PHARMACOGNOSY & PHYTOCHEMISTRY

B. PHARM. V^TH SEM.

UNIT-I

Shailesh pathak

* **PHARMACOGNOSY:-** Pharmacognosy known initially as materia medica, It is defined as the study of crude drugs obtained from plants, animals and mineral.

⇒ The word pharmacognosy is derived from two latin words. Pharmakon = a drug
gignoso = To acquire knowledge
It means knowledge or science of drugs.

⇒ Pharmacognosy is a vital link between Ayurvedic and Allopathic system of medicine.

⇒ Phytochemistry is the study of phytochemicals, which are chemicals derived from plants, particularly the secondary metabolits which are synthesized as a measure for self defense against insects, pests, pathogens, herbivores, U.V. exposure and environmental hazards.

* **Metabolic pathways in higher plants and their determination:-**

Study of basic metabolic pathways and formation of different secondary metabolit through pathway.

* **Metabolite:-** Metabolite is an intermediate or end product of metabolism

* **Metabolism:-** the conversion of the energy in food to energy. like the conversion of food to building blocks for protein, lipid, nucleic acid and carbohydrate

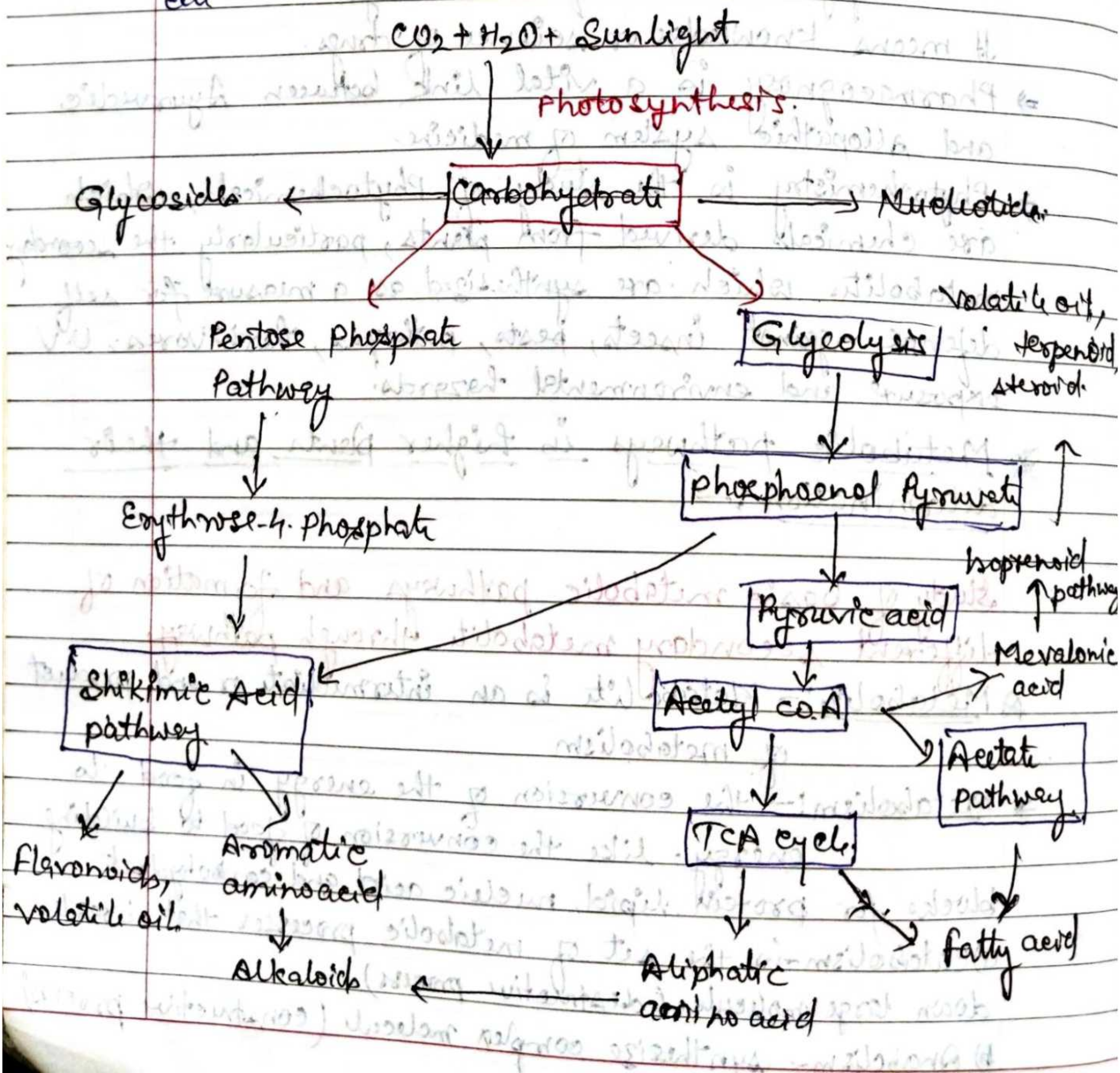
a) Catabolism - is the set of metabolic processes that break down large molecule. (destructive process)

b) Anabolism - synthesize complex molecule (constructive process)

① * Primary metabolite :- Primary metabolites which are directly involved in growth, development and reproduction. Ex. carbohydrate, protein, lipid, Vitamin, amino acids, Harmones. etc

② Secondary metabolites :- Secondary metabolites does not involved in growth & development. act as defense system for plant. Ex. Alkaloid, glycoside, steroids, essential oils etc.

* Metabolic pathway :- conversion of primary to secondary metabolit through a linked series of chemical reaction occuring within cell.



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Secondary metabolite through these pathway:

1. Shikimic acid Pathways
2. Acetate Pathways.
3. Amino acid Pathways.

1) SHIKIMIC ACID PATHWAYS :-

The Shikimate pathway provides an alternative route to aromatic compounds, particularly the aromatic amino acids phenyl alanine, tyrosine and tryptophan.

⇒ This pathways employed by microorganisms and plants but not by animals and accordingly the aromatic A.A feature among those amino acid (AA) for man whom have to obtained in the diet

⇒ A central intermediate in the pathways is shikimic acid, a compound which had been isoleted from plants of Illicium species (Japanese shikimi).

⇒ The pathway from phosphaenol pyruvate (from glycolysis) and d-erythrose-4-phosphate (from the pentose phosphate cycle)

⇒ Phenyl alanine and tyrosine form the basis of C_3 C_6 phenylpropane unit found in many natural product

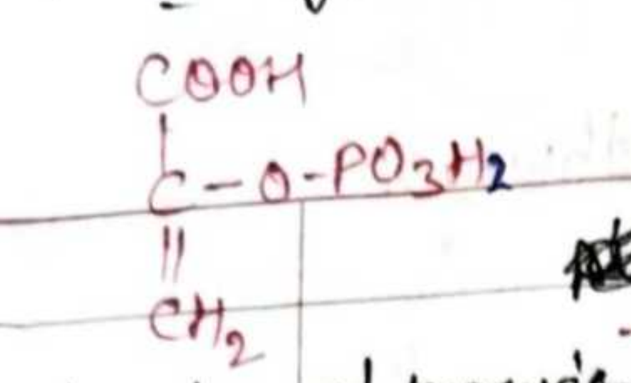
⇒ The shikimic acid pathway contains several branch point, the first of these, dehydroquinic acid. The enzym catalysing the dehydration of dehydroquinic acid are of two kinds

⇒ The formation of chorismic acid is an important branch point the shikimic acid pathways as this compound can undergo three different type of conversion

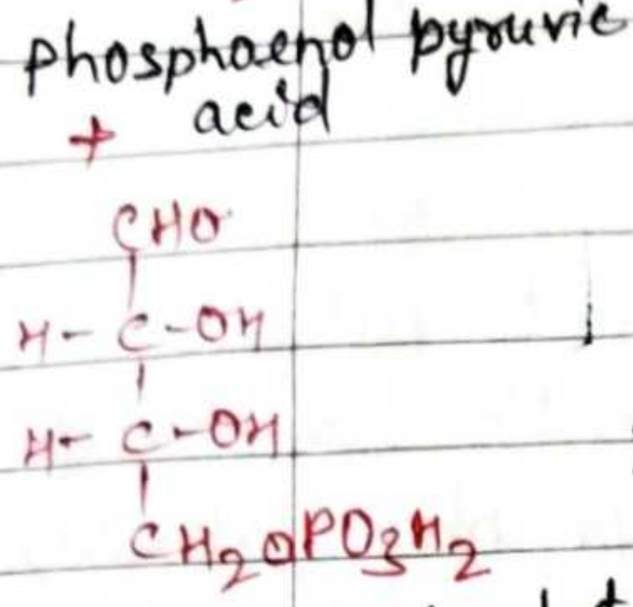
⇒ The name "chorismic" is derived from a Greek word for seperate Indicating the multiple role of this compound.

⇒ In the presence of glutamine chorismic acid converted to anthranillic acid. whereas chorismate mutase catalyses the formation of prephenic acid.

* Biosynthesis of aromatic compound via Shikimic acid pathway

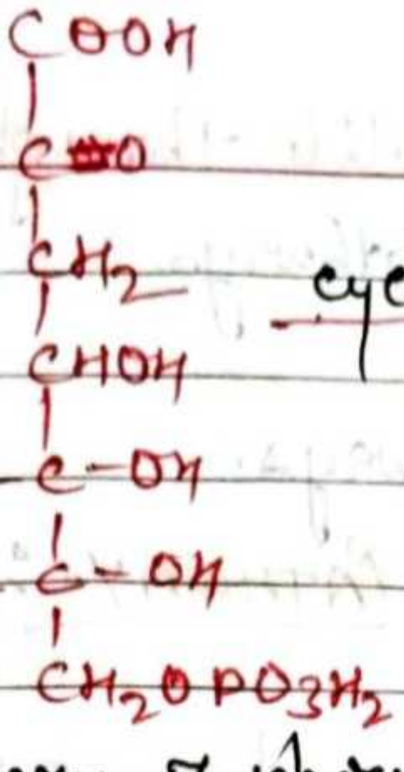


phosphoenol pyruvic acid



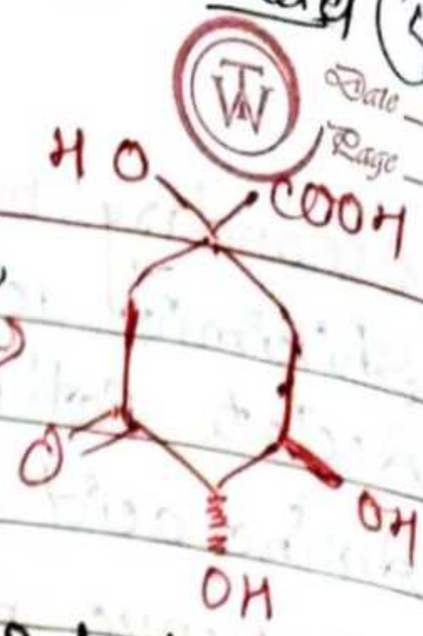
Erythrose-4-phosphate (1)

condensation →



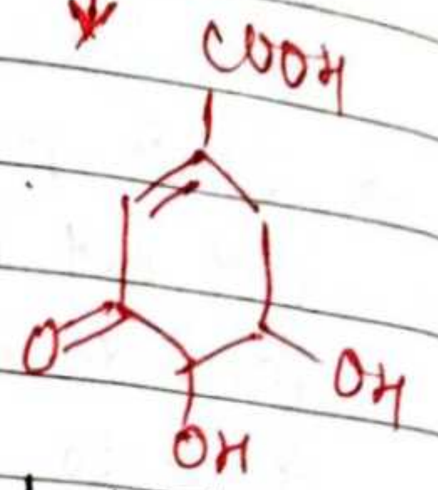
2-Keto-3-deoxy-7-phospho D-glucoheptonic acid (2)

cyclization →

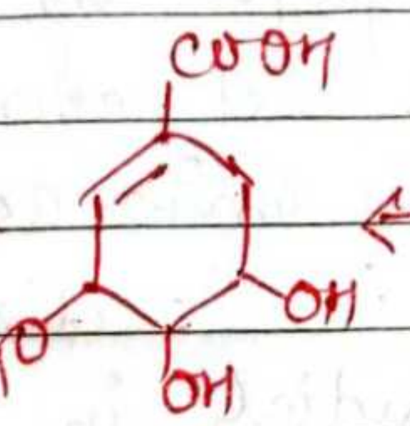


3-Dehydroquinic acid (3)

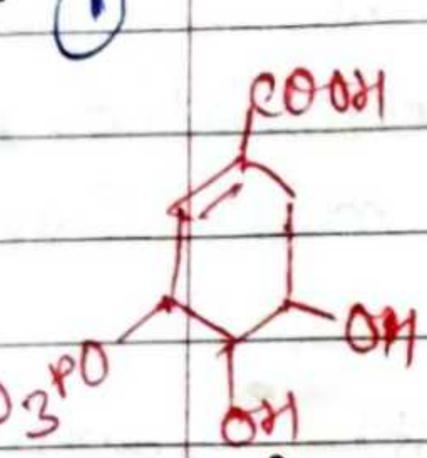
-H2O | dehydrogenation ↓



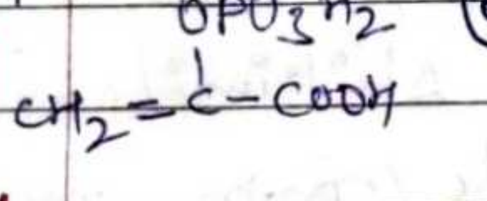
3-Dehydroshikimic acid (4)

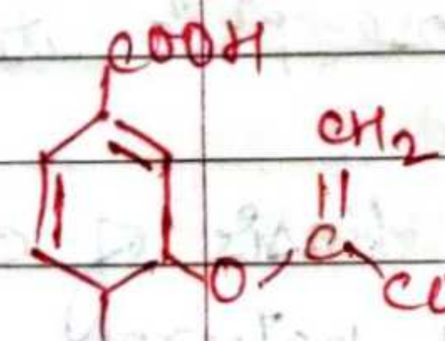


Shikimic acid (5)

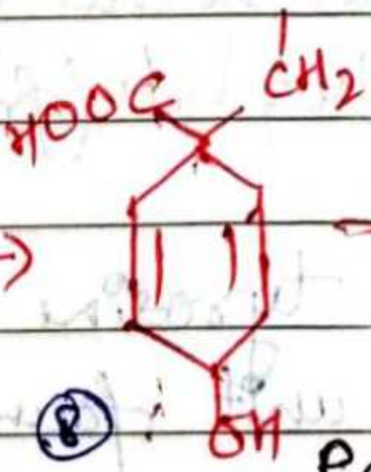


Shikimic acid-3-phosphate (6)

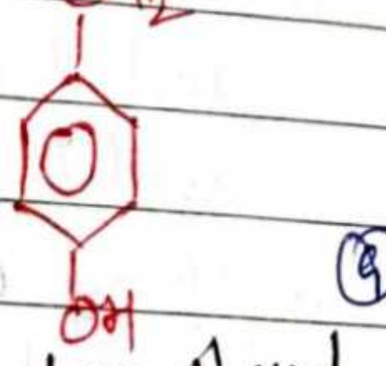
-H2O | 



Chorismic acid (7)

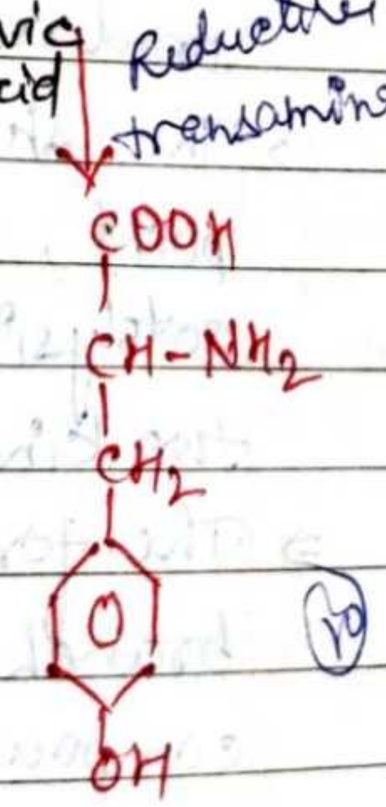


Prephenic acid (8)

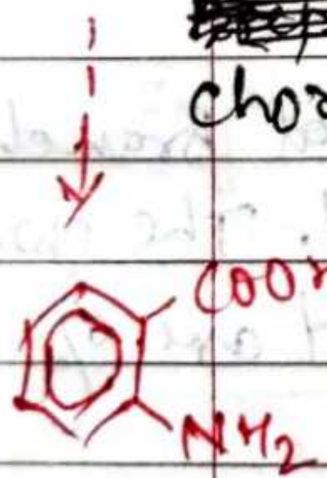


p-Hydroxy phenyl Pyruvic acid (9)

Reductive transamination ↓



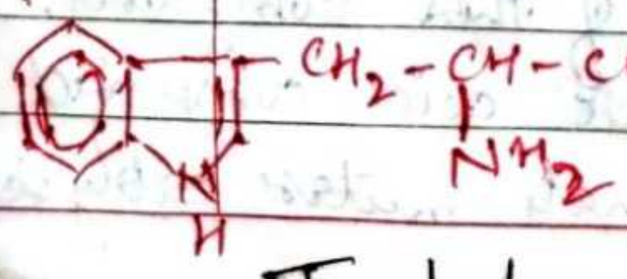
Tyrosine (10)



Anthranilic acid (8)

phosphoribosyl Pyrophosphate

Serine



Tryptophan (9)

Reductive transamination ↓

phenyl pyruvic acid (9)

phenyl alanine (10)

⇒ Then after anthranilic acid is converted into tryptophan.

⇒ Tyrosine and phenylalanine are both synthesized from prephenic acid but by independent pathways.

⇒ In the formation of tyrosine, prephenic acid is first aromatized to 4-hydroxyphenylpyruvic acid, a reaction catalysed by prephenate dehydrogenase. Transamination catalysed by tyrosine aminotransferase then gives tyrosine.

⇒ The biosynthesis of phenylalanine involves first the aromatization of prephenic acid to phenyl pyruvic acid a reaction catalysed by prephenate dehydratase and then transamination catalyse by phenylalanine aminotransferase which gives phenylalanine.

✱ Acetate-Malonate Pathway :- OR ACETATE PATHWAY :-

Acetate pathway is also known as -
- Isoprenoid pathway
- Terpenoid pathway
- HMG CoA reductase pathway.
- Acetate mevalonate pathway

⇒ This secondary metabolite used in the biosynthesis of steroid and Isoprenoids (terpenoids) etc

⇒ Since a long time biochemists aware of the involvement of acetic acid in the synthesis of cholesterol, squalene and rubber like compound.

- The discovery of Acetyl CoA called as 'active acetate' in 1950 further supported the role of acetic acid in biogenetic pathways.
- Later mevalonic acid was found to associated with Acetate. Mevalonic acid further produced Isopentyl pyrophosphate (IPP) and its isomer

Dimethylallyl Pyrophosphate (DMAPP).

- These two main intermediate IPP & DMAPP act the active isoprene unit as a basic building block of Isoprenoid compound.
- Acetate pathways operate functionally with the involvement of acyl carrier protein (ACP) to yield fatty acyl thioester of ACP. The acyl thioesters forms the important intermediate in fatty acid synthesis.

$CH_3-COSCoA$ + $CH_3-\overset{O}{\overset{\|}{C}}-SCoA$

Acetyl CoA — Acetyl CoA

↓ Claisen condensation

$CH_3-\overset{O}{\overset{\|}{C}}-CH_2-\overset{O}{\overset{\|}{C}}-SCoA$

Acetoacetyl CoA

Acetyl CoA | Aldol rex^A

$HOOC-CH_2-\overset{CH_3}{\overset{|}{C}}(OH)-CH_2-\overset{O}{\overset{\|}{C}}-SCoA$

B-Hydroxy-B-methyl glutaryl-CoA (HMG CoA)

2NADPH | HMG CoA Reductase.

$HOOC-CH_2-\overset{CH_3}{\overset{|}{C}}(OH)-CH_2-CH_2OH$

Mevalonic acid

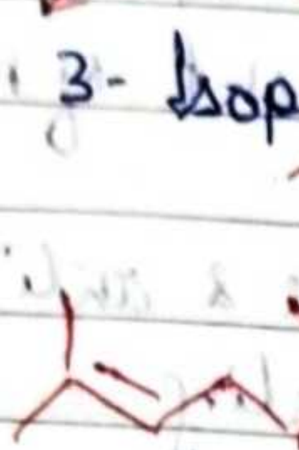
ATP | Phosphorylation

$HOOC-CH_2-\overset{CH_3}{\overset{|}{C}}(OP)-CH_2-OPP$

5-Pyrophosphate-3-phospho mevalonic acid

$-CO_2$ | Decarboxylation

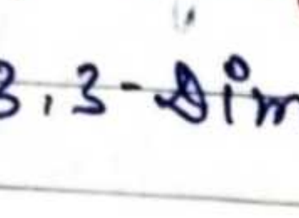
{ IPP, DMAPP } 5C



(R = H_2PO_3)

3- Isopentyl Pyrophosphate (IPP)

↕ Isomerase



3,3-Dimethyl allyl pyrophosphate (DMAPP)

↓ +IPP

($C_{10}PP$)

Geranyl Pyrophosphate (GPP) — monoterpenoid.
(Straight chain, cyclic & bicyclic)

↓ +IPP

($C_{15}PP$)

Farnesyl Pyrophosphate (FPP) → Sesquiterpenes: C15
(Open chain, mono & bicyclic)

×2 ↓ +IPP

$C_{20}PP$ (Diterpenoid)

$C_{30}PP$ ×2

Squalene → Triterpenes

Carotenoids (Tetraterpenoid)

Steroids

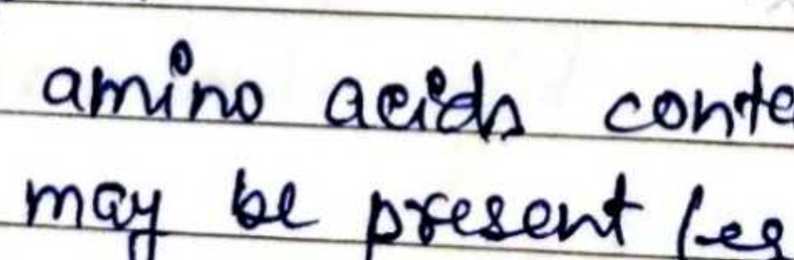
Uses of Terpenoid :-

⇒ Monoterpenoid (C_{10}) used as fragrances, Repellent.
⇒ Sesquiterpenoid (C_{15}) used as treatment of malaria, migraine, bacterial. Infection etc.
⇒ Diterpenoid (C_{20})- It is used in CVS & anti inflammatory.
⇒ triterpenoid (C_{30}) used as wounds healing.
⇒ Steroids, used as: fever, asthma.

* AMINO ACID PATHWAY :-

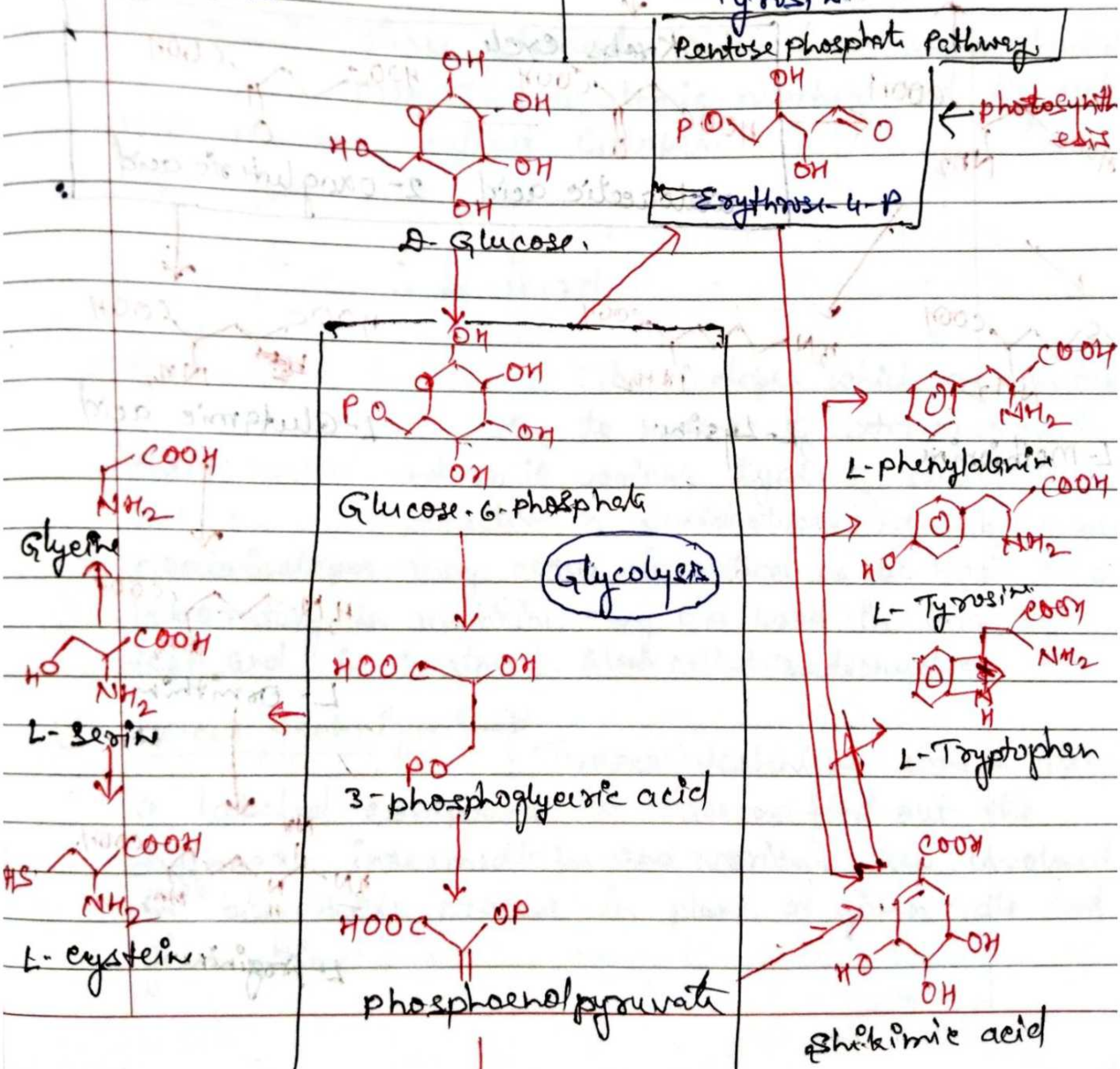
Shailesh Pathak

⇒ Amino acids occur in plants both in free state and as the basic units of proteins and other metabolites.
⇒ They are compound containing one or more amino groups and one or more carboxylic acid groups.
⇒ Most of those found in nature are α- amino acids with an asymmetric carbon atom.



⇒ Some 20 different ones have been isolated from protein, all having an L configuration.
⇒ Some having the D- configuration have been isolated from plants and microorganism, where they may form polypeptide.
⇒ Many amino acids contain only C, H, O & N but other atoms may be present (eg. Sulphur in cystein and Iodine in thyroxin).
⇒ Amino acids are generally soluble in water but only slightly soluble in alcohol.
⇒ A general test is to warm with ninhydrin which the exception of proline, which gives yellow.

Essential amino acid	Non-essential amino acid.
⇒ Not synthesized by body Ex Arginine - Histidine - Isoleucine - Leucine - Lysine - Methionine - Phenylalanine - Threonine - Tryptophan - Valine	⇒ Synthesized by body. Ex. Alanine - Asparagine - Aspartate - Cysteine - Glutamate - Glutamine - Glycine - Proline - Serine - Tyrosine



Phosphaenol pyruvate

L-Valine

L-Alanine

L-Leucine

L-Isoleucine

Pyruvic acid

Deoxyxylulose-5-P

Acetyl CoA

Mevalonic acid

Krebs cycle

oxaloacetic acid

2-Oxoglutaric acid

L-methionine

L-Lysine

L-Glutamic acid

L-Ornithine

L-Arginine

Charu Pathak

* Study of Utilization of radioactive Isotopes in the Investigation of Biogenetic study —

⇒ They are five major techniques which are generally used for the synthetic study of primary and secondary metabolites. They are as follow -

1. Tracer technique
2. Use of isolating organs or tissues
3. Grafting method
4. Use of mutant strains
5. Enzymatic studies

* Isotopes :- Those element which have same chemical properties or atomic number and different mass number called Isotopes.

Eg. $_1H^1$. $_1H^2$ $_1H^3$

$_1P + {}^0N$ $_1P + {}_1N^1$ $_1P + 2N$

* Radioactive Isotopes :- The Isotopes which are unstable due to presence of extra neutrons in their nuclei and emit various types of radiation are called radioactive or radioisotopes. Eg- C_{14} & Ar_{74}

⇒ Radio Isotopes may occur in nature or be made in a laboratory. In medicine, they are used in imaging test and in treatment. Also called radionuclide.

① Tracer technique :- ⇒ Tracer technique which utilizes a labeled compound to trace or find out the different intermediates and various step involved in biosynthetic process in plant at given rate and given time.

⇒ Isotopes may be stable. Eg - ^{2}H, ^{13}C, ^{15}N, ^{18}O. Nucleus may be unstable Eg. ^{1}H, ^{14}C and decay with emission of radiation.

Significance of tracer technique -

- By incorporation of ^{14}C to phenylalanine, the biosynthesis of cyanogentic glycoside can be traced.
- location and quantity of the compound can be determined in biological system.
- for study of Protein, Alkaloid, Amino acid } N atom gives more specific
- for studies on glycosidic linkage - O, N, S & C atom
- for studies on terpenoids - O atom

⇒ Basic steps involved in tracer technique :-

a) Preparation of labeled compound
b) Incorporation of labeled compound to tissue system
c) Seperation or Isolation of labeled compound from tissue
d) Determination of nature of metabolite in various biochemical fraction

a) Preparation of labeled compound :- The labeled compounds may be prepared by use of radioactive isotopes and stable isotopes. Ex- Radioactive Isotopes - ^{14}C, ^{3}H, ^{32}P, ^{131}I, ^{60}Co, ^{36}Cl, ^{24}Na, ^{35}S.

- Radioactive carbon and Hydrogen are mostly used biological investigation. P, S and alkaline earth metal are used for only specific purpose
- The instrument of choice to detect the properties of metabolite is - Scintillation counter, Geiger Muller counter, autoradiography, NMR & MS :- Ionization techniques

b) Introduction of labeled compound to tissue system ⇒

There are six methods used to incorporate labeled compound in to the tissue system.

i) Root feeding - highly preferred method eg. Tobacco.
ii) Stem feeding - cut ends of stem immersed in a solution.
iii) Direct feeding - Applicable to the plant with hollow stem
iv) In-filtration - feeding on plant root in soil
v) Floating method - leaf disc are floating on substrate solution
vi) Spraying technique - sprayed on leaves in aqueous soln. or steroid.

c) Seperation or Isolation of labeled compound or Metabolite

⇒ Depending on the nature of drug and its source different method of extraction is employed.

i) Soft fresh tissue - Infusion, maceration
ii) Hard tissue - decoction & hot percolation
iii) Unorganised drug - maceration
iv) Fat & oil - non polar solvent
v) Plant phenol - polar solvent
vi) alkaloid, glycoside, flavonoid - slightly polar solvent.
vii) Fractional crystallization - partition column chromatography

⇒ Infusion is the process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol by allowing the material to remain suspended in the solvent over time (a process often called steeping)

⇒ Maceration - This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside container, the menstrum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days.

Solvent use for extraction

⇒ Decoction is a method of extraction by boiling herbal or plant material (which may include stems, roots, bark and rhizomes) to dissolve the chemicals of the material.

⇒ The percolation is the most common procedure for the preparation of tinctures and fluid extract. The percolator is a conical vessel with a top opening in which is placed a circular drilled lid allowing the pass of liquid and subjecting the material placed on it to a slight pressure.

d) Determination of nature of metabolites :-

Depending on nature of isotopes various instrumentation techniques is used for determination of chemical nature of intermediate and final product for radioactive isotopes

- GM-Counter
- Scintillation
- Ionization chamber

⇒ for stable isotopes

- Mass spectroscopy
- NMR. gives nature of carbon
- Autoradiography - location of radioisotope in biological system

- GM counter (Geiger Muller counter) - for detecting the presence of and measuring ionization radiation such as α & β- particle.
- Scintillation counter - It involves the admixture of radio labeled metabolite with the scintillation containing solution and measurement of photon emitted by a complex series of events