. They are not poisonous	2. Some of these compounds are poisonous.
3. Examples for primary metabolites are carbohydrates, fats and proteins.	Examples for secondary metabolites are alkaloids, tannins, resins, gums and latex etc.
4. Primary metabolites are produced during the growth phase of cell.	4. Secondary metabolites are produced during the non-growth phase of the cell.

Thank You

UNIT-I

Shikimic Acid Pathway

Presented By

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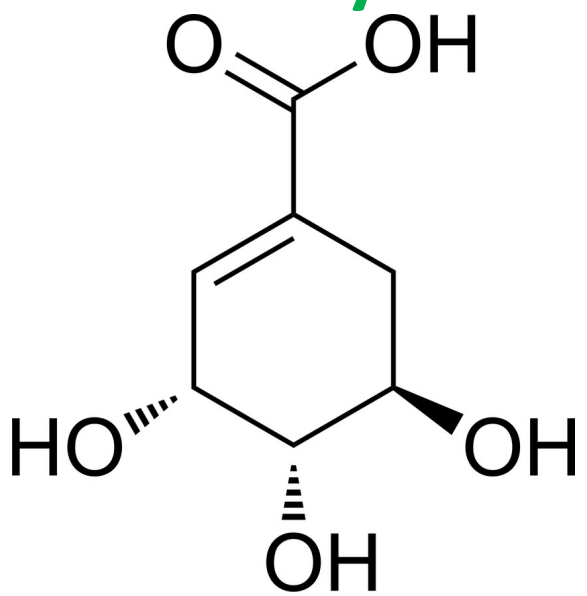
Associate Professor

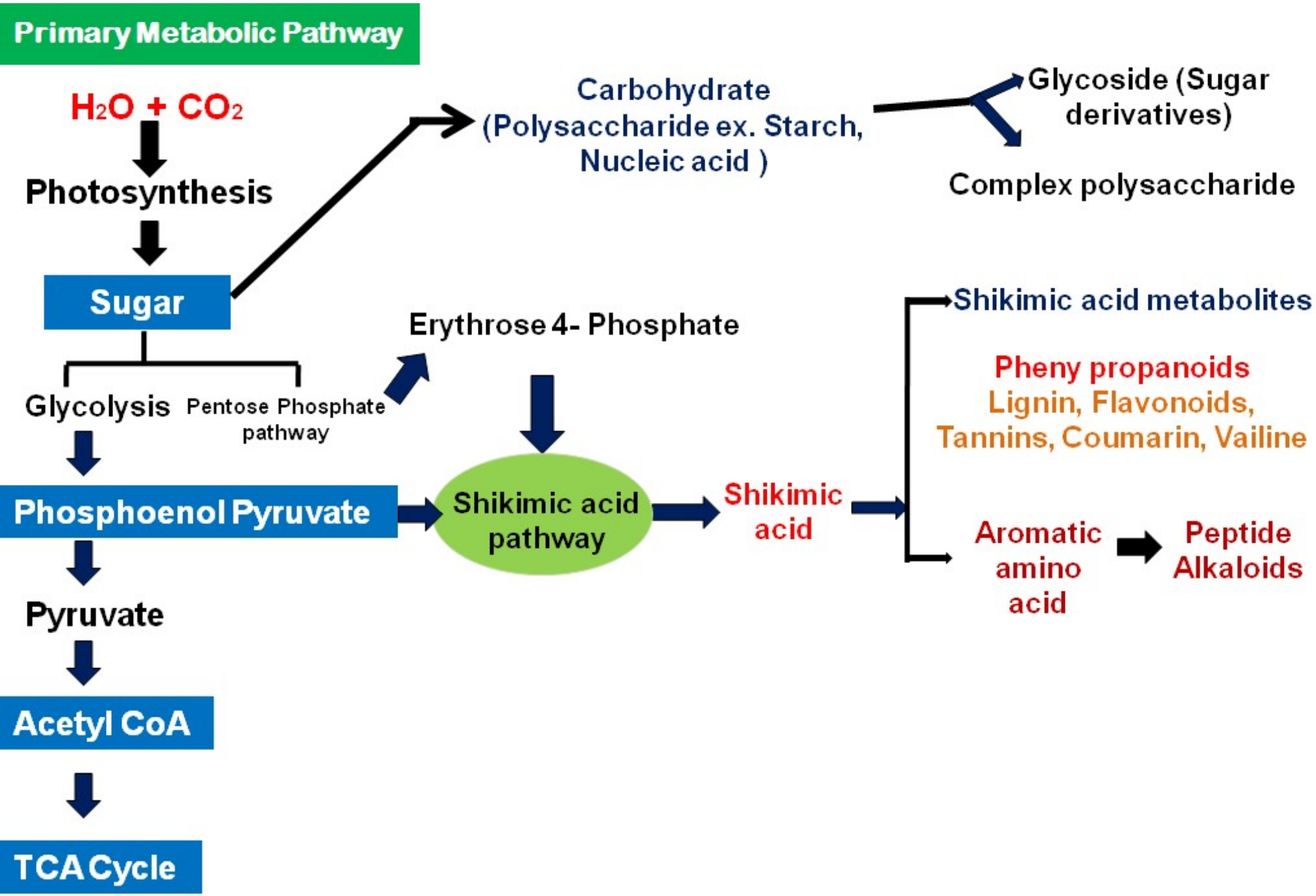
Dept. Of Pharmacognosy

Jijamata College of Pharmacy, Nandurbar.

Shikimic Acid Pathway

- Shikimic Acid Pathway is also known as biosynthesis of **aromatic amino acid and it is a metabolic pathway.**
- It is one of the most important pathway for the genesis of aromatic amino acid (**like phenylalanine, tyrosine and tryptophan**)
- Its name comes from the Japanese flower **Shikimi** (**Japanese Star Anise, *Illicium anisatum***) from which Shikimic acid was first isolated in **1885 by Johan Fredrik Eykman.**




Primary Metabolic Pathway
H2O + CO2
Photosynthesis
Sugar
Carbohydrate
(Polysaccharide ex. Starch,
Nucleic acid)
Glycoside (Sugar
derivatives)
Complex polysaccharide
Glycolysis
Pentose Phosphate
pathway
Erythrose 4- Phosphate
Phosphoenol Pyruvate
Shikimic acid
pathway
Shikimic
acid
Shikimic acid metabolites
Pheny propanoids
Lignin, Flavonoids,
Tannins, Coumarin, Vailine
Aromatic
amino
acid
Peptide
Alkaloids
Pyruvate
Acetyl CoA
TCA Cycle

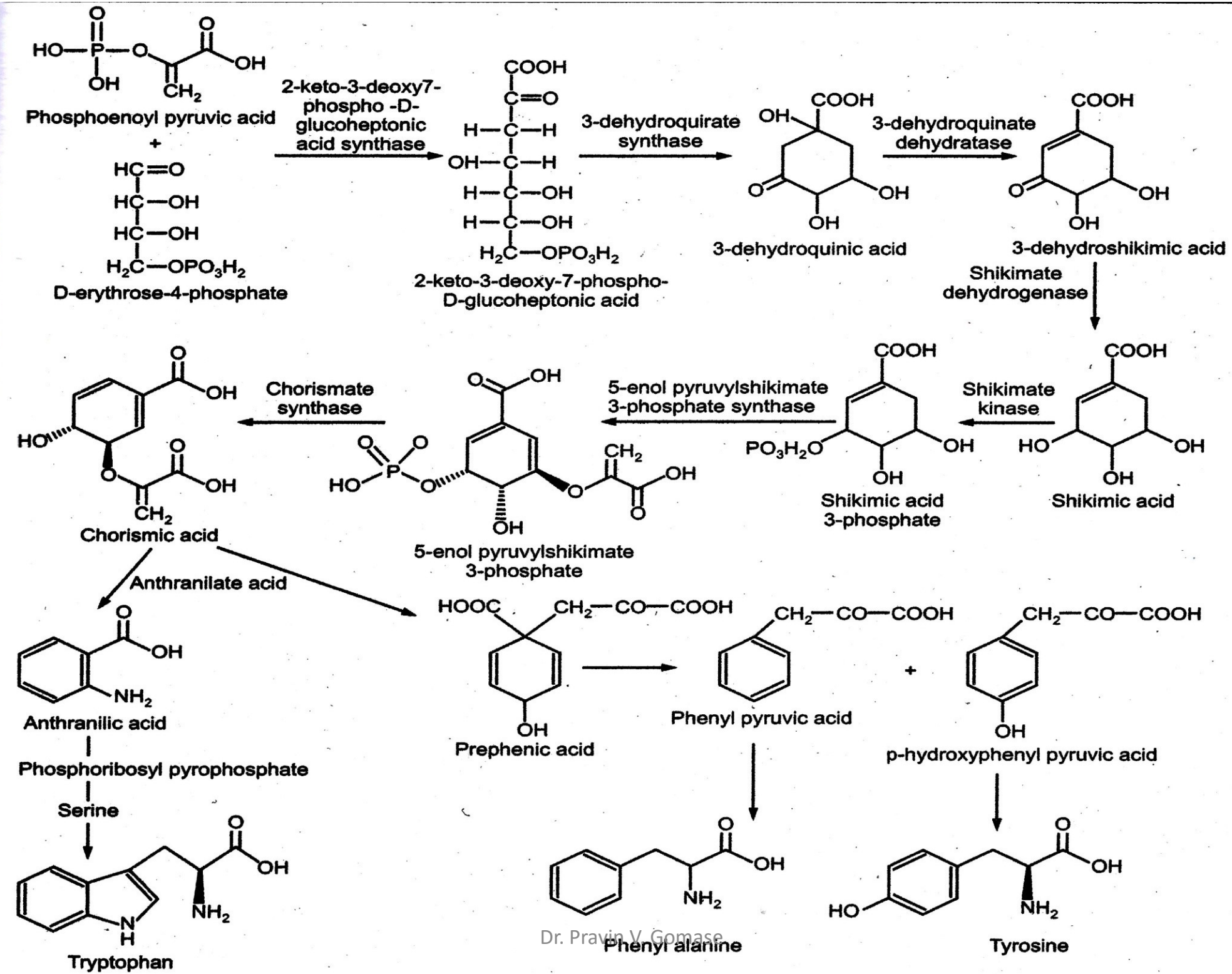
- The **Shikimic acid pathway** is a key intermediate from carbohydrates for the biosynthesis of **C_6-C3** units **(phenyl propane derivative)**
- The Shikimic acid pathway converts simple carbohydrates precursors derived from glycolysis and the pentose phosphate pathway to the aromatic amino acids.

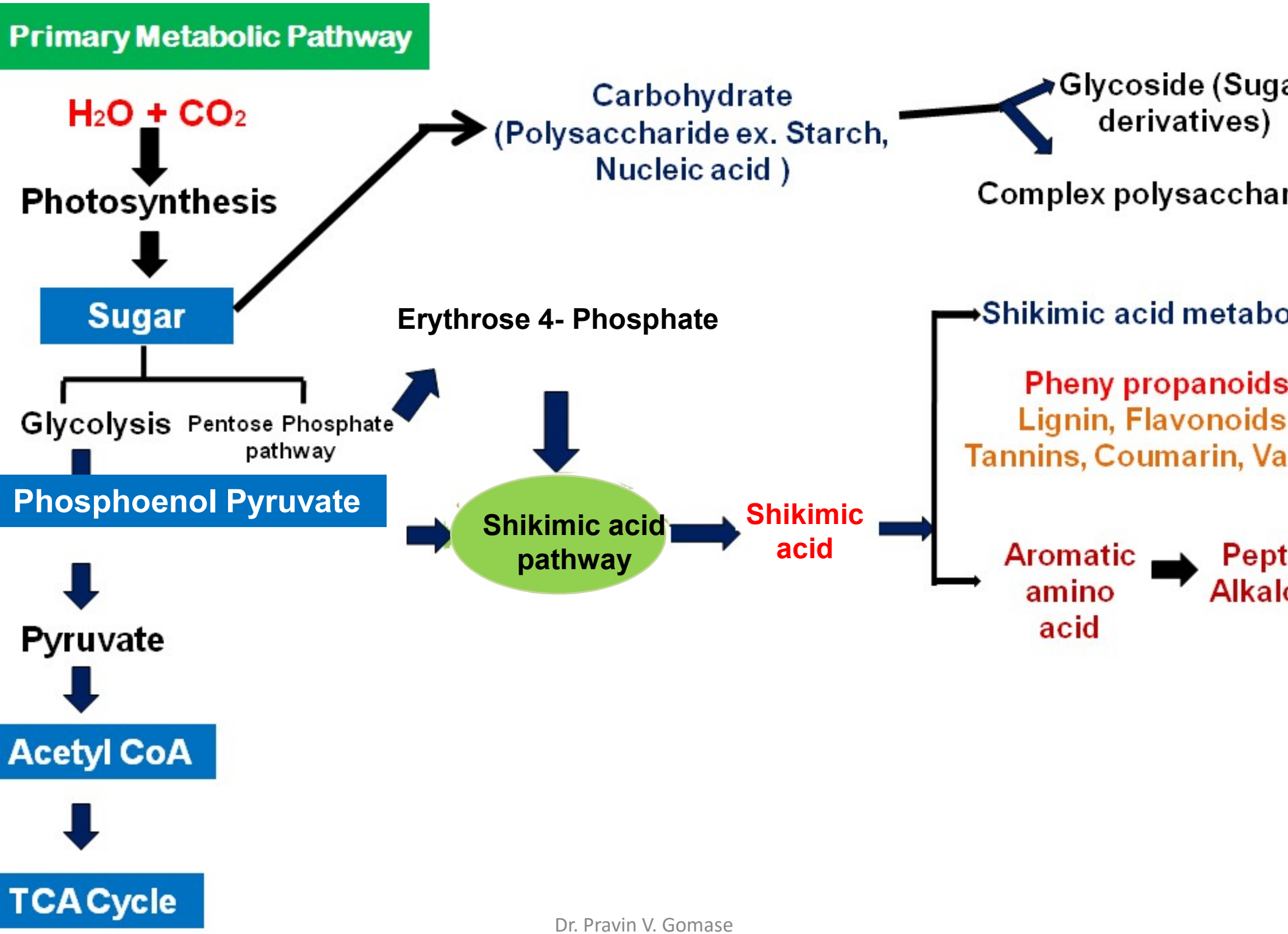
Occurring

- The shikimic pathway is a 7 step metabolic route used by bacteria, fungi, algae, parasites and plants for the biosynthesis of aromatic amino acids **(phenylalanine, tyrosine and tryptophan)**

- This pathway is not found in animals therefore phenylananine and tryptophan represent essential amino acids that must be obtained from the animals diet.

- Animals can synthesize tyrosine from phenylalanine and therefore is not an essential amino acid except for individual unable to hydroxylate phenylalanine to tyrosine.




Primary Metabolic Pathway
H2O + CO2
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Sugar
Carbohydrate
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pathway
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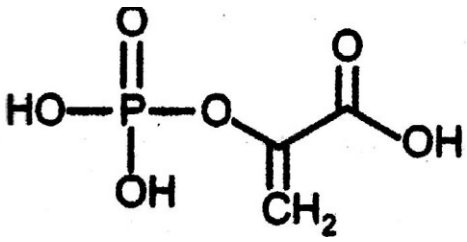
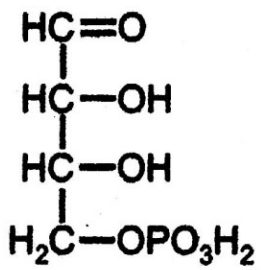
Phosphoenol Pyruvate
Shikimic acid
pathway
Shikimic
acid
Shikimic acid metabo
Pheny propanoids
Lignin, Flavonoids
Tannins, Coumarin, Va
Aromatic
amino
acid
Pept
Alkal
Pyruvate
Acetyl CoA
TCA Cycle

Steps involved in Shikimic acid Pathway

- **Step 1:** **Phosphoenol pyruvate** and **erythrose-4-phospahte** react to from **2- keto-3-deoxy-7-phospho-D-glucoheptonic acid (DAHP)** in a reaction catalyzed by the enzyme **DAHP synthase.**
- **Step 2:** **2- keto-3-deoxy-7-phospho-D-glucoheptonic acid (DAHP)** is then transferred to **3-dehydroquinate (DHQ)** or **3-dehydroquinic acid** in a reaction catalyzed by **DHQ synthase.**
- Although this reaction required **NAD** as a cofactor, the enzyme mechanism regenerates it, resulting in the net use of no NAD.

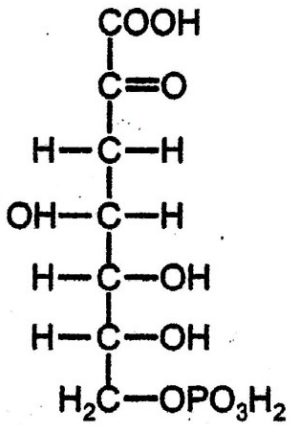


Phosphoenol pyruvate



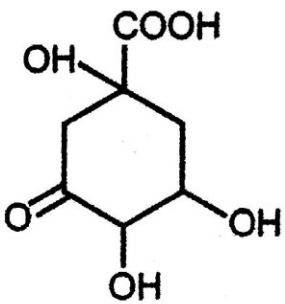
Erythrose-4-phospahte

DAHP synthase



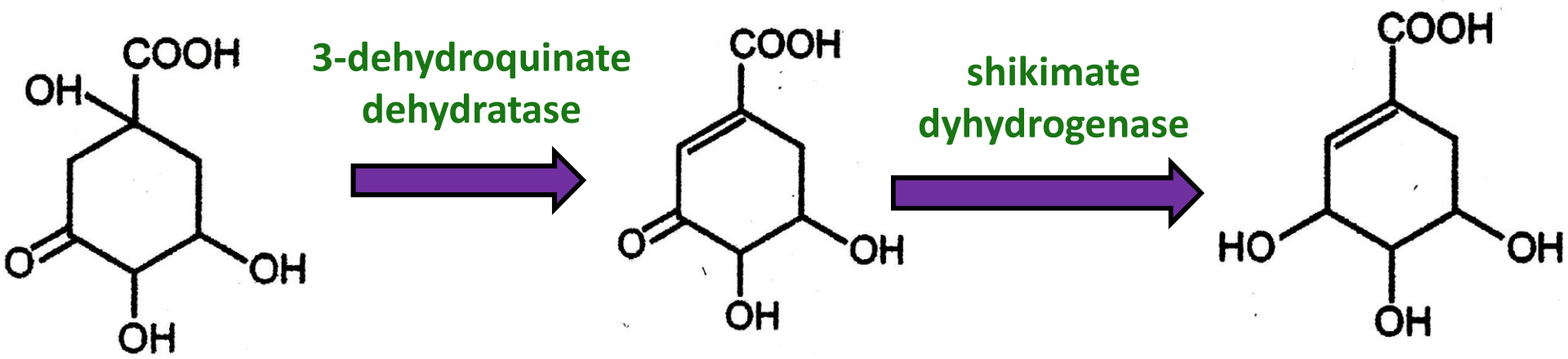
2- keto-3-deoxy-7-phospho-D-glucoheptonic acid (DAHP)

DHQ synthase



3-dehydroquinic acid (DHQ)

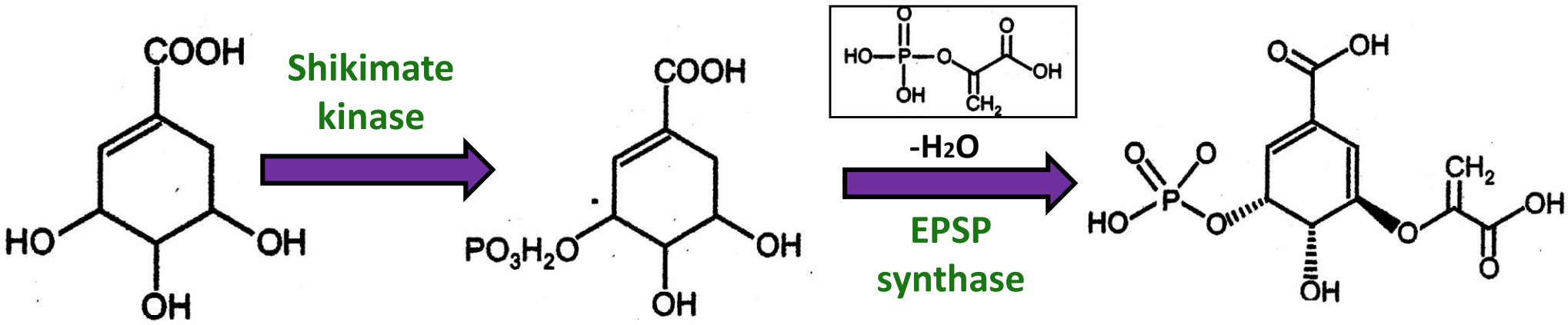
- **Step 3: DHQ** is dehydrated to **3-dehydroshikimic acid** by the enzyme **3-dehydroquinate dehydratase**.
- **Step 4:** which is reduced to **Shikimic acid** by the enzyme **shikimate dyhydrogenase** which uses Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as a cofactor.



3-dehydroquinic acid **3-dehydroshikimic acid** **Shikimic acid**

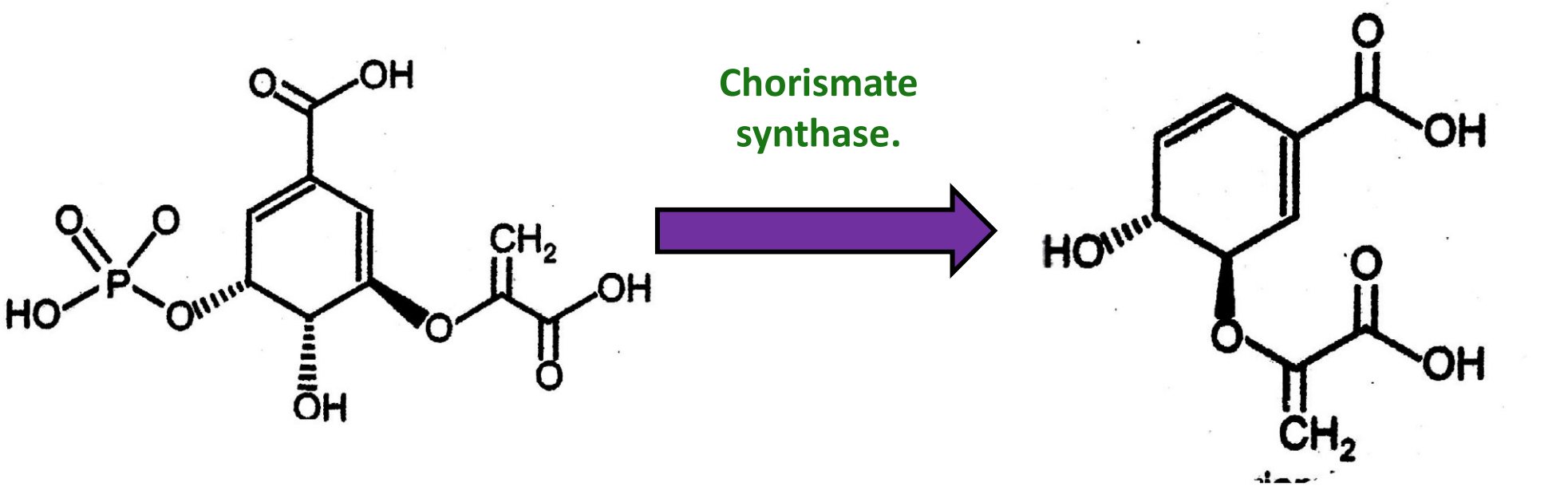
- **Step 5:** The next enzyme involved is **Shikimate kinase** an enzyme that catalyzes the ATP dependent phosphorylation of shikimate to form **Shikimic acid 3-Phosphate** from **Shikimic acid.**

- **Step 6: Shikimic acid 3-Phosphate** is then coupled with **phosphoenol pyruvate** to give **5-enol pyruvylshikimate-3- phosphate** via the enzyme **5-enol pyruvylshikimate-3-phosphate (EPSP) synthase.**



Shikimic acid | **Shikimic acid 3-Phosphate** | **5-enol pyruvylshikimate- 3-phosphate**

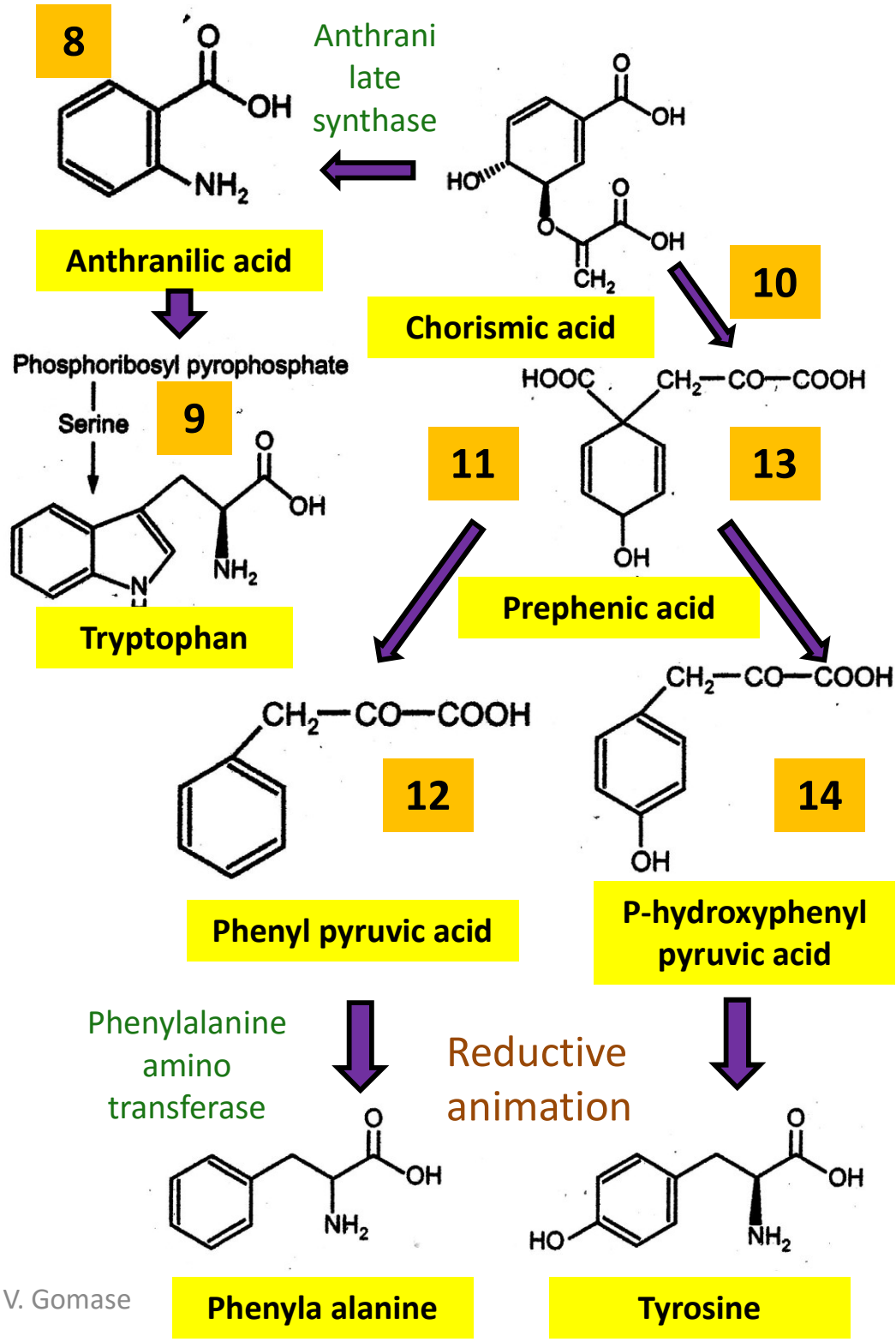
- **Step 7**: Then **5-enolyruvyl shikimate-3-phosphate** is transferred in to **Chorismic acid** via the enzyme **chorismate synthase.**



5-enol pyruvylshikimate- 3- phosphate

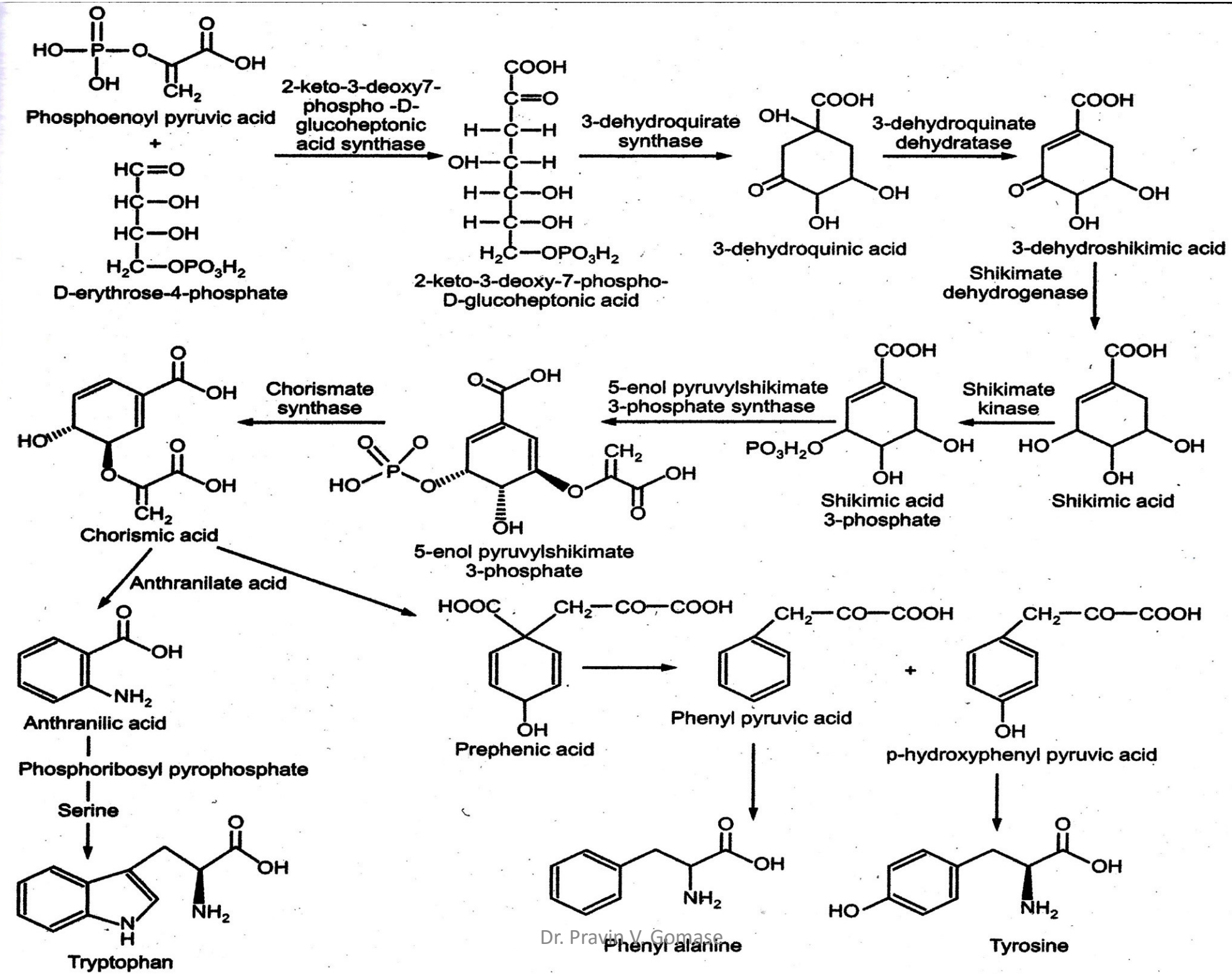
Chorismic acid

- **Step 8,9,10**: **Prephenic acid** is then synthesized by a Claisen rearrangement of chorismate by **Chorismate Mutase.**
- **Step 11, 12,13, 14**: Prephenate is oxidatively decarboxylated with retension of the hydroxyl group by **Prephenate dehygrogenase** to give **p-hydroxyphenyl pyruvat**, which is transaminated using glutamate as the nitrogen source to give **tyrosine** and **alpha-ketoglutarate**.



- **Summary and conclusion:**
- In Phenyl propanoids biosynthesis some starting material like phenolic phenyl alanine and tyrosine as a precursor can be used.
- Phenyl propanoids are used to produced the **flavonoids, Coumarin, tannins and lignin.**
- Galic acid biosynthesis (Galic acid) formed from 3-dehydroshikimate by the action of the enzyme shikimate dehydrogenase to produce 3,5-didehydroshikimate.
- The latter compound spontaneously rearranges to gallic acid.

- **Shikimic acid is a precursor for:**
- Indole, indole derivatives and aromatic amino acid tryptophan and tryptophan derivatives such as the psychedelic compound dimethyl tryptamine and many alkaloid and other aromatic metabolites.



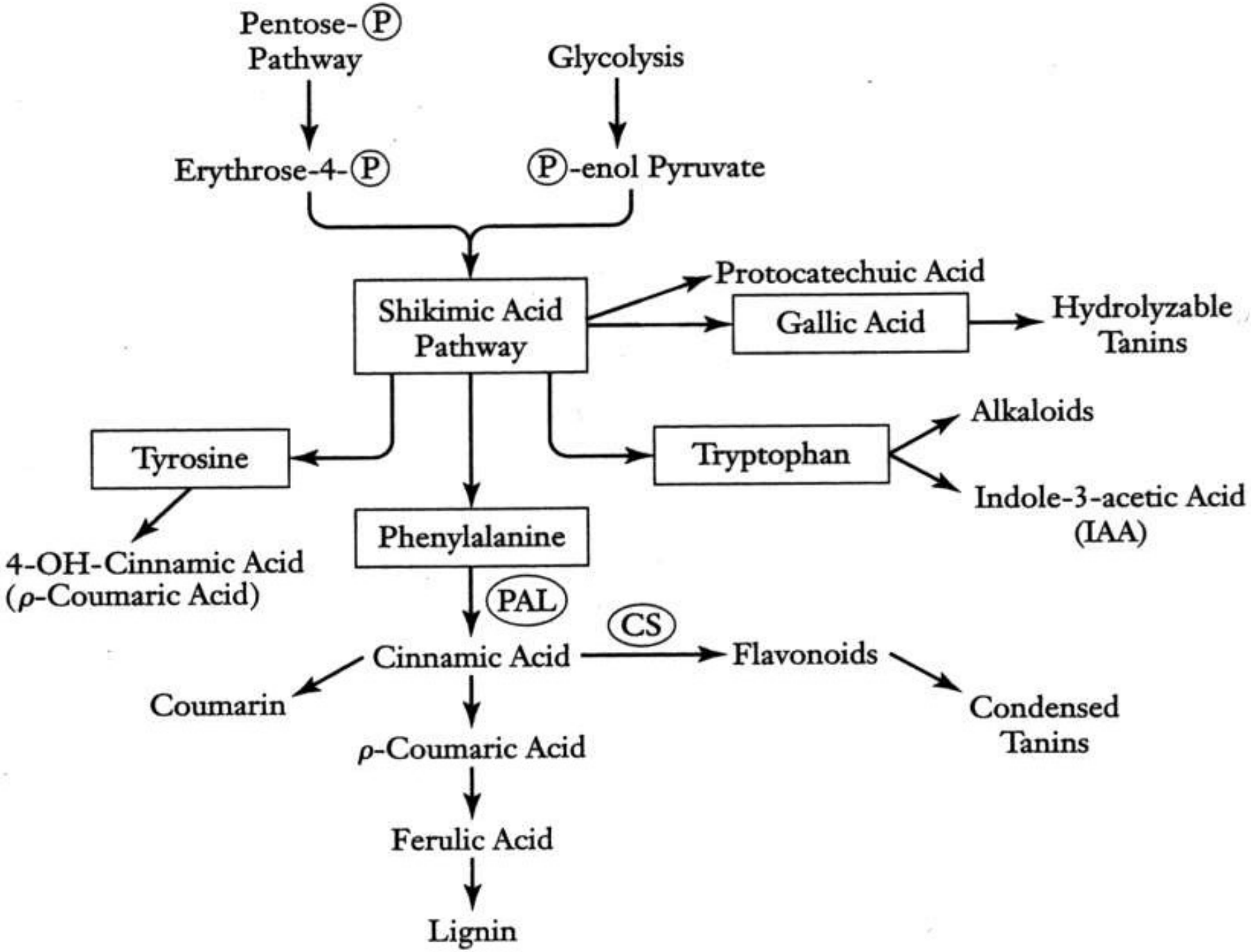


FIGURE 14.14 The central role of the shikimic acid pathway in the synthesis of various primary and secondary metabolites. PAL = phenylalanine ammonia lyase. CS = chalcone synthase.

Uses

- In the pharmaceutical industry, shikimic acid from the Chinese star anise (*Illicium verum) is used as a base material for production of* ***"oseltamivir" (Tamiflu).***

Thank You

UNIT-I

Utilization of Radioactive Isotopes in Biogenetic studies

Presented By

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Isotopes

Iso= Same (Equal), Topes=Place

- **They occupy same place in periodic table. "Elements with same atomic number but different atomic weight**
- **(Same number of protons but differ in neutrons).**
- **Example-** $^{12}C_6 \longrightarrow {}^{13}C_6 \longrightarrow {}^{14}C_6$ (Isotopes)

Atomic mass = No. of Protons + No. of Neutrons

Atomic Number = No. of protons

Hydrogen Isotopes:

$^{1}H_1$	$^{2}H_1$	$^{3}H_1$
[1P]	[1P, 1N]	[1P, 2 N]
Hydrogen	Deuterium	Tritium

Two types of Isotopes

1. Radioactive Isotopes (Radioisotopes)

Radio(Radiation)+Isotopes ⟶ Unstable Isotopes

The isotopes which emits the radiation are called **Radioisotopes.**

Decay with the emission of radiation (α, β, γ radiation).

Example - ^{3}H, ^{14}C, ^{35}S, ^{131}I, ^{24}Na, ^{42}K, ^{35}S, ^{35}P, ^{131}I

- For biological Investigation- Carbon and Hydrogen
- For Metabolic studies- S, P and alkali and alkaline earth metals are used.
- For studies on proteins, alkaloids and amino acids- labelled nitrogen atom give more specific information.
- ^{3}H compound is commercially available.

2. Stable Isotopes:

- Stable isotopes are non-radioactive forms of atoms (they do not emit radiations).
- Although they do not emit radiations, their unique properties enable them to be used in a broad variety of applications, including water and soil management, environmental studies, nutrition assessment studies and forensics.

Examples- ^{2}H, ^{13}C, ^{15}N, ^{18}O

- Used for labeled compounds as possible intermediates in biosynthetic pathways
- Usual method of detection are: - MASS Spectroscopy [^{15}N, ^{18}O]
- NMR Spectroscopy [^{2}H, ^{13}Cl]

Radiolabelled Tracers (Radio labelled compound)

- **When one or more atom of chemical compound replaced by radioisotopes used- for the study of the biosynthetic pathway, is known as Radiotracers.**

Radiotracer Technique: The technique which utilises radioactive labelled compound to find out or to trace various precursors and intermediates involved at different stages of biosynthetic pathway at given rate and time.

In this technique, different isotope, mainly the radioactive isotopes which are incorporated into the presumed precursor of plant metabolites and used as marker in the biogenic studies.

Steps in Tracer Technique

1. **Selection of Radioisotopes**
2. **Preparation of Radioisotopes**
3. **Introduction/Insertion of Radiolabelled compound in biological system (Plant part)**
4. **Seperation and determination of labelled compound in various biochemical reaction**

Selection of Radioisotopes

Selection based on according to half life

$^{10}C_6$ half life few second

$^{11}C_6$ half life few minute

$^{14}C_6$ half life around six thousand years (mainly used)

$^{3}H_1$ (Tritium) half life 12 years

2. Preparation of labelled compound:

(a) Growing chlorella in atmosphere of $^{14}CO_2$

(b) Nuclear Reactor/Acceralator

$$^{14}N_7 + {}^{1}n_0 \longrightarrow {}^{14}C_6 + {}^{1}P_1$$

(c)Tritium gas: Tritium labelled compound ($^{3}H_1$) are commercially available. Tritium labelling is effected by catallytic exchange in aqueous media by hydrogenation of unsaturated compound with tritium gas.

(d) by the use of Organic Synthesis:

$$CH_3MgBr + {}^{14}CO_2 \longrightarrow CH_3{}^{14}COOHMgBr + H_2O$$

$$CH_3{}^{14}COOH + MgOHBr$$

Radiolabelled

Acetic acid

Insertion of Radiolabelled compound in plant part

Precaution

- **The precursor should react at necessary site of synthesis in plants.**
- **Plant at the experiment time should synthesize the compound under investigation.**
- **The dose given is for short period.**

1. **Root feeding: When the root is biosynthetic site (Ex- Tobacco and Datura alkaloid).**
2. **Stem Feeding: Cut end of stem immersed in water, nutrient and radiolabelled compound.**
3. **Direct Injection: Which have hollow stem (Umbelliferous fruits).**
4. **Infilteration: It is also called Wick Feeding method.**
5. **Floating method: Substrate solution which contain Radioactive compound.**
6. **Spray technique**

Separation or Isolation of Radiolabelled compound and detection of radioisotope labelled compound

Depends on Nature of drugs and Sources of drugs-

Soft tissue- Infusion, Maceration

Hard Tissue- Decoction, hot percolation

Unorganised drugs- Maceration

Alkaloid, Glycoside, Flavonoids- Slightly polar solvent

Applications of Tracer Technique

1. **Study of squalene cyclization by use of ^{14}C, ^{3}H labelled mevalonic acid.**
2. **Interrelationship among 4-methyl sterols and 4,4-dimethyl sterols, by use of ^{14}C acetate.**
3. **Terpenoid biosynthesis by chloroplast isolated in organic solvent by use of 2-^{14}C mevalonate.**
4. **Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin.**
5. **Origin of carbon and nitrogen atoms of purine ring system by use of ^{14}C or ^{15}N labelled precursor.**
6. **Study of formation of scopoletin by use of labelled phenylalanine.**
7. **By use of ^{45}Ca as tracer, found that the uptake of calcium by plants from the soil (CaO and $CaCO_2$).**
8. **By adding ammonium phosphate labelled with ^{32}P of known specific activity the uptake of phosphorus is followed by measuring the radioactivity as label reaches first in lower part of the plant, than the upper part i.e. branches, leaves, etc.**

Thank You

Dr. Pravin V. Gomase