Roll No. | | | | | | | | | | |

SHAMBHUNATH INSTITUTE OF PHARMACY

Subject Code : RPH839 Subject: Chemistry of Natural Products

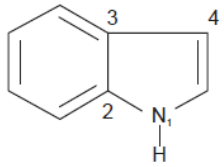
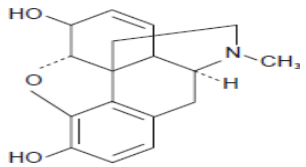
B.Pharm. 8th SEMESTER

FIRST SESSIONAL EXAMINATION, EVEN SEMESTER, (2019-2020)

Time –1hr 30 min **Maximum Marks – 30**

SECTION – A

1. Attempt all questions in brief. **(1*5 = 5)**

Q N	QUESTION	Marks	CO	BL
a.	**Give the tests for Protein.** Ans: a) Biuret test b) Million's test c) Xanthoprotein test **d)** Ninhydrin Test	1	1	2
b.	**Which the nucleus is present in Reserpine and Morphine.** Ans: Indole nucleus is present in Reserpine and Morphine contains Phenanthrene nucleus.  Indole	1	2	4
c.	**Differentiate between secondary and primary metabolites.** Ans: The primary metabolites like sugars, amino acids and fatty acids that are needed for general growth and physiological development of plant are widely distributed in nature and are also utilized as food by man. The secondary metabolites such as alkaloids, glycosides, flavonoids, volatile oils etc are biosynthetically derived from primary metabolites.	1	2	2
d.	**Write the structure and biological source of Morphine.** Ans: Morphine is obtained from the air dried milky latex obtained by incision from the unripe capsules of *Papaver somniferum* Linn, belonging to family Papaveraceae.  Morphine	1	2	2

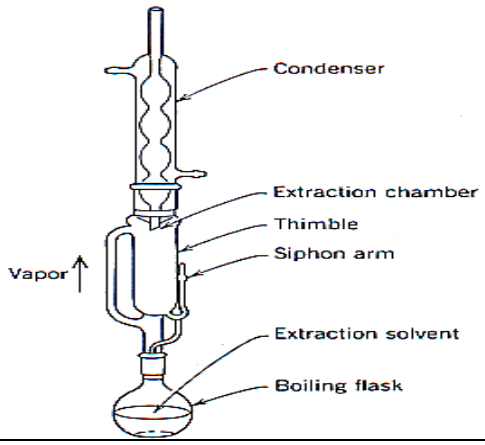
e.	**What are the methods of extraction?** Ans: Methods of Extraction of Medicinal Plants are: • Maceration • Infusion • Digestion • Decoction • Percolation • Hot Continuous Extraction (Soxhlet) • Counter-current Extraction • Ultrasound Extraction (Sonication)	1	1	1

SECTION - B

2. Attempt any <u>TWO</u> of the following. (2*5 = 10)

Q N	QUESTION	Marks	CO	BL
a.	**Write the application of spectroscopy in structural determination of Natural products.** Ans: **Spectroscopy** The isolated and purified plant constituents should be identified and its chemical nature should be determined. The plant compounds could be identified by their spectral characteristics. Spectroscopy is the use of absorption, emission or scattering of electromagnetic radiation by atoms or molecules (or atomic or molecular ions) to qualitatively or quantitatively study the atoms or molecules or to study the physical process of a compound. **Ultraviolet-Visible Absorption Spectroscopy** Different organic molecules with certain functional groups (chromophores) that contain valence electrons of low energy can absorb ultraviolet (UV) or visible (VIS) radiation at different wavelengths. Hence the absorption spectrum of a certain molecule will show a number of absorption bands corresponding to structural groups within the molecule. **Infrared Spectroscopy** This is done by IR spectrophotometer and the plant compounds used is either in liquid, e.g. chloroform, as a mull with nujol oil or in the solid state, mixed with potassium bromide to form a thin disc. The term 'infra red' covers the range of the electromagnetic spectrum between 0.78 and 1,000 μm. **Nuclear Magnetic Resonance Spectroscopy** The nuclear magnetic resonance is a theoretically complex but powerful tool for providing information about the structure of a molecule in a solution. Proton NMR spectroscopy provides a means of determining the structure of an organic compound by measuring the magnetic moments of its hydrogen atom. **Mass Spectroscopy** In mass spectrometry, the sample in gas or liquid or solid state is introduced to the spectrometer followed by ionization, mass analysis, and ion detection/data analysis. We could get the exact molecular weights of the compounds in microgram amounts of sample. Volatilization of the sample (liquid or solid state) is done either prior to ionization or along with the ionization.	5	1	3
b.	**Discuss about the Biogenetic investigation techniques.** **Ans:** Biosynthetic pathway in plants can be investigated by means of following techniques: - **1-Use of isolated organ 2- Grafting methods 3-Use of mutant strain 4-Tracer technique 5-Enzymatic studies**	5	2	2

	ISOLATED ORGAN/TISSUE: This method is based on using isolated parts of plant e.g., stem, roots. This technique is useful in the determination of site of biosynthesis of particular compounds. Roots and leaves for the study of Nicotiana and Datura, petal disc for the study of rose oil, tropane alkaloids in the root of solanaceae family. **Grafting methods**: This method is used for the study of alkaloid formation by grafted plants. Tomato scions grafted on Datura produce alkaloids, while Datura scion grafted on Tomato produce less quantity of alkaloids. This shows that main site of alkaloid biosynthesis is root,.Use of mutant strains: In this mutant strains of microorganisms are produced with the lack of certain enzymes..Gibberella mutant is used to produce isoprenoid compounds, *Lactobacillus acidophillus* is used for mevalonic acid pathway for isoprenoid biosynthesis **TRACER TECHNIQUE:** It can be defined as technique which utilizes a labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time. OR In this technique different isotope, mainly the radioactive isotopes which are incorporated into presumed precursor of plant metabolites and are used as marker in biogenic experiments. The labelled compound can be prepared by use of two types of isotopes. **RADIOACTIVE ISOTOPES:** • [e.g. 1H, 14C, 24Na, 42K, 35S, 35P, 131I decay with emission of radiation] • For biological investigation – carbon & hydrogen. • For metabolic studies – S, P, and alkali and alkaline earth metals are used. • For studies on protein, alkaloids, and amino acid – labelled nitrogen atom give more specific information.3H compound is commercially available **Stable isotopes:** • [e.g. 2H, 13C, 15N, 18O] • Used for labeling compounds as possible intermediates in biosynthetic pathways. • Usual method of detection are: – Mass spectroscopy [15N, 18O] • NMR spectroscopy [2H, 13C] • Tracing of biosynthetic pathway: e.g. By incorporation of radioactive isotope of 14C into phenylalanine, the biosynthetic cyanogenetic glycoside prunasin, can be detected. Location & quantity of compound containing tracer: 14C labelled glucose is used for determination of glucose in biological system Different tracers for different studies: For studies on alkaloids, proteins nitrogen and amino acid (Labelled nitrogen give specific information than carbon). For terpenoids O atom and glycosides O, N, S & C atom used Convenient and suitable technique.			
c.	**Define Chromatography and enlist different techniques of chromatography.** Ans: CHROMATOGRAPY Chromatography is widely used for the separation & identification of components of a mixture. Separation of chemical compounds is carried out by mobile phase and stationary phase. Chromatography can be classified according to mechanism of separation as: • adsorption chromatography, • partition chromatography, • ion exchange chromatography, • size exclusion chromatography • affinity chromatography.	5	1	1

	PAPER CHROMATOGRAPHY The principle is partition mainly the stationary phase is moisture present in the cellulose fibers and mobile vary as we using. The components separated based on their solubility The ratio between the distance travelled on the paper by a component of the test solution & the distance travelled by the solvent is termed the RF value. **THIN LAYER CHROMATOGRAPHY (TLC)** TLC is an e.g. of adsorption chromatography, the stationary phase being a thin layer adsorbent held on a suitable backing. Separation of the compounds present in the plant extract depends on the differences in their adsorptive/desorptive behaviour in respect of the stationary phase. **COLUMN CHROMATOGRAPHY** It is a method used to purify individual chemical compounds from mixtures of compounds the principle of separation is adsorption. **GAS CHROMATOGRAPHY (GC)** **It** is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases, either because the compounds are normally gases or they can be heated and vaporized into a gaseous state. **High-performance liquid chromatography (HPLC)** High performance liquid chromatography is a powerful tool in analysis. It uses the same principles as in thin layer chromatography and column chromatography.			
d.	**Explain Hot continuous extraction process with labeled diagram.** **Ans: Hot Continuous Extraction (Soxhlet)** In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.  	5	1	2

SECTION - C

3. Attempt any ONE part of the following : (1*5 = 5)

Q N	QUESTION	Marks	CO	BL
a.	**Write the extraction, isolation and structure elucidation of Reserpine.**	5	2	2

RESERPINE

PROPERTIES OF RESERPINE: It occurs as a white or pale buff to slightly yellow, odourless, crystalline powder. It darkens slowly on exposure to light but more rapidly when in solution. It is practically insoluble in water and solvent ether but soluble in acetone, chloroform and alcohol. It is freely soluble in acetic acid. They must be protected from light during storage.

ISOLATION OF RESERPINE:

The roots are powdered and moistened with 10% $NaHCO_3$ solution and extract with benzene, until the extract gives a weak positive reaction with HgI_2. The extract is concentrated and ether is added to benzene solution. This mixture is concentrated with dilute HCl and the acid layer is separated. The acid solution is washed with ether and filtered. The solution is rendered alkaline ammonia and is extracted with chloroform. The chloroform extract is washed with 10% solution of sodium carbonate and the extract is evaporated to dryness. The residue is dissolved in anhydrous methanol, a crystal of reserpine is formed and the liquid is cooled when reserpine crystallizes out.

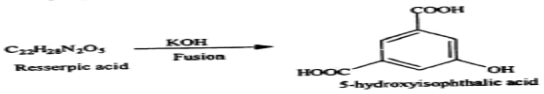
ELUCIDATION OF RESERPINE:

1. **Molecular formula of reserpine is $C_{33}H_{40}N_2O_9$.**
2. **Reserpine heated with HI, it gives 5 moles of methyl iodide (Zeisel method). Presence of five methoxyl groups.**
3. **Nature of the nitrogen atom: As reserpine is a weak base, it indicates that both the nitrogen atoms should be present in its ring systems. Further, reserpine does not have any hydroxyl group but it forms a monoacetyl derivative indicating that one of the nitrogen atoms is present as an –NH- group. Reserpine also forms at methiodide with methyl iodide, indicating that the second nitrogen atom must be in tertiary state.**
4. **Reserpine is alkaline hydrolysis to give methanol. 3, 4, 5- trimethoxybenzoic acid and reserpic acid.**
5. **Structure of reserpic acid:**

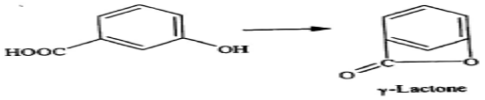
(i) **Molecular formula of reserpic acid is $C_{22}H_{28}N_2O_5$.**

(ii) **Reserpic acid on oxidation with potassium permagnate to gives 4-methoxy-N-oxalyl anthranilic acid. Presence of Indole nucleus.**

(iii) **Reserpic acid on fusion with potassium hydroxide gives 5-hydroxyisophthalic acid. One of the acidic groups of isophyjalic acid must be the acidic group of reserpic acid itself, the hydroxyl and carboxyl groups in reserpic acid are meta to each other.**



(iv) **Reserpic acid is heating with Ac_2O to form γ-lactone.**



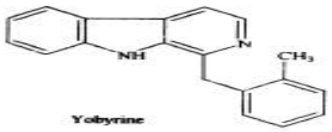
(v) **Dehydrogenation: When methyl reserpate is dehydrogenated with selenium, it yields a hydrocarbon of molecular formula $C_{19}H_{16}N_2$ as one principal product. So for knowing the carbon frame work of reserpic acid and hence reserpine it is essential to know the structure of this compound, named yobyrine.**

Structure of hydrocarbon Yobyrine:

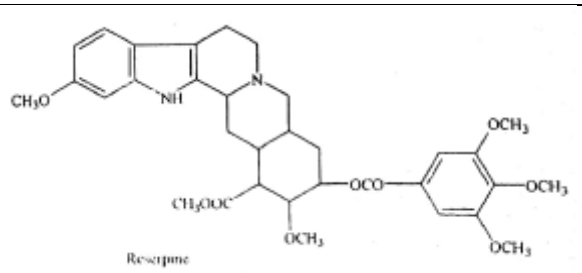
- **Yobyrine distilled with zinc dust, it yields 3-ethyl Indole and Isoquinoline.**
- **Yobyrine is oxidised with permagnate, it yields phthalic acid.**

- **Yobyrine is oxidised with chromic acid yields o-toluic acid.**

- **Yobyrine gives condensation products with aldehydes, suggesting that the presence of a pyridine ring with a $–CH_2–$ substituent adjacent to the nitrogen.**
- **On the basis of the fact, the following structure has been postulated for yobyrine:**

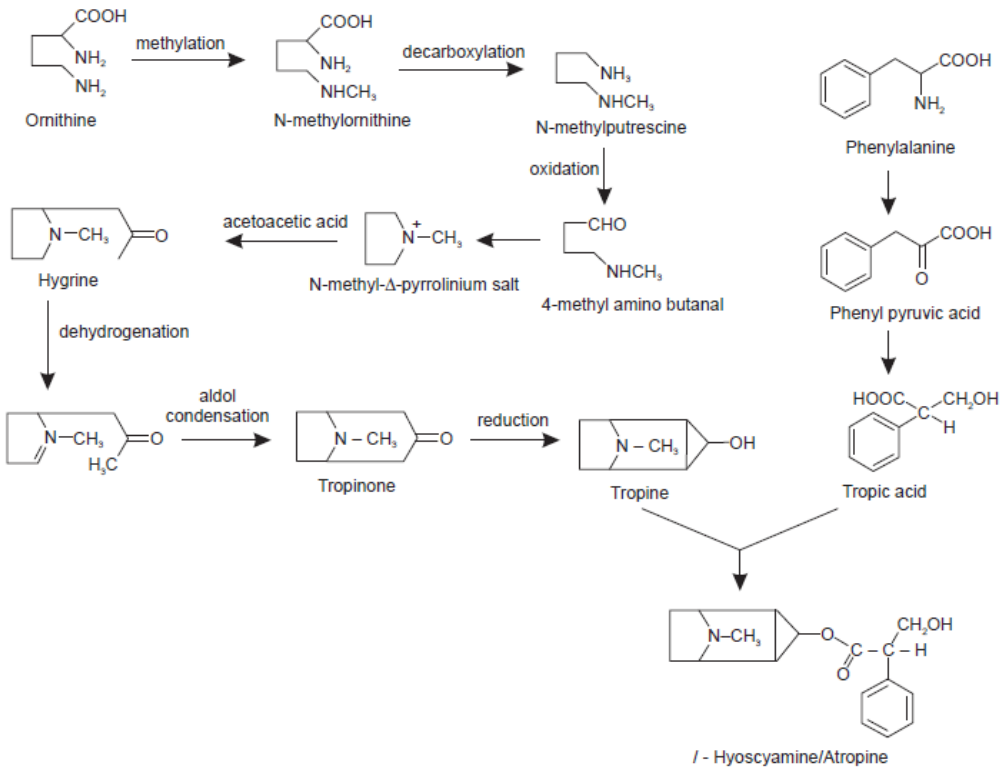
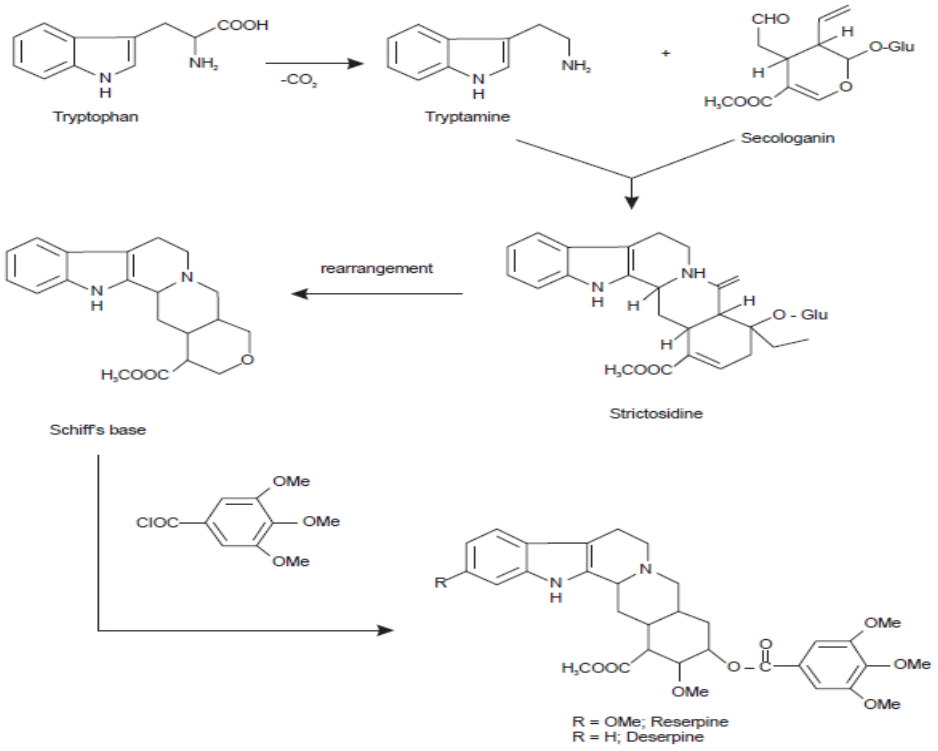


- **The above structure of yobyrine has been confirmed by its synthesis:**

	 Reserpine			
b.	**Write the extraction, isolation and structure elucidation of Quinine.** **Isolation of Quinine from cinchona bark:** Cinchona bark powder → Treated with 10% Na_2CO_3 solution → Dry cinchona bark powder → Kept overnight → Dried alkaline powder → Reflex with benzene for 6 hrs → Filtrate, Hot → Extracted with dilute H_2SO_4 → Aqueous quinine in solution → Made slightly alkaline (7.5 pH) with sodium carbonate → Alkaloidal solution (7.5 pH) boil with activated charcoal and filter → Filtrate of quinine sulphate Kept at 20 °C for overnight and filter → Boil residue with water and make Alkaline with sodium carbonate solution → Decantation → Precipitate of quinine settles down **ELUCIDATION OF QUININE:** 1. Molecular formula is $C_{20}H_{24}N_2O_2$. 2. Quinine reacts with two molecule of methyl iodide to form a diquaternary salt presence of ditertiary base. 3. Quinine heated with hydrochloric acid, quinine eliminate one carbon atom as methyl chloride; presence of one methoxyl group. 4. Quinine forms a monoacetate and monobenzoate, one hydroxyl group present. 5. Quinine oxidised with chromium trioxide produce quinine; hydroxyl group is secondary alcohol. $C_{20}H_{24}N_2O_2 \xrightarrow{CrO_3} C_{20}H_{22}N_2O_2$ (Quinine → Quininone) 6. Presence of quinoline group: Quinine is fused with concentrated KOH, produced mixture of 6-methoxyquinoline and lepidine (4-methylquinoline) along with other product. Presence of quinoline nucleus. 6-methoxyquinoline; Lepidine 7. Presence of meroquinene: Oxidation of quinine with chromic acid produces quininic acid. $C_{20}H_{24}N_2O_2 \xrightarrow[H_2SO_4]{CrO_3} C_{11}H_9NO_3$ (Quinine → Quininic acid) Controlled oxidation of quinine with chromic acid yields quininic acid and meroquinene. $C_{20}H_{24}N_2O_2 \xrightarrow[\text{Controlled oxidation}]{CrO3} C_{11}H_9NO_3 + C_9H_{15}NO_2$ (Quinine → Quininic acid + Meroquinene) 8. (Structure of Quininic acid:) (a) Molecular formula is $C_{11}H_9NO_3$. (b) When heated with soda-lime, quininic acid is decarboxylated to a methoxyquinoline. $C_{11}H_9NO_3 \xrightarrow[(NaOH+CaO)]{\text{Soda lime}}$ 6-methoxyquinoline	5	2	2

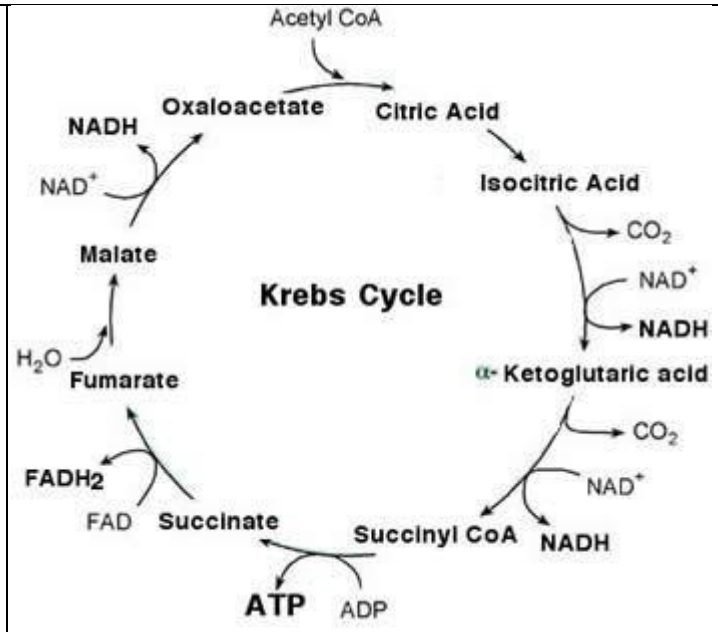
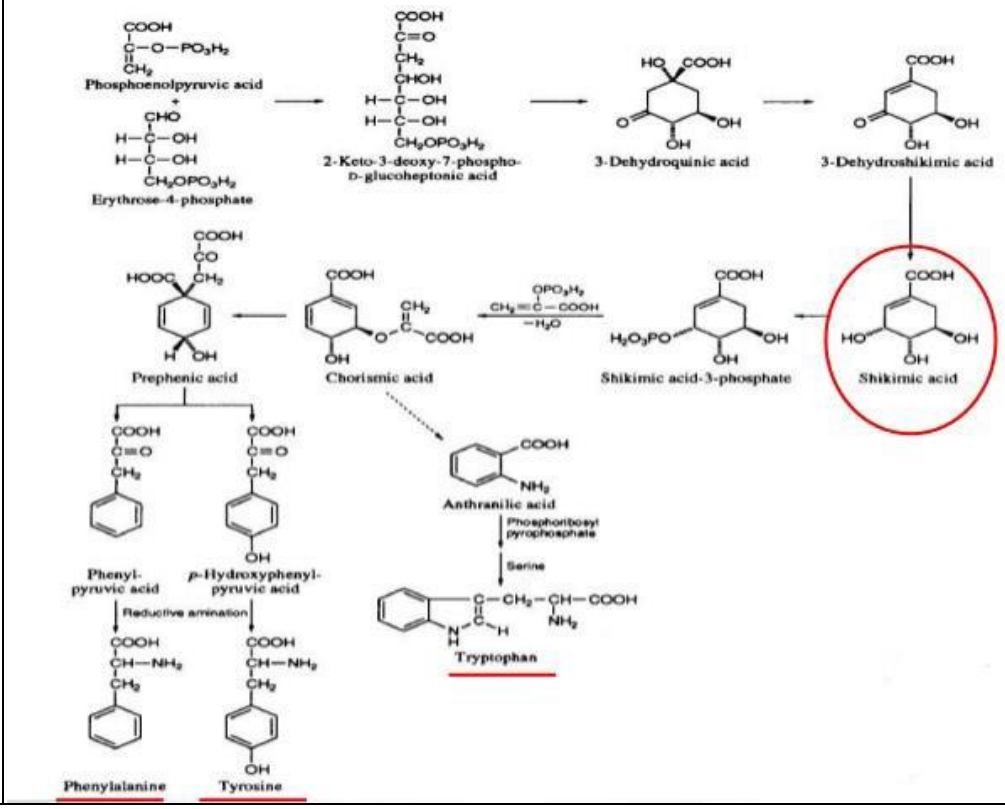
4. Attempt any ONE part of the following : **(1*5 = 5)**

Q N	QUESTION	Marks	CO	BL
a.	**Discuss the biogenesis of Ornithine derives alkaloid.** **Alkaloid derived from ornithine**: Ornithine is incorporated into both pyrrolidine specifically and asymmetrically into pyrrolidine ring of tropane nucleus, the α-carbon of ornithine becoming the C_1 of tropine nucleus. The remaining three carbon atoms are derived from acetate, thus completing piperidine moiety. Methionine serves as the methyl group donor, whereas phenyl alanine is the precursors of the	5	2	2

	tropic acid. The different alkaloids derived from ornithine.  			
b.	**Discuss the biogenesis of tryptophan derived alkaloid.**  Fig. 13.16 Biosynthesis of reserpine	5	2	2

5. Attempt any ONE part of the following : (1*5 = 5)

Q N	QUESTION	Marks	CO	BL
a.	**Discuss the biosynthesis of TCA.**	5	2	2

				
b.	**Discuss Shikimic acid pathway.** 	5	2	2