Name Of Unit	Metabolic Pathways in Higher Plants and Their Determination
Subject /Course	Pharmacognosy and Photochemistry-II
Subject/Course ID	BP 504T
Class: B.Pharm.	Semester 5th
Course Coordinator	Mr. Anubhav Gupta

Learning Outcome

To outline the metabolic pathway in higher plants and their biogenetic studies.

Module Content Topic

- The basic metabolic pathways producing secondary metabolites .
- Shikimic acid pathway
- Shikimic acid pathway produced secondary metabolites .
- The Acetate (acetate-malonate) pathway producing Fatty acids .
- The Acetate-Mevalonte (Isoprenoid) pathway
- The Acetate-Mevalonte (Isoprenoid) pathway produced secondary metabolites .
- The Amino acid Pathway
- Radioactive isotopes in the investigation of biogenetic studies- Tracer techniques .

STUDY OF UTILIZATION OF RADIOACTIVE ISOTOPES IN THE INVESTIGATION OF BIOGENETIC STUDIES

BIOGENETIC STUDIES

It is a branch of biology focused on understanding the biosynthetic pathways of living organisms, particularly how plants synthesize and transform natural products.

The primary goal is to draw out the sequence of biochemical reactions (biosynthetic pathways) that plants and other organisms use to create specific compounds.

These studies often employ tracer techniques using radioisotopes.

APPLICATIONS:

- **Tracing Metabolic Pathways:**

  Identifying the sequence of chemical reactions involved in the formation of plant metabolites, such as cynogenetic glycosides.

- **Understanding Plant Metabolism:**

  Gaining insights into the complex biochemical processes that occur within plants.

- **Characterizing Natural Products:**

  Determining the origins and structural components of various natural products.

BIOGENETIC STUDIES

There are 5 techniques used for the investigation of biosynthetic pathway of primary and secondary metabolites.

1. Tracer technique
2. Use of isolated organ and tissues
3. Grafting method
4. Use of Mutant strains
5. Enzymatic studies

Out of the above 5 methods, in **Tracer technique** method, radioactive isotopes are used for the investigation of biogenetic pathways.

TRACER TECHNIQUE

Tracer techniques utilizes radioactive isotope labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time.

Isotopes

Elements with identical chemical properties or same atomic no. but different atomic weights/ mass no. are called isotopes. In other words, isotopes are atoms of same element whose nuclei contain same no. of protons but different no. of neutrons.

Stable isotopes

They are stable and **do not** emit radiation, **e.g-** 2H, 13C, 15N, 18O

Radioactive isotopes

They are unstable and emit radiations. The phenomenon of emitting radiation is called radioactivity and such isotopes are called radioactive isotopes.

Significance of tracer techniques

- High sensitivity
- More effective
- Simple administration and isolation.
- Shows accurate results when enough metabolic time & technique is used.
- **Position & Quantity** of compound containing tracer isotope 14C marked glucose is used for glucose determination in the biological system.
- For different studies, different tracers can be used.
- Biosynthetic pathway can be **traced by incorporating radioactive** isotopes into the precursor or starting material.
- **Location and quantity** can be determined in biological system.

Different trace elements used for different studies

- For studies on nitrogen and amino acid, Labelled nitrogen gives specific information than carbon.
- For studies on protein, alkaloids and amino acid, nitrogen atom gives more specific information than carbon.
- For studies on glycosidic linkage- O, N, S and C atom.
- For studies on terpenoids- O atom.

Steps involved in tracer techniques

1. Preparation of labelled compound
2. Incorporation of labelled compound
3. Separation and isolation of labelled compound
4. Determination of nature of metabolites in various biochemical fractions.

1. PREPARATION OF LABELLED COMPOUND

In biological investigation, the use of bioactive isotopes enables the metabolism of compounds to be followed in living organisms for detection and estimation of soft and easily absorbed radiation from labelled compound.

- Labelled compounds may be prepared by use of radioactive isotopes and stable isotopes e,g- Radioactive isotopes- ^{14}C, ^{3}H, ^{32}P, ^{131}I
- Stable isotopes- ^{2}H, ^{15}N, ^{13}C, ^{18}O
- Radioactive carbon and hydrogen are mostly used in biological investigation.
- Radioactive isotopes having long half-life are used.

Criteria for selection of trace elements:

- Starting concentration of trace element must be sufficient to withstand dilution in the course of metabolism.
- Physical and chemical nature of compound must be known.
- Should not damage the tissue system
- Should have low radiation energy.

2. INCORPORATION OF LABELLED COMPOUND TO TISSUE SYSTEM

i) **Root feeding** Hydroponic feeding of roots e.g- Tobacco.

ii) **Stem feeding** Through the cut ends of stem immersed in a solution.

iii) **Direct injection** This method is used in plants with hallow stem. e.g- Umbelliferae and capsule plants (opium poppy). Microsyringe is used to inject labelled compound solution.

iv) **Infiltration (wick feeding)** A thread is drawn through the stem which is dipped into radioactive solution or a flap can be cut in stem and this dipped in the solution.

v) **Floating method** When a small amount of material is available, this method is used. Leaf disc/chopped leaves are floated on labelled compound solution.

vi) **Spraying technique** Compounds have been absorbed after being sprayed on leaves. e.g- steroids.

3. SEPARATION AND ISOLATION OF LABELLED COMPOUND

- **Soft tissue (Fresh)-** Infusion, Maceration
- **Hard tissue-** Decoction and hot percolation
- **Unorganized drug-** Maceration with solvent
- **Fat and oil-** Non-polar solvent
- **Alkaloids, Glycosides, Flavonoids-** Slightly polar solvent
- **Plant phenol-** Polar solvent

4. DETERMINATION OF NATURE OF METABOLITES IN VARIOUS BIOCHEMICAL FRACTIONS

a. GEIGER-MULLER COUNTER

- It is a type of particle detector that measures ionizing radiation, e.g. alpha, beta particles or gamma rays.
- It works by measuring the conductance of electrical charges when a particle or photon of radiation makes the gas conductive by ionization.
- Ionization is produced in low-pressure gas, usually helium, neon or argon with halogens added in the Geiger-Muller tube.

Fig A: Geiger-Muller Counter

b. SCINTILLATION OR LIQUID SCINTILLATION COUNTER

- Scintillation is the emission of a flash of light in transparent materials when exposed to an ionizing radiation.
- A scintillation detector consists of a scintillator crystal (NaI, CsI, SrI_2, LiI, LiF, LaBr, $LaCl_3$, $CeBr_3$, BGO, etc.) coupled with an electronic light sensor, which is traditionally a photomultiplier tube (PMT).
- The latter converts every light photon emitted by the scintillator into an electrical pulse that provides a meaningful information about the energy deposited by the incident radiation.
- Samples shall be dissolved or suspended in a "cocktail" containing a solvent (aromatic organics such as benzene or toluene), typically some form of a surfactant, and small amounts of scintillators.

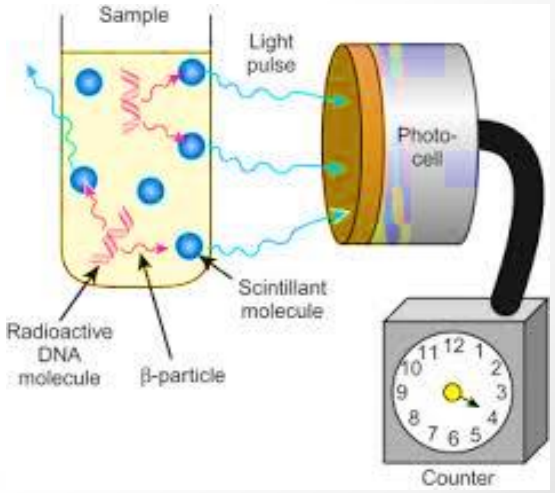


Fig A: Scintillation Counter

c. MASS SPECTROPHOTOMETER

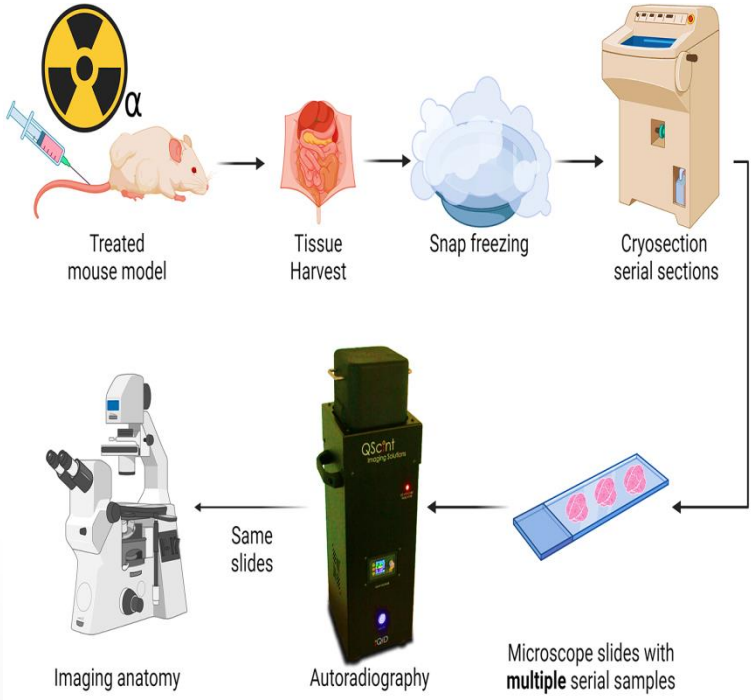
- Mass spectrometry (MS) is an analytical technique used to measure the mass-to-charge ratio of charged particles.
- It is used to determine the mass of the particles, to determine the elemental composition of the sample or molecule.
- It also helps in elucidation of the chemical structures of molecules, such as peptides and other chemical compounds.

d. NMR SPECTROPHOTOMETER

- NMR spectroscopy gives the magnetic properties of certain atomic nuclei to determine the physical and chemical properties of the atoms or molecules they contain.
- It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information on the structure, dynamics, reaction status and chemical environment of the molecules.

e. AUTORADIOGRAPHY

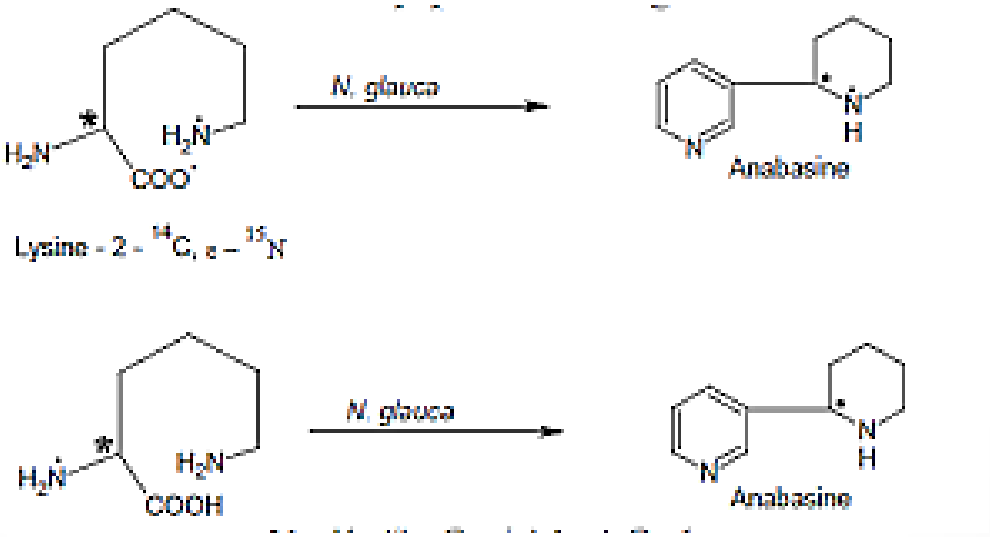
- It is a tool for examining the distribution of radioactive material in a plant object, e.g. histological tissue, chromatography sheet.
- This method uses a photographic film or emulsion (X-ray film) as an ionizing radiation detector.
- The specimen is in close contact with the emulsion for a period.
- The pattern of delivery of radioactive substances can be elucidated with the aid of the autograph collected.



METHODS OF TRACER TECHNIQUES

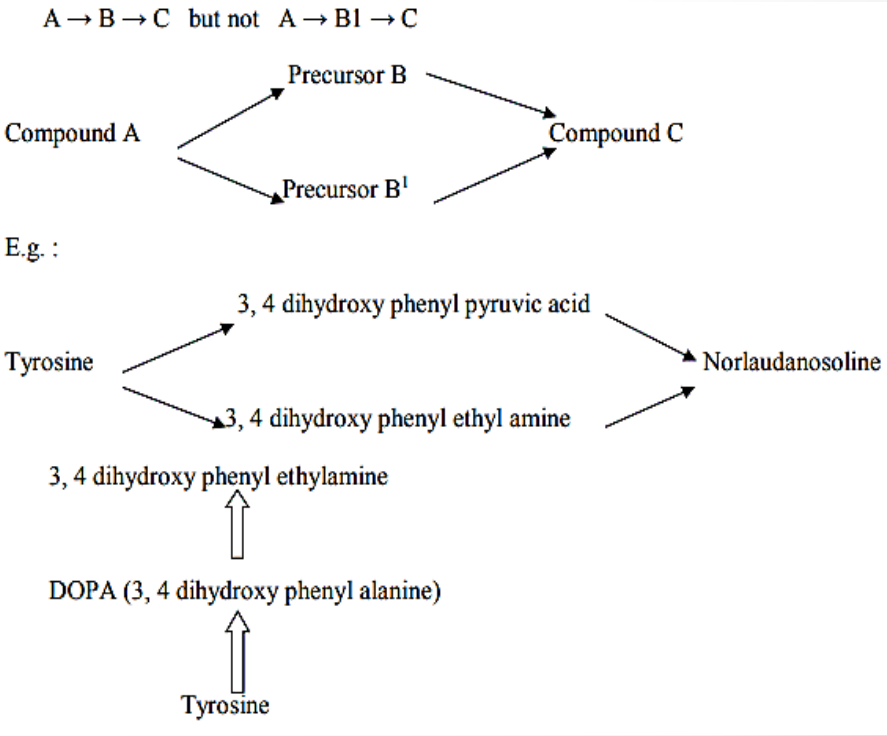
1. PRECURSOR PRODUCT SEQUENCE

The presumed precursor of the constituent under investigation on a labelled form is fed into the plant and after a suitable time the constituent is isolated, purified and radioactivity is determined.



2. COMPETITIVE FEEDING

- Competitive feeding helps in determining which of two potential compounds (A or B) is the actual intermediate in a metabolic pathway, where a precursor (P) forms a product (C) via a normal intermediate.
- By feeding a radioactive tracer along with each potential intermediate to separate plant groups, we observe which group shows radioactivity in the final product, revealing the true biosynthetic pathway
- This method is particularly useful for studying the biosynthesis of alkaloids and other secondary metabolites in plants.



3. SEQUENTIAL ANALYSIS

- Principle of this method is to grow a plant in an atmosphere of 14CO2 & then analyze the plant at given time interval to obtain the sequence in which various correlated compound become labeled.
- Degradation of isolated radioactive compounds is important, because some units of molecule may become labelled more rapidly than others.
- This method is used in the elucidation of path of carbon in photosynthesis.

Application

Exposure period to 14CO2 as short as 5 min. have been used to obtain evidence of biosynthetic sequence as

Piperitone → (-)-menthone → (-)-menthol in Mentha piperita