PHARMACOGNOSY-II

UNIT 1 NOTES

BASIC METABOLIC PATHWAYS

- SHIKIMIC ACID PATHWAY
- ACETATE PATHWAY
- AMINO ACID PATHWAY
- STUDY OF UTILISATION OF RADIOACTIVE ISOTOPES IN BIOGENETIC STUDIES

IMPERFECT PHARMACY

METABOLIC PATHWAYS

- Metabolism in plants refers to all the complex set of chemical reactions that occur within plant cell to maintain life, grow & reproduce
- Metabolic Pathways in Plants refers to series of interconnected biochemical reactions in plant cells, where a specific molecule is converted through a series of steps into another molecule.
- Metabolites are small compounds that are intermediates or end products of metabolic processes in living organism.

TYPES OF METABOLITES

Metabolites are of two types :

① Primary Metabolites
② Secondary Metabolites

PRIMARY METABOLITE

- These are the metabolites that are directly involved in growth & development of living organism.
- They play central role in basic metabolic pathway.
- example : sugars, amino acids lipid etc

SECONDARY METABOLITE

- These are the metabolites that are not essential for growth & development of organism
- They are often byproducts of primary metabolism
- example : Alkaloids, Glycosides Flavanoids, Tannins etc.

SHIKIMIK ACID PATHWAY

- The Shikimic Acid Pathway is a metabolic pathway for the biosynthesis of certain Aromatic Amino Acids (Phenylalanine, Tyrosine, Tryptophane).
- Shikimic Acid is a key intermediate in this pathway.
- The pathway occurs in plants & certain microorganism but not in animals.

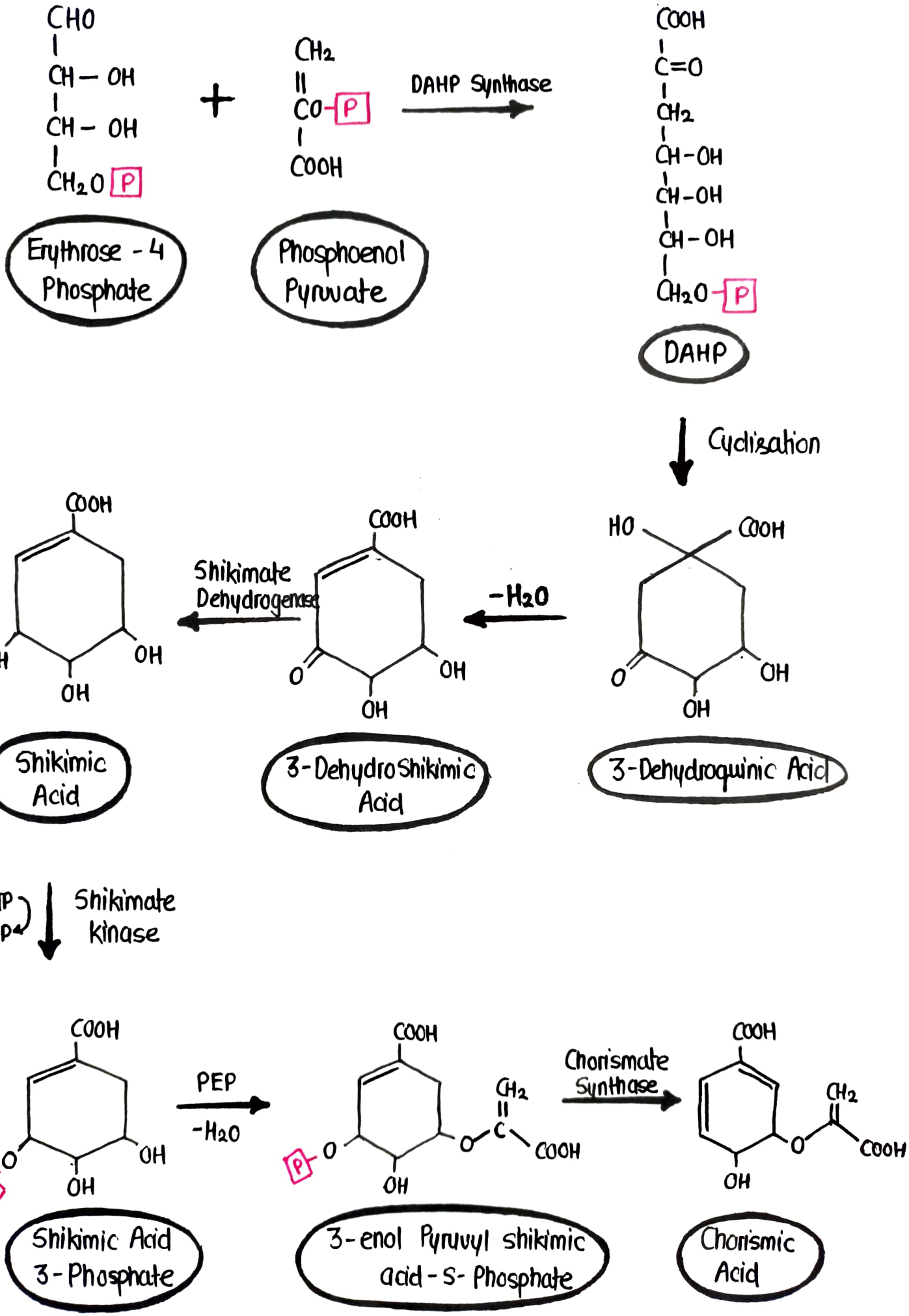
KEY STEPS

The pathway have following key steps:

① **Starting Material**: The pathway begins with Phosphoendpyruvate (PEP) and Erythrose 4 Phosphate.

② **Conversion**: Through a series of enzymatic reactions, these substrates are converted into Shikimic Acid

③ **Further Reactions**: Shikimic Acid undergoes additional transformations to produce the 3 aromatic amino acids i.e. Phenylalanine, Tyrosine, Tryptophan.


CHO
CH – OH
CH – OH
CH2O P
Erythrose - 4 Phosphate
CH2
CO P
COOH
Phosphoenol Pyruvate
DAHP Synthase
COOH
C=O
CH2
CH-OH
CH-OH
CH-OH
CH2O P
DAHP
Cyclisation
HO
COOH
O
OH
OH
3-Dehydroquinic Acid
-H2O
Shikimate Dehydrogenase
3-Dehydro Shikimic Acid
Shikimic Acid
ATP
ADP
Shikimate kinase
PEP
-H2O
Shikimic Acid 3-Phosphate
Chorismate Synthase
3-enol Pyruvyl shikimic acid-5-Phosphate
Chorismic Acid

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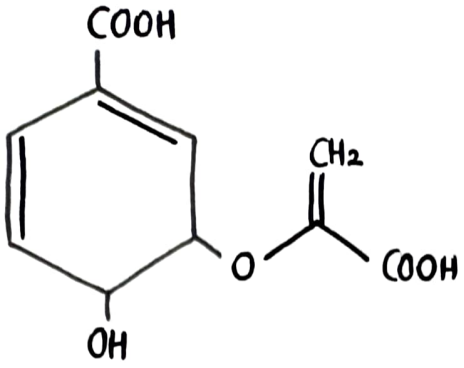
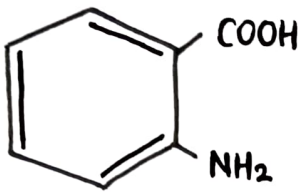


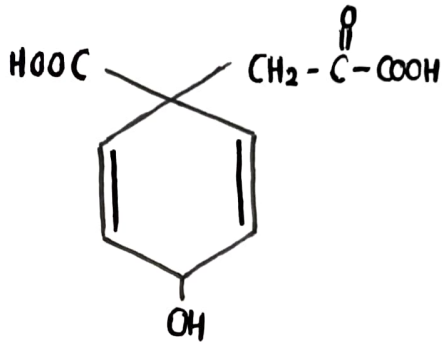
Chorismic Acid

Anthranilic Acid Synthase

Chorismate Mutase



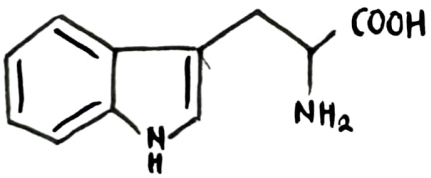
Anthranilic Acid



Prephenic Acid

Phosphoribo-
-syl Pyropho-
-sphate

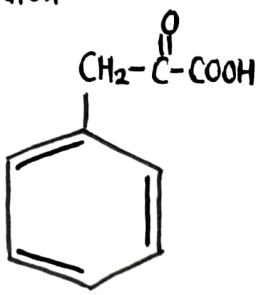
Serine



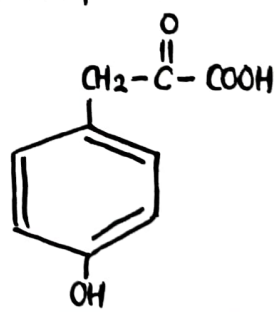
TRYPTOPHAN

Decarboxylation & Dehydration

Dehydrogenation & Decarboxylation

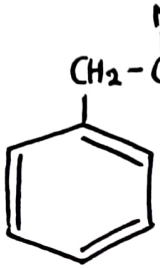


Phenyl Pyruvic Acid



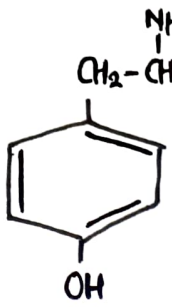
4-Hydroxy Phenyl Pyruvic acid

Reductive Transamination



PHENYLALANINE

Reductive Transamination



TYROSINE

Important key steps in Shikimic Acid Pathway

The shikimic acid pathway is crucial for the biosynthesis of aromatic amino acids in plants, fungi & some bacteria, Here are key steps:

① Formation of Dehydroquinate

The pathway begins with condensation of Phosphoenol pyruvate (PEP) & Erythrose 4 Phosphate (E4P) to form 3- Dehydroquinate via the enzyme DAHP Synthase.

② Reduction To Shikimic Acid

3- Dehydroquinate is then converted to Shikimic Acid through a series of enzymatic reactions, including the action of 3- Dehydroquinate dehydratase & shikimate dehydrogenase.

③ Formation of Chorismic Acid

Shikimic acid undergoes further transformations to produce Chorismic Acid via the action of several enzymes including Shikimate kinase & Chorismate Synthase.

④ Biosynthesis Of Aromatic Amino Acids

Chorismic Acid serves as a precursor for synthesis of 3 amino acids

- Phenylalanine
- Tyrosine
- Tryptophan

SIGNIFICANCE

- It is crucial for production of aromatic amino acids i.e., Phenyalanine, Tyrosine & Tryptophan
- The pathway leads to synthesis of important secondary metabolites, including, flavanoids, Alkaloids etc.
- Aromatic Amino Acids derived from this pathway are vital for plant growth, influencing processes such as cell division & differentiation.
- Shikimic acid is a precursor for synthesis of various pharmaceutical products i.e. Antiviral drugs etc.

ACETATE PATHWAY

- The acetate pathway, also known as acetyl Co-A pathway, is a key metabolic route involved in the synthesis & breakdown of Acetyl Co-A, a critical molecule in cellular metabolism.
- It is also one of the important pathway for synthesis of different types of secondary metabolites.

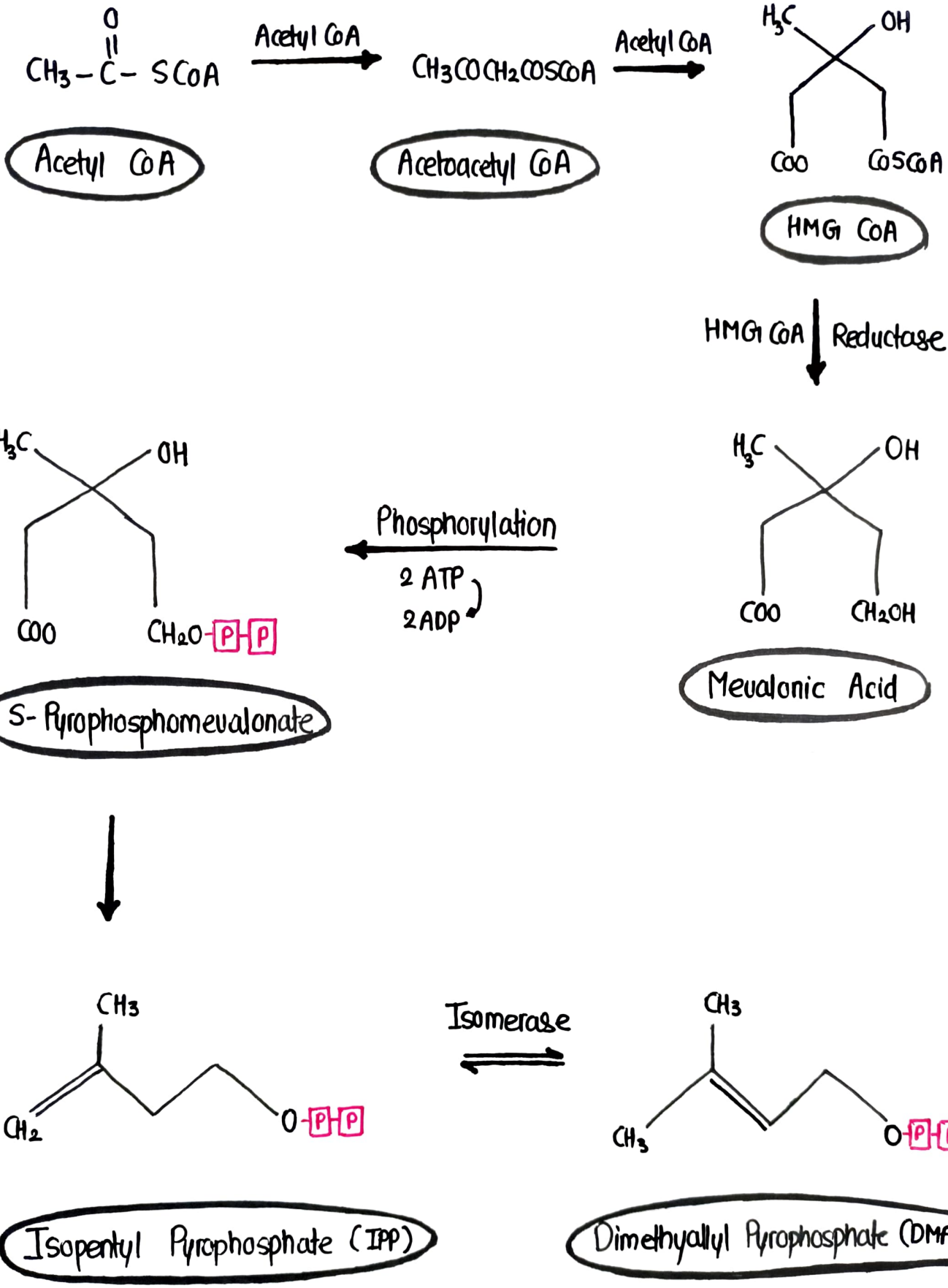
ROUTES FOR ACETATE PATHWAY

There are two main route of acetate pathway :

① Acetate Mevalonate Pathway
② Acetate Melonate Pathway

ACETATE MEVALONATE PATHWAY

- The acetate - mevalonate pathway is a biosynthesis pathway crucial for the synthesis of cholesterol, steroid hormones & other isoprenoid (terpenoids) compound.
- The pathway starts from Acetyl-CoA and proceeds through several steps to produce Mevalonate, which is eventually converted into isoprenoid units like Isopentyl Pyrophosphate (IPP) and Dimethylallyl Pyrophosphate (DMAPP).


Acetyl CoA
Acetyl CoA
Acetyl CoA
Acetyl CoA
Acetoacetyl CoA
HMG CoA
HMG CoA Reductase
Mevalonic Acid
Phosphorylation
2 ATP
2 ADP
S-Pyrophosphomevalonate
Isomerase
Isopentyl Pyrophosphate (IPP)
Dimethyallyl Pyrophosphate (DMAPP)

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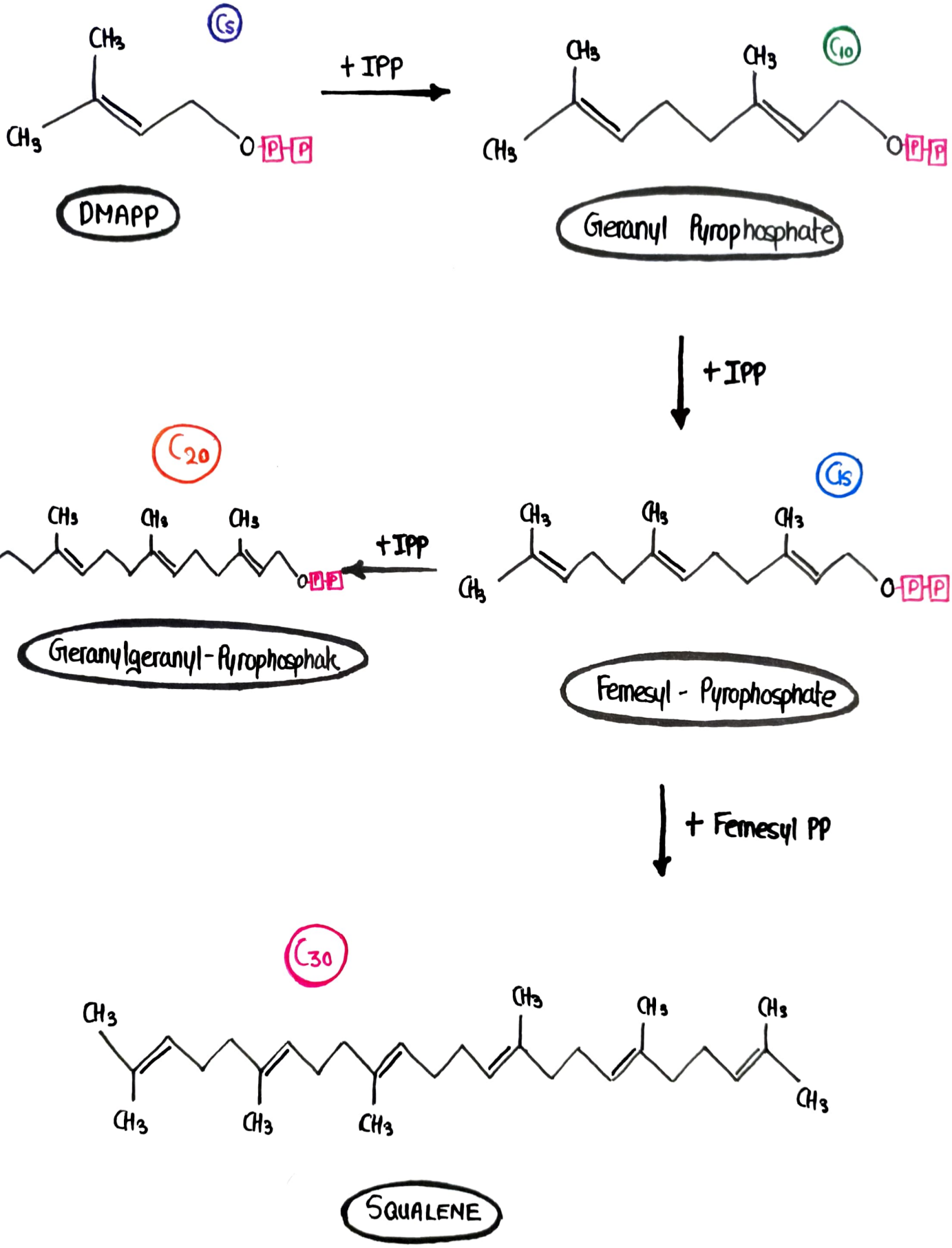

C5
CH3
CH3
O
P
P
DMAPP
+ IPP
C10
CH3
CH3
CH3
O
P
P
Geranyl Pyrophosphate
+ IPP
C15
CH3
CH3
CH3
CH3
O
P
P
Fernesyl - Pyrophosphate
+IPP
C20
CH3
CH3
CH3
CH3
CH3
O
P
P
Geranylgeranyl - Pyrophosphate
+ Fernesyl PP
C30
CH3
CH3
CH3
CH3
CH3
CH3
CH3
CH3
CH3
SQUALENE

KEY STEPS IN ACETATE MEVALONATE PATHWAY

The acetate mevalonate pathway is crucial for biosynthesis of isoprenoids & sterols.
Here are main steps involved :

① Acetyl CoA Formation

Acetyl Co-A is generated from breakdown of carbohydrates, fats & proteins.

② Condensation

Two molecules of Acetyl CoA condense to form Acetoacetyl-CoA, catalyzed by enzyme Thiolase.

③ Formation of HMG CoA

Acetoacetyl Co-A condense with another Acetyl CoA to form 3-Hydroxyl-3-methylglutaryl-CoA (HMG CoA), facilitated by HMG CoA synthase.

④ Reduction to Mevalonate

HMG CoA reduced to Mevalonate by the enzyme HMG-CoA reductase, which is a key regulatory step in pathway.

⑤ Phosphorylation of Mevalonate

Mevalonate is phosphorylated twice to form Mevalonate-5-Pyrophosphate.

⑥ Decarboxylation

Mevalonate-5-Pyrophosphate undergoes decarboxylation to produce Isopentyl Pyrophosphate (IPP).

Isomerisation

IPP can isomerize to form Demethylallyl Pyrophosphate (DMAPP).

Formation of Geranyl Pyrophosphate

IPP & DMAPP condense to form Geranyl Pyrophosphate.

Formation of Farnesyl Pyrophosphate

Geranyl Pyrophosphate condense with IPP to form Farnesyl Pyrophosphate.

Synthesis of Squalene

To FPP molecule condense to form Squalene, which a precursor to & sterols.

Cyclization & Modification

Squalene undergoes cyclization & further modifications to produce sterols & other isoprenoid compounds.

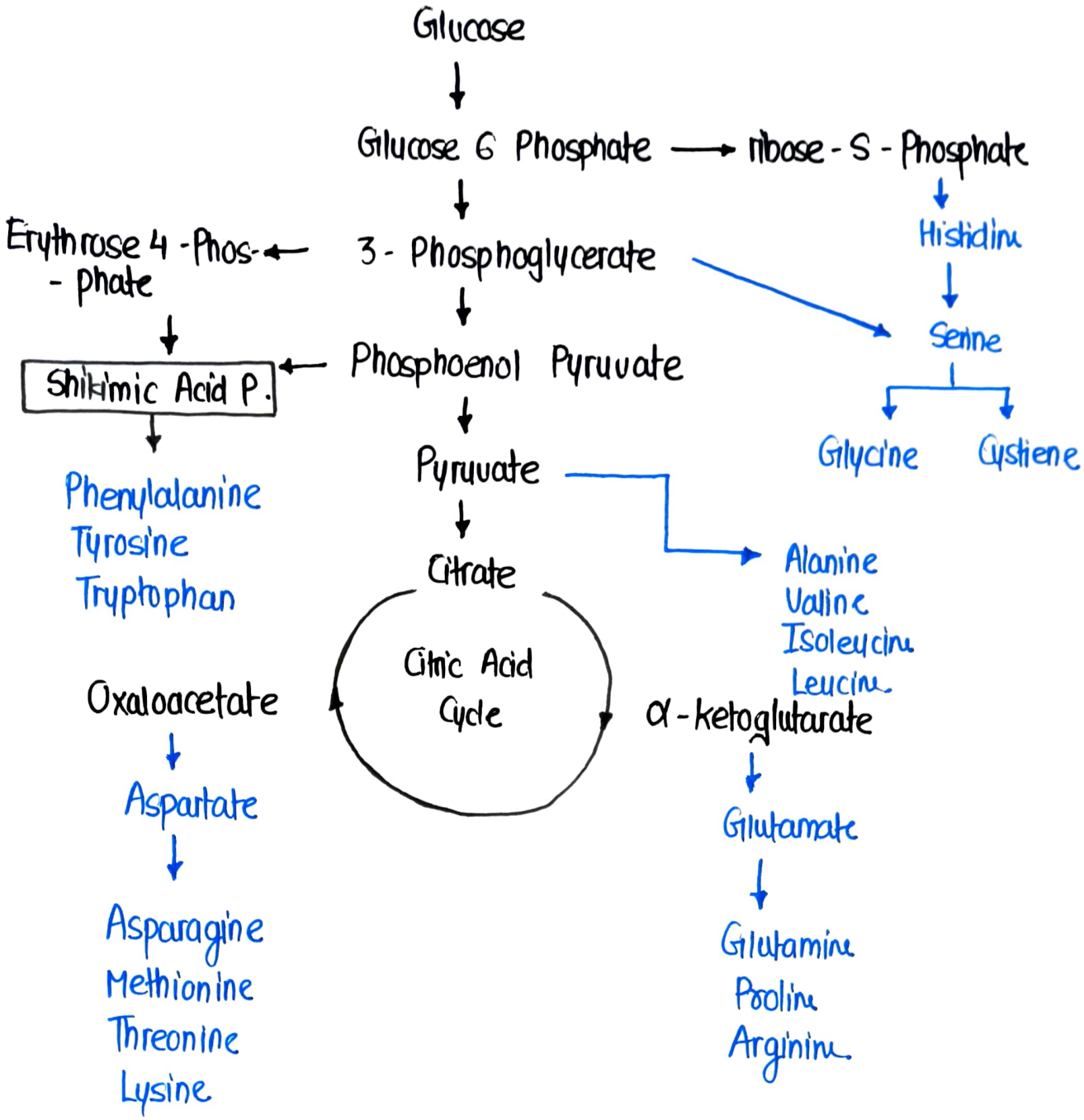
SIGNIFICANCE

- It is crucial for producing isopoenoids, which includes essential molecules like Terpenes, Carotenoids & Steroid Hormones.
- The pathway leads to cholesterol synthesis, vital for cell membrane integrity., hormone production & overall cellular functions
- key enzymes in this pathway, particularly HMG CoA reductase serves as regulatory points and are targets for Cholestrol-lowering drugs

Amino Acid Pathway

Amino acids are organic compounds containing Amine & Carboxyl functional group, along with a side chain group specific to each amino acid.
Amino acid pathway is set of biochemical pathway through which amino acids are produced.
All amino acids are derived from intermediate in Glycolysis, Citric Acid Cycle or Pentose Phosphate Pathway.
Whereas most bacteria & plants can synthesize all 20 amino acids required for protein synthesis, Humans can only synthesize half of them.

Essential Amino Acids	Non Essential Amino Acids
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Lysine	Cysteine
Methionine	Glutamate
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
Leucine	Tyrosine


Glucose
Glucose 6 Phosphate
ribose - 5 - Phosphate
Histidine
Erythrose 4 - Phos- - phate
3 - Phosphoglycerate
Serine
Glycine
Cystiene
Shikimic Acid P.
Phosphoenol Pyruvate
Phenylalanine
Tyrosine
Tryptophan
Pyruvate
Citrate
Alanine
Valine
Isoleucine
Leucine
Citric Acid Cycle
Oxaloacetate
α - ketoglutarate
Aspartate
Glutamate
Asparagine
Methionine
Threonine
Lysine
Glutamine
Proline
Arginine

Investigation Of Biogenetic Studies

Various Intermediates and steps that are involved in biosynthetic pathway in plants can be investigated by following techniques:

- Tracer Technique
- Use of isolated organ & tissues
- Grafting Methods
- Use of Mutant Strains
- Enzymatic Studies

Tracer Technique

- Tracer Technique involves the use of radioactive istopes that are crucial in biogenetic studies to track & understand various biological processes at molecular & cellular levels.
- Radioactive isotopes play a crucial role in biogenetic studies, aiding the understanding of biologolical poocesses at molecular level.
- Their unique prooperties allow researchers to trace & visualize complex biochemical pathways.

Isotopes

- Isotopes are made up of two words, Iso → same & Topes → Place.
- Means they occupy same place in periodic table i.e., having same atomic number but different atomic weight.
- They same no of prooton but different numbers of Neutron.
- Example : ${}_6C^{12}$, ${}_6C^{13}$, ${}_6C^{14}$

  ${}_1H^1$, ${}_1H^2$, ${}_1H^3$

Types Of Isotopes

Isotopes are of mainly two types

① Radioactive Isotopes / Unstable Isotopes

② Stable Isotopes

Radioactive Isotopes

- In radioactive isotopes, Neutron & proton combination is very unstable in nature & have excess energy
- They emit radiation in the form of α, β & γ particles as they decay into more stable forms
- They are the ones used for biological investigation.
- Example : ^{3}H, ^{14}C, ^{35}S, ^{131}I etc.

Stable Isotopes

- They are stable in nature
- They do not emit radiations.
- Example : ^{2}H, ^{13}C, ^{15}N etc.

Steps Involved In Tracer Techniques

- Preparation of Labelled Compound
- Incorporation of Labelled Compound
- Separation & Isolation of Labelled Compound
- Determination of Natural Metabolites

Preparation Of Labelled Compound

- For biological investigation, first we prepared labelled compound by using radioactive isotopes or stable isotopes.
- Most commonly, we used radiactive carbon & hydrogen.
- The radiactive isotopes used for preparation of labelled compounds must have long half life.
- They should not damage the tissue system.

Incorporation Of Labelled Compound

- In this step, we basically insert the labelled compound in plants part
- The precursors should react at necessary site of synthesis in plants.
- There are different methods as follows :

① **Root Feeding :** This method is prefered in plants in which the roots are the sites of biosynthesis e.g. Tobacco

② **Stem Feeding :** In this labelled compounds administered into the plant through stem as a solution foom.

③ **Direct Injection :** This method is preffered for plants having hollow stem e.g. opium

④ **Infiltration :** In this a thread is drawn through the stem which is dipped into radioactive solution.

⑤ **Spraying :** In this method compounds have been absorbed after being sprayed on leaves.

Separation & Isolation of Labelled Compound

For separation & isolation, different methods are used depending on nature of drug & its sources.

- Soft Tissues : Infusion, Maceration.
- Hard Tissues : Decoction & Hot Percolation
- Unorganized Drug : Maceration with solvent
- Fat & Oil : Non Polar Solvent.

Determination Of Nature of Metabolite

The following different types of detectors are used to determine the nature of metabolites :

- Geiger - Muller Counter
- Liquid Scintillation Chamber
- Ionization Chamber
- Mass Spectrophotometer
- NMR Spectrophotometer
- Autoradiography

THANK YOU

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