1. write a detailed note on Shikimic Acid pathway. with its structure and Significance.

Q-1

⇒ Shikimi Acid Pathway –

Shikimic Acid Pathway

- Biosynthetic Pathway in plants + microorganisms
- Produces aromatic amino acids 4 secondary metabolites
- Not present in animals → Target for herbicides

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Starting Materials

- Erythrose-4-Phosphate (E4P)
  - from pentose phosphate pathway
  - 4-carbon sugar phosphate
- Phosphoenol pyruvate (PEP)
  - from glycolysis
  - High energy compound

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Pathway steps (7 steps)

Step 1: E4P + PEP → 3-Deoxy-D-arabino-heptulosonic 7-phosphate (DAHP) [DAHP synthase enzyme]

Step 2: DAHP ⟶ 3-Dehydroquinate (DHQ) [DHQ synthase]

Step 3: DHQ → 3-Dehydroshikimate → shikimic acid [Shikimate dehydrogenase]

"Shikimic Acid = key intermediate"

step-6: Shikimate-3-phosphate + PEP → 5-Enolpyruvylshikimate-3-phosphate (EPSP)
[EPSP synthase]

Step 7:- EPSP → chorismate
[chorismate synthase]

Chorismate = Branch Point compound

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Reaction Network (RN)
(Simplified flow)

Linear Part:

E4P + PEP → DAHP → DHQ → Dehydroshikimate → shikimate → Shikimate-3-P → EPSP → chorismate

Key enzymes:
DAHP synthase,
EPSP synthase

Branching from Chorismate:

- Prephenate → phenylalanine + Tyrosine
- Anthranilate → Tryptophan
- p-Aminobenzoate (PABA) → folate
- Isochorismate → vitamin K, ubiquinone

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Significance & Applications

1. Essential for plants:
   - Produces 3 aromatic amino acids:
     - Phenylalanine
     - Tyrosine
     - Tryptophan
   - Animals get these from diet

2. Herbicide Target:
- glyphosphate (Roundup) inhibits EPSP synthase
- Blocks pathway → plant dies
- selective toxicity (animal lack pathway)

3. Pharmaceutical Importance:
- Source of many plant secondary metabolites:
  - Alkaloids
  - flavonoids
  - Lignins
  - Tannins
- Tamiflu (oseltamivir) synthesized from shikimic acid.

4. Antibiotic Target:
- Some antibiotics target bacterial shikimic pathway.
- Bacteria have this pathway

5. Industrial Applications:
- fermentation production of shikimic acid
- precursor for drug synthesis

② Write a detailed note on Tracer Technique.

[OR]

write detailed note on Radioactive isotopes in the investigation of biogenetic studies / pathways.

→ Tracer Technique (Radioactive Isotopes) →

Tracer Technique – Radioactive Isotopes.
- uses labeled compound to track pathways
- Radioactive isotopes act as "molecular spies"
- Essential for studying biosynthetic routes.

What is A Tracer?

Radioactive Tracers:
- ^{14}C, ^{3}H, ^{32}P
- Emit radiation
- Detected by counters
- Highly sensitive

Stable Isotope Tracers:
- ^{13}C, ^{15}N, ^{2}H
- No radiation
- Detected by MS (mass spec)
- Safer to handle
- modern preference

Commonly used Radioactive Isotopes

1. Carbon-14 (^{14}C):
   - Half-life: 5730 years
   - β- emitter
   - used for: carbon skeleton studies
   - Example: Acetate-2-^{14}C → cholesterol study

2. Tritium (^{3}H):
   - Half-Life: 12.3 years
   - β-emitter
   - used for: Hydrogen transfer studies
   - High specific activity

3. Phosphorus-32 (^{32}P):
- Half-life: 14.3 days
- β- emitter
- used for: Phosphorylation studies
- Nucleic acid metabolism

4. Sulfur-35 (^{35}S):
- Half-life: 87.5 days
- β- emitter
- used for: sulfur-containing compounds
- protein synthesis studies

Experimental Procedure

1. Feeding
- Administer leveled precursor to living system
- can be:
  - Injection
  - Root uptake
  - Leaf applicational

2. Incubation & Harvesting
- Allow time for metabolism
- Harvest tissue at intervals
- Quick freeze to stop metabolism

3. Extraction + Separation
- Extract metabolites using solvents
- Separate using:
  - Chromatography (TLC, column)
  - Electrophoresis
  - Distillation
- monitor radioactivity during separation

4. Detection & Quantification:
- Scintillation counting (for β-emitters)
- Autoradiography (visual detection on film)
- Geiger-Muller counter
- Calculate incorporation rate

Applications In Biogenetic Studies / Pathways

1. Pathway Elucidation
- Determine step-by-step pathway
- Identify intermediates
- Locate branch points

2. Precursor Product Relationships
- Prove which precursor leads to which product
- Establish biosynthetic sequence

3. Metabolic Rate studies:
- measure turnover rates of metabolites
- study rate-limiting steps
- Determine pool sizes.

4. Enzyme mechanism studies:
- Identify active sites
- Study substrate binding
- Investigate catalytic mechanisms

5. Transport Studies:
- Track nutrient uptake
- Study translocation within organisms

- Investigate membrane transport

6. Degradation Pathways:
   - Study catabolic routes
   - Identify degradation products
   - Investigate detoxification mechanisms

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Classic Examples

Mevalonate Pathway (Terpenoids):
- Acetate-1-^{14}C & Acetate-2-^{14}C feeding
- Proved IPP & DMAPP as building blocks
- Established terpenoid origin

Shikimate Acid Pathway (Aromatic AAs):
- Glucose-U-^{14}C → Shikimic acid
- Proved erythrose origin
- Established pathway steps.

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Advantages & Limitations

Advantages:
1. High sensitivity (detect picomole amounts)
2. Specific labeling possible
3. Can study in intact living systems
4. Quantitative data obtainable
5. Time-course studies possible

Limitations:
1. Radiation hazards
2. Special facilities needed
3. Regulatory restrictions

4. Expensive
5. Disposal problems

Modern Trend: stable isotopes (^{13}C, ^{15}N) with MS are now preferred for safety.