DIRECTORATE OF DISTANCE & CONTINUING EDUCATION

MANONMANIAM SUNDARANAR UNIVERSITY

TIRUNELVELI- 627 012

OPEN AND DISTANCE LEARNING (ODL) PROGRAMMES

(FOR THOSE WHO JOINED THE PROGRAMMES FROM THE ACADEMIC YEAR 2023–2024)

M. Sc. Chemistry

Course Material

Elective V – Pharmacognosy and Phytochemistry

Course Code: SCHE31

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PHARMOCOGNOSY AND PHYTOCHEMISTRY

UNIT-I: Pharmacognosy and Standardization of Herbal drugs:

Introduction, definition, development classification and Source of Drugs: Biological, mineral, marine, and plant tissue cultures. Study of pharmacognostic of a crude drug. Biosynthesis: Shikimic acid pathway and acetate pathway. Systematic analysis of Crude drugs. Standardization of Herbal drugs. WHO guidelines, Sampling of crude drug, Methods of drug evaluation. Determination of foreign matter, moisture Ash value. Phytochemical investigations-General chemical tests.

UNIT-II: Extraction Techniques:

General methods of extraction, types – maceration, Decoction, percolation, Immersion and soxhlet extraction. Advanced techniques- counter current, steam distillation, supercritical gases, sonication, Micro waves assisted extraction. Factors affecting the choice of extraction process.

UNIT-III: Drugs containing Terpenoids and volatile oils:

Terpenoids: Classification, Isoprene rule, Isolation and separation techniques, General properties Camphor, Menthol, Eucalyptol. Volatile Oils or Essential Oils: Method of Preparations, Classifications of Volatile oils, Camphor oil, Geranium oil, Citral - Structure uses. Pentacyclic triterpenoids: amyrines; taraxasterol: Structure and pharmacological applications.

UNIT-IV: Drugs containing alkaloids:

Occurrence, function of alkaloids in plants, pharmaceutical applications. Isolation, Preliminary Qualitative tests and general properties. General methods of structural elucidation. Morphine, Reserpine, papaverine - chemical properties, structure and uses. papaverine - structure, chemical properties and uses.

UNIT-V: Plant Glycosides and Marine drugs:

Glycosides: Basic ring system, classification, isolation, properties, qualitative analysis. Pharmacological activity of Senna glycosides, Cardiac glycosides - Digoxin, digitoxin, Steroidal saponins glycosides- Diosgenin, hecogenin. Plant pigments: Occurrence and general methods of structure determination, isolation and synthesis of quercetin and cyanidin chloride. Marine drugs -Selected Drug Molecules: Cardiovascular active substances, Cytotoxic compounds, antimicrobial compounds, antibiotic compounds, Anti-inflammatory agents. Marine toxins.

Recommended Text Books:

1. Gurdeep R Chatwal (2016), Organic chemistry of Natural products, Volume I&II, 5th edition, Himalaya publishing House.

2. S.V.Bhat, B.A. Nagasampagi, M.Sivakumar (2014), Chemistry of Natural Products, Revised edition, Narosa Publishers.

Reference Books

1. Jeffrey B. Harborne (2012), Phytochemical methods: A Guide to Modern Techniques of Plant Analysis, 4th edition, Indian reprint, Springer.

2. Ashutoshkar (2007), Pharmacognosy and Pharmacobiotechnology, 2nd edition, New Age International (P) Limited, New Delhi.

Unit – I

Pharmacognosy and Standardization of Herbal drugs

Introduction

India is a mother hub for development of Ayurveda, Unani, Siddha; Homoeopathy and other natural herbs based health science (Ayush). Ayush Pharmaceutical industry is having great potential and opportunities for development in future. Mainly in following herbal medicinal plants and their value added products well accepted in domestic and international market e.g. Ayurvedic medicines, Unani medicines, Siddha medicines, Homoeopathic medicines, herbal nutraceuticals, herbal cosmoceutical, herbal health drinks, dietary health supplements, medicinal plants / crude drugs, herbal extracts / concentrates, herbal veterinary medicines, health foods, Ayush health care management, Ayurvedic panchakarma centre and health spa. Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle i.e. shelflife, storage, distribution and use by various parameters. As we all know in our Ayurvedic system of medicines drug standardisation of Ayurvedic formulation is a big challenge. Clear cut guidelines have not been developed so far. So it is necessary to promote ISM manufacturing industry people for drug standardization work. Ministry of Ayush, Government of India recently established Pharmacopoeial Commission of Indian medicines and Homoeopathy (PCIM and H) for setting up drug standard of ASU and H Medicines.

World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose.

As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection),

the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic.

Standardization as defined by American Herbal Product association: “Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes.

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs.

The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compounds would serve as an additional parameter in assessing the quality of the sample. Phytochemical standardization encompasses all possible / information generated with regard to the chemical constituents present in an herbal drug.

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Definition of Pharmacognosy

Pharmacognosy is the study of the physical, chemical, biochemical, and biological properties of drugs, and the search for new drugs from natural sources.

Development

- In the next few years, strychnine (1817), emetine (1817). Brucine (1819), piperine (1819), quinine (1820) and colchicine (1820) were isolated.

- Pelletier – french pharmacist, reported the isolation of strychnine from ignatius beans and later from nux vomica seeds in 1818.
- Stass and Otto – a new extraction process for alkaloid was developed in 1852.
- Posselt and Reimann – isolated nicotine from tobacco leaves in 1828.
- Neumann – isolated cocaine in 1860.
- Hardy and Gallows – isolated ouabain in 1877.
- Gerrard and Hardy – isolated pilocarpine in 1875.
- Nagai – isolated ephedrine in 1887.
- Kuersten – isolated podophyllotoxin in 1891.
- 20th Century - Isolation of ergometrine, digoxin, reserpine, theophylline and quinidine are the significant discoveries

Source of Drugs:

The following are the natural sources of Drugs:

- Biological sources (i.e. from Terrestrial and Marine living things)
  - Plants
  - Animals
  - Microorganisms: Fungi, Algae, Bacteria
- Mineral sources
- Biotechnology: Tissue culture/ Recombinant DNA Technology

Biological Sources

Plant Sources

Plant source is the oldest source of drugs. Most of the drugs in ancient times were derived from plants. Almost all parts of the plants are used i.e. leaves, stem, bark, fruits and roots. The number of species of flowering plants is estimated to be 2 to 2.5 lakhs falling in about 300 families and 10000 genera. Only a small percentage of the total species have been studied scientifically for the presence of any therapeutic activity and isolation of the responsible bioactive compound isolated. Investigators face numerous hurdles and problems in the systematic investigation of all the species and as a result thousands of species are still not investigated thoroughly. In many areas of the world, plants used in folklore medicine have been recorded. In other regions of the world such information has not been recorded or

lost. Ethno botanists across the world have been trying to gather and record such valuable information before it is completely lost or forgotten. The search for new drug needs a team work of experts from various domains such as botany, pharmacognosy, pharmacology, phytochemistry, medicine etc. Majority of the natural drugs from plant sources are derived from Spermatophytes (seed bearing plants). Thy phyla Angiosperme is the dominant one while the phyla Gymnospermae yields few useful drugs such as Turpentine oil, Colophony, ephedrine etc. Male Fern from Pteridophyta provides Taenicidal (tape worm killing) agents In Angiospermae, Dicotyledon plants provide more drugs than the Monocotyledon plants which yield limited drugs such as Squill, Lemon grass oil, Aloes etc. Examples of drugs obtained from plants include Quinine, Atropine, Cocaine, Morphine, Codeine, Ergotamine, Reserpine, Caffeine, Sennosides, Glycyrrhizin, volatile oils, fixed oils etc.

Animal Sources

Gelatin is obtained from ox and sheep, Wool fat from sheep, Beeswax from honeycomb, Cochineal from insects are some examples of drugs obtained from land animals. Spermaceti, Shark liver oil, Cod liver oil, halibut liver oil are some of the drugs obtained from marine animals.

Microbial Sources

Well-known antibiotics produced by a group of microorganisms known as actinomycetes yielding antibiotics such as actinomycin, amphotericin, chloramphenicol, erythromycin, kanamycin, neomycin, gentamicin, streptomycin and tetracycline. Aspergillate group of fungi produce antibiotics such as penicillin, griseofulvin and cephalosporin. Among the bacteria, genus Bacillusproduces antibiotics such as polymyxin B and bacitracin. Ergot alkaloids also are obtained from a resting stage of a fungus. Algae are source of limited number of drugs such as Agar and Alginate

Mineral Sources

Several silicates such as Kaolin, Bentonite, Diatomite and compounds of Na, K, Al, Ca, Mg etc. are obtained from Mineral sources including Sulphur and Iodine.

Marine, and Plant Tissue Cultures

Recombinant DNA technology involves cleavage of DNA by enzyme restriction endonucleases. The desired gene is coupled to rapidly replicating DNA (viral, bacterial or plasmid). The new genetic combination is inserted into the bacterial cultures which allow production of vast amount of genetic material. Important example is Human insulin is produced by modification of porcine insulin or by bacteria using recombinant DNA technology. Few others include Somatotrophin, Erythropoietin, Human blood coagulation factors etc. Scopolamine, Podophyllotoxin, Paclitaxel, Rosmarinic acid, Vanillin and Shikonin are some of the examples of compounds produced from plant cell cultures.

Classification of Drugs

The flora and fauna of mother earth has a great diversity. The number of plant species divided in about 300 families and 10,500 genera are supposed to be about 2–2.5 lacs. At least 100–150 species of medicinal plants are currently cultivated and about 30–40 of them are the large-scale field corps. Drugs of the animal and mineral origin have also been used since the beginning and even today many such crude drugs are important, commercial products. All these drugs of natural origin have been used as the curative agents and even in this age of scientific discoveries and invention, natural drug have been the primary choice as a source of drug. Human inquisitiveness has gone beyond the terrestrial regions and exploited the seas and oceans which contain about 5 lacs species of marine organisms. Therapeutically active constituents found in these organ-isms open yet another great natural source of drugs of unending search. Crude drugs can be regarded as the substances either used directly or indirectly as a drug which have not been changed or modified in its chemical composition. The crude drugs of natural origin can be divided into two main categories as organized crude drugs and unorganized crude drugs.

Organized drugs

Organized Drugs Organized drugs consist of the cellular organization in the form of anatomical features. These are mostly the crude drugs from plant sources. Almost all of the morphological plant parts or the entire plant itself can be called as an organized drugs. A long list can be made of such crude drugs. To mention few of them, like, Cinchona bark,

Sandalwood, Quassia wood, Senna, Digitalis leaves, Nux vomica seeds, Rauwolfia roots and many other examples of above-mentioned groups or crude drugs exemplified by some other morphological organs can be quoted as the example of organized crude drugs Microscopical and anatomical studies are preeminent for such crude drugs. These can be used directly in medicine or can be used by modifying or by extracting the active ingredient from it. The simple medicines prepared from these drugs are herbal teas, extracts, tinctures, etc., and it may be extensively processed for the isolation and purification of pure therapeutically active constituent which is ultimately responsible for the action of the drug.

Unorganized Drugs:

The unorganized drugs do not have the morphological or anatomical organization as such. These are the products which come directly in the market but their ultimate source remains the plants, animals or minerals. Microscopical studies are not required for such crude drugs. These includes products like plant exudates as gums, oleogums, oleogumresins, plant lattices like that of opium, aloetic juices like aloes or dried extracts of black and pale catechu, agar, alginic acid, etc., are products coming under this group. Other products like essential oils, fixed oils, fats and waxes obtained from vegetable or animal sources, although hydro-distilled or extracted from plant, become the direct commodity for use. Unorganized crude drugs may be miscellaneous mineral products like shilajit. These products may be solid, semisolid or liquid and the physical, chemical and analytical standards may be applied for testing their quality and purity.

Classification:

The most important natural sources of drugs are higher plant, microbes and animals and marine organisms. Some useful products are obtained from minerals that are both organic and inorganic in nature. In order to pursue (or to follow) the study of the individual drugs, one must adopt some particular sequence of arrangement, and this is referred to a system of classification of drugs. A method of classification should be: a) simple, b) easy to use, and c) free from confusion and ambiguities. Because of their wide distribution, each arrangement of classification has its own merits and demerits, but for the purpose of study the drugs are classified in the following different ways:

1.Alphabetical classification

2.Taxonomical classification

3.Morphological classification

4. Pharmacological classification

5. Chemical classification

6. Chemotaxonomical classification

Study of Pharmacognostic of a Crude Drug

Crude drugs of natural origin that is obtained from plants, animals and mineral sources and their active chemical constituents are the core subject matter of pharmacognosy. These are also used for the treatment of various diseases besides being used in cosmetic, textile and food industries. During the first half of the nineteenth century apothecaries stocked the crude drugs for the preparation of herbal tea mixtures, all kinds of tinctures, extracts and juices which in turn were employed in preparing medicinal drops, syrups, infusions, ointments and liniments.

The second half of the nineteenth century brought with it a number of important discoveries in the newly developing fields of chemistry and witnessed the rapid progress of this science. Medicinal plants became one of its major objects of interest and in time, phytochemists succeeded in isolating the pure active constituents. These active constituents replaced the crude drugs, with the development of semisynthetic and synthetic medicine, they became predominant and gradually pushed the herbal drugs, which had formerly been used, into the background. It was a belief that the medicinal plants are of no importance and can be replaced by man-made synthetic drugs, which in today's scenario is no longer tenable. The drug plants, which were rapidly falling into disuse a century ago, are regaining their rightful place in medicine. Today applied science of pharmacognosy has a far better knowledge of the active constituents and their prominent therapeutic activity on the human beings. Researchers are exploiting not only the classical plants but also related species all over the world that may contain similar types of constituents. Just like terrestrial germplasm, investigators had also diverted their attention to marine flora and fauna, and wonderful marine natural products and their activities have been studied. Genetic engineering and tissue culture biotech-nology have already been successful for the production of genetically engineered molecules and biotransformed natural products, respectively.

Lastly, crude drugs and their products are of economi-cal importance and profitable commercial products. When these were collected from wild sources, the amount collected

could only be small, and the price commanded was exorbitantly high. All this has now changed. Many of the industrially important species which produced equally large economic profits are cultivated for large-scale crop production. Drug plants, standardized extracts and the therapeutically active pure constituents have become a significant market commodity in the international trade. In the light of these glorious facts, scope of pharmacognosy seems to be enormous in the field of medicine, bulk drugs, food supplements, pharmaceutical necessities, pesticides, dyes, tissue culture biotechnology, engineering and so on.

Scope for doctoral graduates in pharmacognosy is going to increase in the coming years. The pharmacognosist would serve in various aspects as follows:

Academics: Teaching in colleges, universities, museums and botanical gardens.

Private industry: Pharmaceutical companies, consumer products testing laboratories and private commercial testing laboratories, the herbal product industries, the cosmetic and perfume industries, etc.

Government: Placement in federal agencies, such as the Drug Enforcement Agency, the Food and Drug Administration, the U.S. Department of Agriculture, Medicinal plant research laboratories, state agencies like forensic laboratories, environmental laboratories, etc.

Undoubtedly, the plant kingdom still holds large number of species with medicinal value which have yet to be discovered. Lots of plants were screened for their pharmacological values like, hypoglycaemic, hepatoprotective, hypotensive, antiinflammatory, antifertility, etc. pharmacognosists with a multidisciplinary background are able to make valuable contributions in the field of phytomedicines.

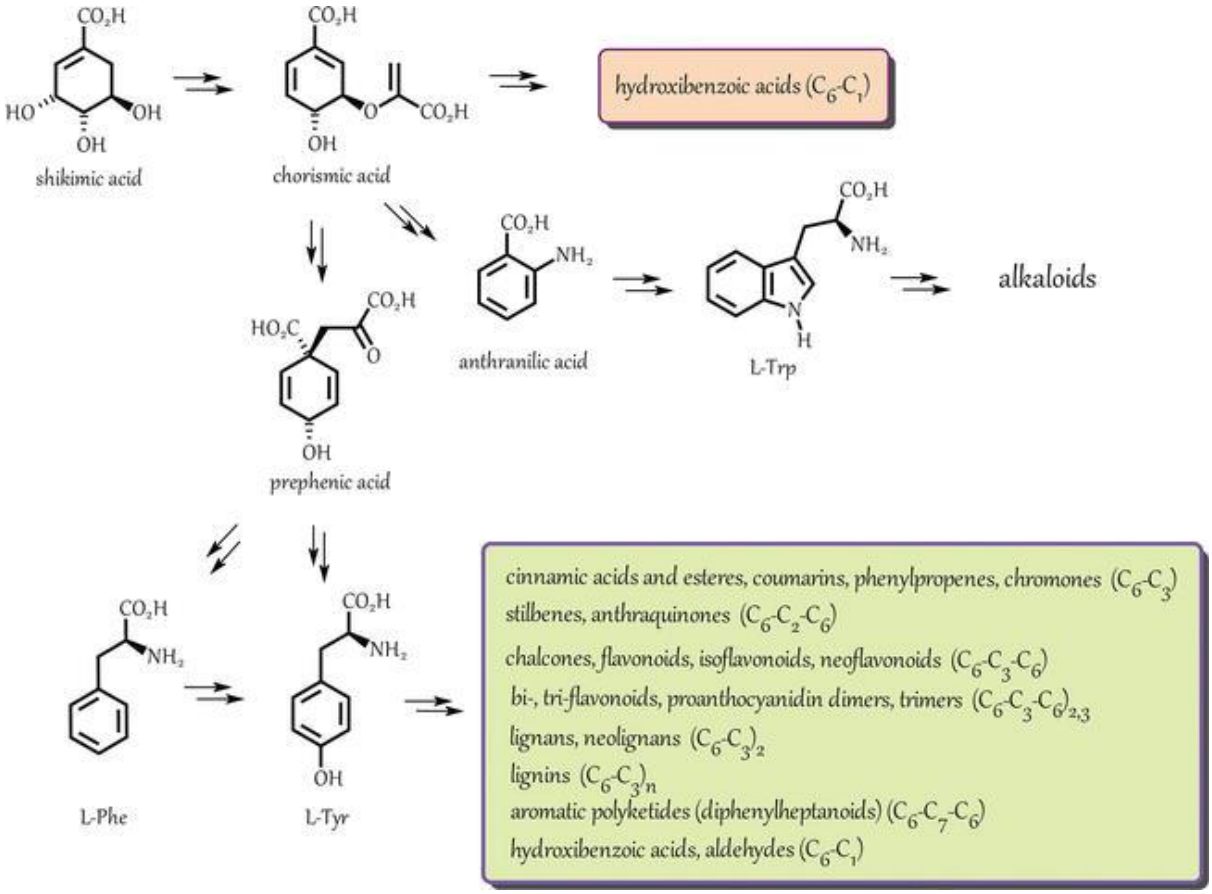
Biosynthesis

The secondary metabolism is a biosynthetic source of several interesting compounds useful to chemical, food, agronomic, cosmetics, and pharmaceutical industries. The secondary pathways are not necessary for the survival of individual cells but benefit the plant as a whole. Another general characteristic of secondary metabolism is that found in a specific organism, or groups of organisms, and is an expression of the individuality of species. The secondary metabolism provides chemical diversity to organic molecules with low molecular weight that are related by the respective pathways; such organic molecules are called secondary metabolites. The secondary metabolites are often less than 1% of the total carbon in plant molecules. These organic molecules isolated from terrestrial plants are the most

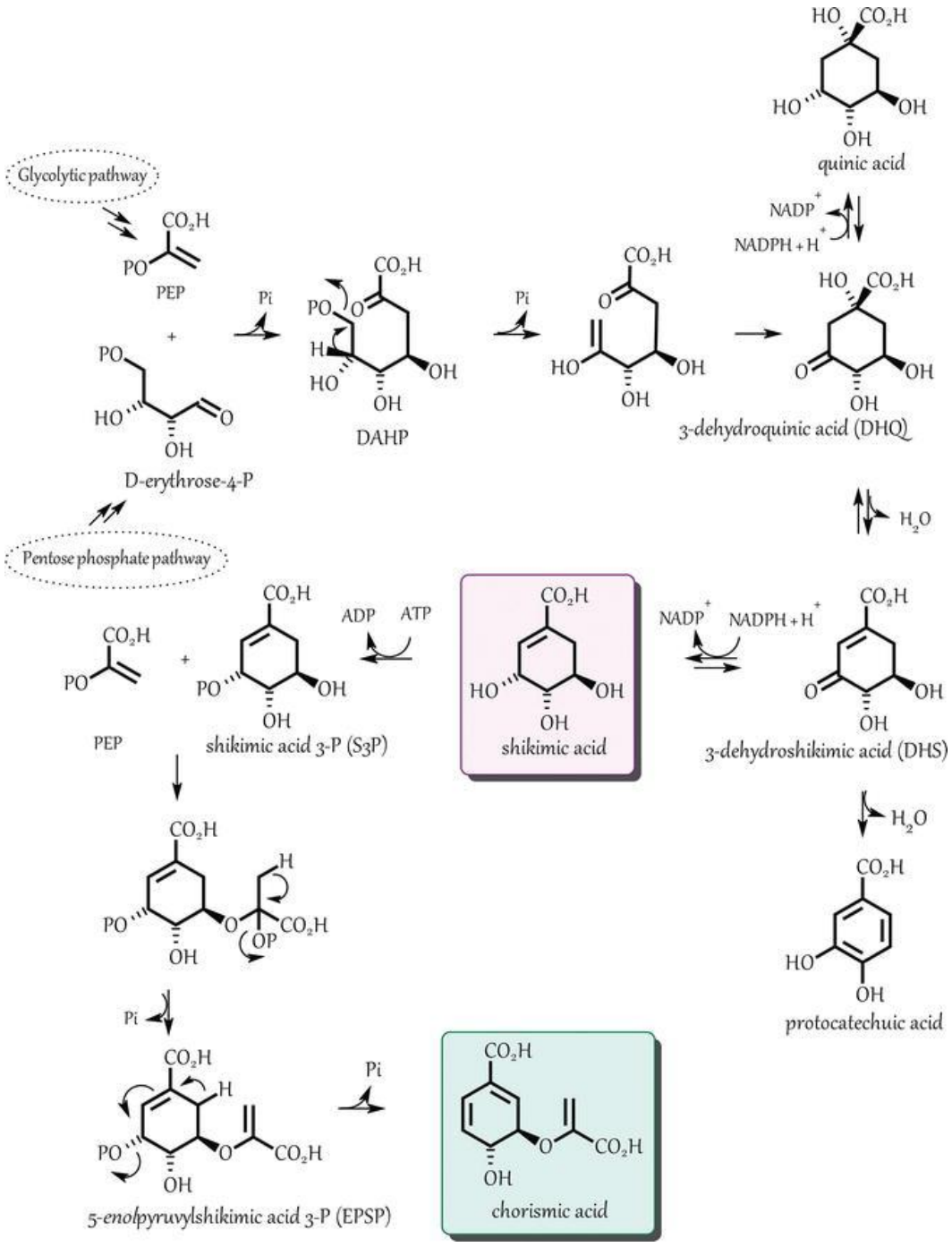
studied, and their syntheses have an important role in the protection against pathogens, unfavorable temperature and pH, saline stress, heavy metal stress, and UVB and UVA radiation. Secondary metabolism reflects plant environments more closely than primary metabolism. There are three principal kinds of secondary metabolites biosynthesized by plants: phenolic compounds, terpenoids/isoprenoids, and alkaloids and glucosinolates (nitrogen- or sulfur-containing molecules, respectively). Phenolic compounds are biosynthesized by the shikimate pathway and are abundant in plants. The shikimate pathway, in plants, is localized in the chloroplast. These aromatic molecules have important roles, as pigments, antioxidants, signaling agents, electron transport, communication, the structural element lignan, and as a defense mechanism.

Shikimic Acid Pathway

The shikimate biosynthesis pathway provides precursors for aromatic molecules in bacteria, fungi, apicomplexan, and plants, but not in animals. Shikimic acid is named after the highly toxic Japanese *shikimi* (*Illicium anisatum*) flower from which it was first isolated. This biochemical pathway is a major link between primary and secondary metabolism in higher plants. In microorganisms, the shikimate pathway produces aromatic amino acids L-phenylalanine (L-Phe), L-tyrosine (L-Tyr), and L-tryptophan (L-Trp), molecular building blocks for protein biosynthesis. But in plants, these aromatic amino acids are not only crucial components of protein biosynthesis; they also serve as precursors for diverse secondary metabolites that are important for plant growth. These secondary metabolites are called phenolic compounds and are synthesized when needed by the plant. These molecules play an important role in the adaptation of plants to their ecosystem, and their study advances biochemical techniques and molecular biology [3, Bourgaud]. The principal aromatic phenolic compounds synthesized from L-Phe and L-Tyr are cinnamic acids and esters, coumarins, phenylpropenes, chromones (C_6-C_3), stilbenes, anthraquinones (C_6-C_2-C_6), chalcones, flavonoids, isoflavonoids, neoflavonoids (C_6-C_3-C_6), and their dimers and trimers, respectively (C_6-C_3-C_6)$_{2,3}$, lignans, neolignans (C_6-C_3)$_2$, lignans (C_6-C_3)$_n$, aromatic polyketides, or diphenylheptanoids (C_6-C_7-C_6). L-Trp is a precursor of alkaloids in the secondary metabolism. Additionally, diverse hydroxybenzoic acids and aromatic aldehydes (C_6-C_1) are biosynthesized via branch points in the shikimate pathway, Phenolic compounds biosynthesized from the shikimate pathway have structural versatility.



The shikimate pathway consists of seven sequential enzymatic steps and begins with an aldol-type condensation of two phosphorylated active compounds, the phosphoenolpyruvic acid (PEP), from the glycolytic pathway, and the carbohydrate D-erythrose-4-phosphate, from the pentose phosphate cycle, to give 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP). The seven enzymes that catalyze the pathway are known: 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (DAHPS; EC 4.1.2.15, now EC 2.5.1.54), 3-dehydroquinate synthase (DHQS; EC 4.2.3.4), 3-dehydroquinate dehydratase/shikimate dehydrogenase (DHQ/SDH; EC 4.2.1.10/EC 1.1.1.25), shikimate kinase (SK; EC 2.7.1.71), 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS; EC 2.5.1.19), and chorismate synthase (CS; EC 4.2.3.5).



The shikimate pathway has special characteristics that are present only in bacteria, fungi, and plants. The absence of the pathway in all other organisms provides the enzymes catalyzing these reactions with potentially useful targets for the development of antibacterial agents and herbicides. For example, 5-*enol*pyruvylshikimate 3-phosphate synthase (EPSP-synthase) catalyzes the transfer of the enolpyruvyl (carboxyvinyl) moiety from PEP to shikimic acid 3-phosphate (S3P).

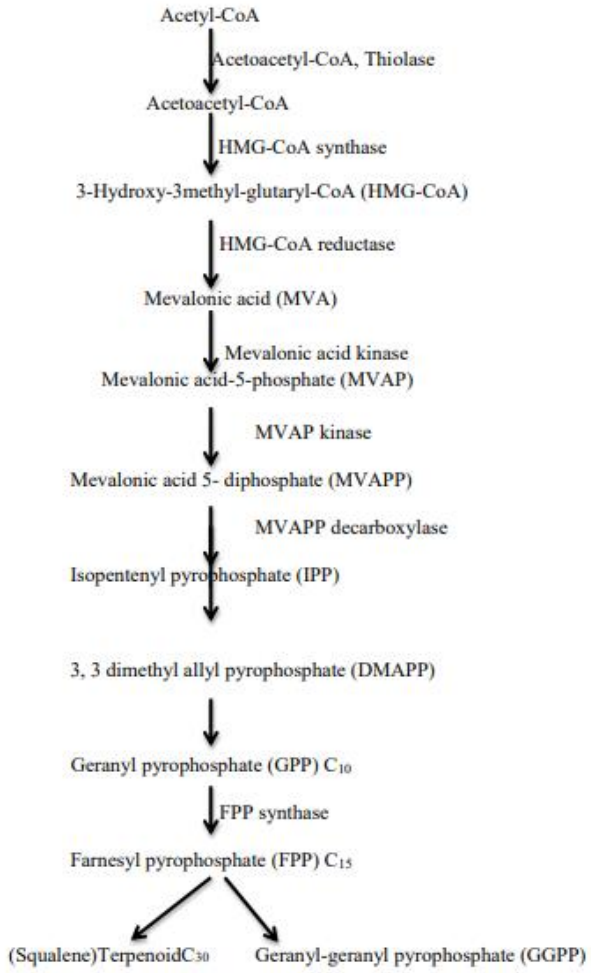
In the second reaction step, DAHP loses phosphate (Pi); the enolic-type product is cyclized through a second aldol-type reaction to produce 3-dehydroquinic acid (DHQ). The 3-dehydroquinate synthase (DHQS) catalyzes this cyclization in the shikimate pathway. The DHQ dehydrates to produce 3-dehydroshikimic acid (DHS) (3-dehydroquinate dehydratase); this compound has a conjugated double carbon-carbon. The protocatechuic and the gallic acids (C_6-C_1) are produced by branch-point reactions from DHS. The fourth step in the pathway is a reduction reaction of DHS with reduced nicotinamide adenine dinucleotide phosphate (NADPH). The fifth section of the pathway is the activation of shikimic acid with adenosine triphosphate (ATP) (shikimate kinase, SK) to make shikimic acid 3-phosphate (S3P). The sixth chemical reaction is the addition of PEP to S3P to generate 5-*enol*pyruvylshikimic acid 3-phosphate; the enzyme that catalyzes this reaction step, 5-e*nol*pyruvylshikimate 3-phosphate synthase (EPSPS), has been extensively studied. The reason for this interest is because glyphosate [*N*-(phosphonomethyl)glycine] is a powerful inhibitor of EPSPS, so glyphosate has been used as a broad-spectrum systemic herbicide. It is an organophosphorus molecule, phosphonic acid, and glycine derivative that has a similar molecular structure to PEP.

Acetate pathway

A metabolic pathway is a biochemical reaction occurring within the cell. The reactants, products, and intermediates of an enzymatic reaction are known as metabolites. Therefore plants are living and solar-powered in which manufactures both primary and secondary metabolites from air, water, minerals and sunlight. The primary metabolites are needed for normal growth & development of plants and also utilized as food by man. The secondary metabolites are biosynthetically derived from primary metabolites. Since a long time it was believed that acetic acid is involved in the synthesis of cholesterol, squalene and rubber-like compounds. The discovery of acetyl coenzyme A further supported the role of acetic acid in biogenetic pathways. Later, mevalonic acid was found to be associated with the acetate. The pathway begins with acetyl CoA molecule produced from pyruvic acid, which is the end product of glycolysis. First two molecules of acetyl CoA forms acetoacetyl CoA through Claisen condensation. 3 rd molecule of acetyl CoA forms β-hydroxy β-methylglutarylCoA by aldol addition. Next on reduction gives rise to mevalonic acid, which is the main precursor for biosynthesis of terpenoids. Mevalonic acid on ATP mediated phosphorylation gives

mevalonic acid diphosphate. Which on decarboxylation gives the 1st isoprene unit, isopentyl pyrophosphate (IPP)? By the isomerase enzyme, the IPP gives 2 nd isoprene unit Dimethyl allyl pyrophosphate (DMAPP). Electrophilic addition of IPP with DMAPP via enzyme prenyl transferase yield C10 unit, geranyl pyrophosphate (GPP), which is the precursor for synthesis of monoterpenes. Combinations of another IPP unit with GPP give rise to form farnesyl pyrophosphate (FPP), C15 unit which acts as a precursor for the synthesis of sesquiterpenes. Further addition of IPP unit gives C20 geranyl geraniol pyrophosphate (GGPP) to produce a range of Diterpenes. On further addition of IPP unit gives C25 geranyl farnesyl pyrophosphate called sesquiterpenes. The tail to tail addition of two FPP units yields C30 unit, triterpene. Similarly 2 units of GGPP yield C40 unit, tetraterpene. The acetate mevalonate pathway thus works through IPP and DMAPP via squalene to produce two different skeleton containing compounds, that is, steroids and triterpenoids. It also produces vast range of monoterpenoids, sesquiterpenoids, diterpenoids, carotenoids, polyprenols, and also the compounds like glycosides and alkaloids in association with other pathways.

Schematic Diagram



Standardization of Herbal drugs

Phytotherapeutic agents are normally marketed as standardized preparations in the form of liquid, solid (powdered extract), or viscous preparations. They are prepared by maceration, percolation or distillation (volatile oils). Ethanol, water, or mixtures of ethanol and water are used for the production of fluid extracts. Solid or powered extracts are prepared by evaporation of the solvents used in the process of extraction of the raw material. Some phytotherapeutic agents are greatly concentrated in order to improve their therapeutic efficacy.

The identification, purity and quality of herbal drugs are determined by reference given in a pharmacopoeia. Pharmacopoeia prescribes like Analytical, physical and structural standards for the herbal drugs. A significant identification and examination of crude drugs is important in processes of herbal formulation because of more diversity and changes in their chemical nature or characters.

To reduce this problem all pharmacopeias have certain standards. Alkaloids content dragendorff test, Fat content Acid value Iodine value, saponification value molish test carbohyadrates Millon tests Amino acid Volatile oil Hemolytic activity Assay for Phosphate/ Aluminium/ Camphor /Potassium /Lead/ Iron/Gold/ Calcium. The Standardization of crude drug materials is done by authentication: Stage of collection, parts of the plant collected, identity like phytomorphology, microscopical and histological analysis (characteristic of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibers, vessels etc.), Leaf constant: palisade ratio, vein islet number, vein termination, stomatal number, and stomatal index. Other histological test are trichomes, Stomata, quantitative microscopy, taxonomical identity, foreign matter, organoleptic evaluation, ash values and extractive values, moisture content determination, chromatographic and spectroscopic evaluation, heavy metal determination, pesticide residue, microbial contamination, radioactive contamination.

The herbal formulation in general can be standardized schematically as to formulate the medicament using raw material collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. All the routine physical, chemical and pharmacological parameters are checked for all the batches in order to select the final finished product and to validate the whole manufacturing process.

The stability parameters for the herbal formulations which include physical, chemical and microbiological parameters are as follow: Physical parameters include color, odor, appearance, clarity, viscosity, moisture content, pH, disintegration time, friability, hardness, flow ability, flocculation, sedimentation, settling rate and ash values. Chemical parameters include limit tests, chemical tests, chemical assays etc. Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC, GC, UV, GC-MS, fluorimetry etc.

Microbiological parameters include total viable content, total mold count, total enterobacterial and their count. Limiters can be utilized as a quantitative or semi quantitative tool to ascertain and control the amount of impurities like the reagents used during abstraction of various herbs, impurities coming directly from the manufacturing vessels and from the solvents etc.

Systematic analysis of Crude drugs

The systematic analysis of crude drugs involves a comprehensive evaluation to assess their identity, quality, and purity. It includes macroscopic (organoleptic) examination, which uses sensory observations like shape, size, color, odor, and taste; and microscopic analysis, where transverse sections or powders of the drug are studied to identify structural features. Chemical evaluation involves qualitative tests for detecting active constituents like alkaloids, glycosides, flavonoids, and tannins, along with quantitative methods for determining their amounts. Additionally, physical parameters such as moisture content, ash values, and extractive values are measured to ensure the drug’s quality and detect impurities or adulteration.

WHO Guidelines

The **World Health Organization (WHO)** has established guidelines for the **standardization of herbal drugs** to ensure their quality, safety, and efficacy. These guidelines cover the entire process, from cultivation to the final product. Here's an overview of key components:

1. Botanical Identity

- Proper identification and authentication of the plant material are essential. This includes scientific nomenclature, botanical description, and plant part used.
- Microscopic and macroscopic analysis should be conducted to confirm the identity of the herbal drug.

2. Good Agricultural and Collection Practices (GACP)

- Guidelines ensure that medicinal plants are grown, harvested, and processed in ways that preserve their medicinal properties. This includes specifying soil type, climate, collection time, and post-harvest handling to prevent contamination.

3. Good Manufacturing Practices (GMP)

- GMP covers the procedures for the production, packaging, and storage of herbal drugs. It emphasizes hygiene, quality control, and documentation at every stage to ensure the final product is safe and consistent.

4. Phytochemical Evaluation

- Standardization involves qualitative and quantitative analysis of the active constituents or marker compounds in the herbal drug using techniques like chromatography (HPLC, GC), spectrophotometry, etc. This ensures batch-to-batch consistency in terms of chemical composition.

5. Physical and Chemical Testing

- These tests include the evaluation of moisture content, ash values, and extractive values. These parameters help in assessing purity, stability, and quality.

- Tests for potential contaminants such as heavy metals, pesticides, aflatoxins, and microbial contaminants (e.g., bacteria, fungi) are also required.

6. Safety and Toxicity Testing

- WHO emphasizes the need for preclinical safety assessments, including acute, sub-acute, and chronic toxicity tests, as well as studies on mutagenicity, genotoxicity, and carcinogenicity to ensure that the herbal drug is safe for human use.

7. Clinical Evaluation

- Clinical trials should be conducted to validate the therapeutic claims of the herbal drug. This includes trials to assess the efficacy and safety in the target population, following proper ethical and scientific guidelines.

8. Stability Testing

- Stability studies are necessary to determine the shelf-life of the herbal drug, considering factors like temperature, light, humidity, and packaging.

9. Labeling and Packaging

- Proper labeling is essential, with information such as the botanical name, part used, method of extraction, concentration, dosage, storage conditions, and expiration date clearly indicated. Packaging should protect the product from environmental factors that might degrade its quality.

10. Documentation

- Comprehensive documentation is required at every step to ensure traceability, batch consistency, and regulatory compliance. This includes maintaining records for raw material sourcing, processing, quality control, and distribution.

These guidelines aim to ensure that herbal drugs are safe, effective, and of consistently high quality.

Sampling of Crude drug

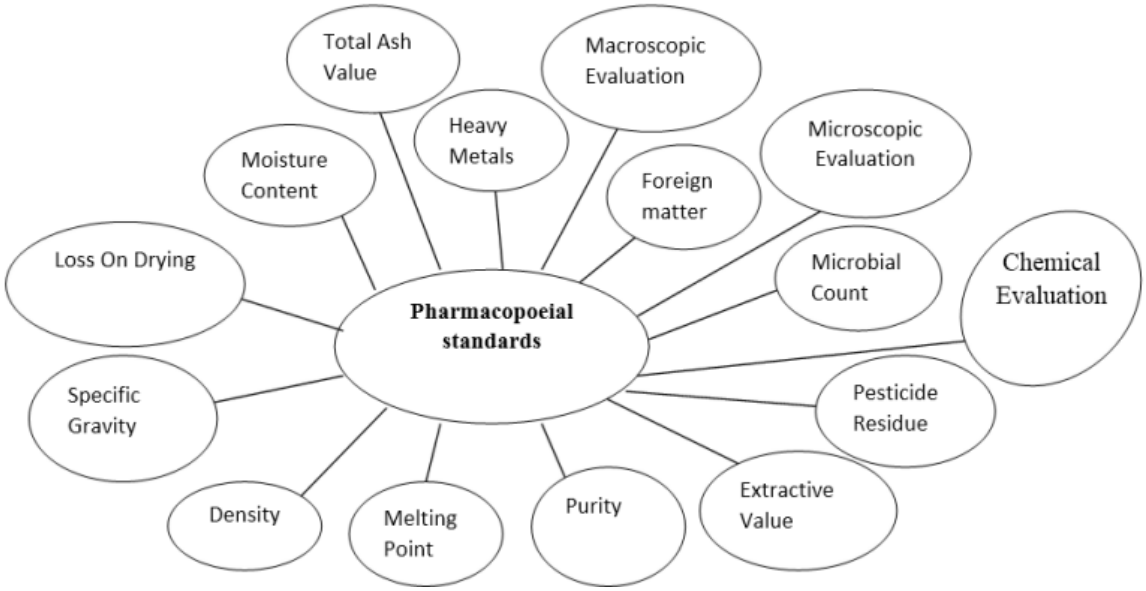
Sampling of crude drugs with a focus on phytochemistry is critical for ensuring that the samples accurately represent the chemical constituents of the whole batch. Phytochemical sampling is aimed at identifying and quantifying the active compounds, which directly contribute to the drug's therapeutic properties. The process typically involves selecting a representative portion of the crude material from different parts or batches to avoid sampling bias, ensuring uniformity in the chemical profile.

Key considerations include the geographical origin, harvesting season, and storage conditions, as these factors can significantly influence the concentration of phytochemicals like alkaloids, terpenoids, and flavonoids. The method of extraction and sample preparation is crucial in preserving these bioactive compounds. For example, improper drying or prolonged exposure to light and air can lead to the degradation of sensitive phytochemicals. Analytical techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Thin-Layer Chromatography (TLC) are often employed to analyze the phytochemical profile during sampling.

By employing rigorous phytochemical sampling protocols, the consistency, potency, and quality of crude drugs are ensured, enabling their safe and effective use in pharmaceutical preparations.

Methods of Drug Evaluation

The identification, purity and quality of herbal drugs are determined by reference given in a pharmacopoeia. Pharmacopoeia prescribes like Analytical, physical and structural standards for the herbal drugs. A significant identification and examination of crude drugs is important in processes of herbal formulation because of more diversity and changes in their chemical nature or characters. To reduce this problem all pharmacopeias have certain standards. Specific test for specific plant material are given below. Alkaloids content dragendorff test, Fat content Acid value Iodine value, saponification value molish test carbohyadrates Millon tests Amino acid Volatile oil Hemolytic activity Assay for Phosphate / Aluminium / Camphor / Potassium / Lead / Iron / Gold / Calcium.



Determination of Foreign Matter

Herbal drugs should be prepared from the confirmed part of the plant. They should be totally free from insects or moulds, including visible and excreta contaminant such as stones, sand, harmful and poisonous foreign matter and chemical residues. Animal objects such as insects and invisible microbial contaminants, which produces toxins, as well as the potential contaminants of herbal medicines. Macroscopic evaluation can easily used to determine the

presence of foreign matter, although microscopy is essential in certain special cases for example starch intentionally added to “dilute” the plant material.

% of foreign Organic Matter = N × W × 94,100 × 100/S × M × P

Where; n = No. of chart particles in 25 field.

S = No. of spores in the same area of 25 fields.

W = Weight in mg of lycopodium taken.

M= weight in mg of the sample

P= number of characteristics particles per mg of the pure foreign matter.

94,100= number of spores per mg of lycopodium.

Moisture Content

The moisture content was determined by heating the green leaves of plants at 90^{o}C in a hot air oven. The difference between initial weight of the green leaves and final weight after drying is the moisture content. The percentage of chemical constituents in crude drug is mentioned on air dried basis.

Moisture Content % = Initial Wt. – Final Wt. × 100 Initial wt.

Ash value

The residue after incineration is the total ash content of the crude drug, which simply represents inorganic salts, naturally found in drug or adhering to it or deliberately added to it, in the form of adulteration. Two types of total Ash value:

1. Water soluble Ash value

2. Acid insoluble Ash value

Extractive values are determined by the amount of soluble and insoluble matter in the plant ash. The more soluble matter, the higher the value.

There are a few ways to test for extractive values. One is to place the ash in water and see how much of it dissolves. This will give you the percentage of soluble matter in the ash.

Another way to test for extractive values is to ignition test. This is done by heating the ash and seeing how much weight is lost during the process. The weight loss will tell you the percentage of insoluble matter in the ash.

Knowing the extractive values of plant ash can be helpful when determining its use. For example, high values may be indicative of a good potash fertilizer while low values may mean it is not as effective. Extractive values can also help determine how well a material will withstand weathering or leaching.

Alcohol Soluble Ash value

The Alcohol Soluble Ash (ASA) value is a measure of the amount of minerals that are soluble in alcohol. This value is important because it helps to determine the extractive values of plants. The ASA value is determined by taking a sample of the plant material and adding it to a solution of alcohol and water.

The mixture is then allowed to stand for a period of time, typically 24 hours. After this time, the solution is filtered and the residue is weighed. The ASA value is then calculated by dividing the weight of the residue by the weight of the original plant material.

The ASA value is important because it helps to determine how much of the plant's mineral content is extractable. This information is valuable for both farmers and processors who need to know how much of the plant's nutrients can be extracted and used.

Alcohol soluble extractive = value (%w/w)

Water Soluble Ash Value

The water soluble ash value is a measure of the amount of inorganic material that is present in the plant ash. This value is important because it can help to determine the extractive values of the plant ash. The water soluble ash value is determined by taking a sample of the ash and adding water to it.

The mixture is then allowed to stand for a period of time, typically 24 hours. After this time, the mixture is filtered and the filtrate is analyzed. The results of this analysis can help to determine the extractive values of the plant ash.

Water soluble ash (%w/w) = (weight of ash – weight of insoluble ash/ weight of sample) ×100

Acid Soluble Ash Value

The Acid Soluble Ash Value is the measure of inorganic material that is soluble in acid and is a good indicator of the quality of the plant ash. This value is important because it helps to determine the extractive values of the plant ash.

The Acid Soluble Ash Value is determined by taking a sample of the ash and adding it to an acid solution. The mixture is then allowed to stand for a period of time before being filtered. The filtrate is then evaporated to dryness and weighed.

Acid insoluble ash (%w/w) = (weight of ash/weight of sample) × 100

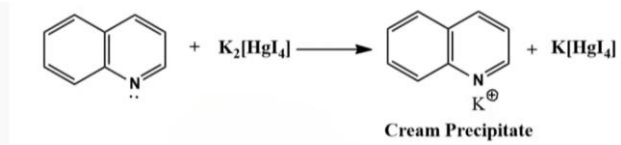
Phytochemical investigations - General chemical tests

General Chemical Test:

1. Alkaloids:

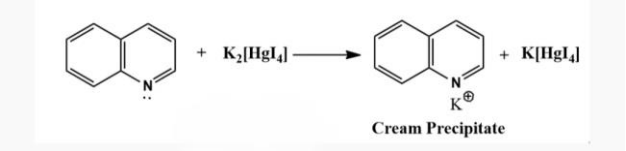
a. Mayers Test :

To 0.5ml of filtrate add 2 to 3 drops of mayers reagent, if there is a formation of pale yellow or white cream precipitate alkaloids is present. Potassium Mercuric Iodide is known as mayers reagent.



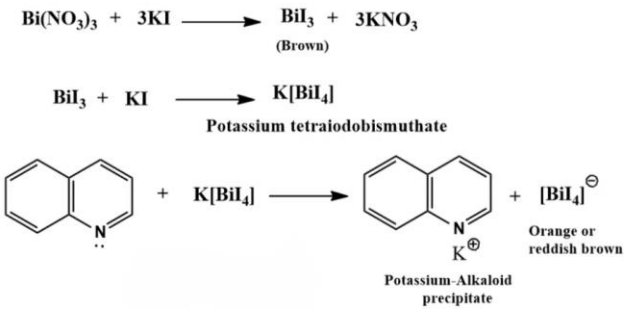
b. Wagner's Test :

To 0.5ml filtrate add 2 to 3 drops of Wagner's reagent, if there is a formation of reddish-brown precipitate alkaloids is present. Iodine solution [KI + I_2] is Wagner's reagent.



c. Dragendroff's Test:

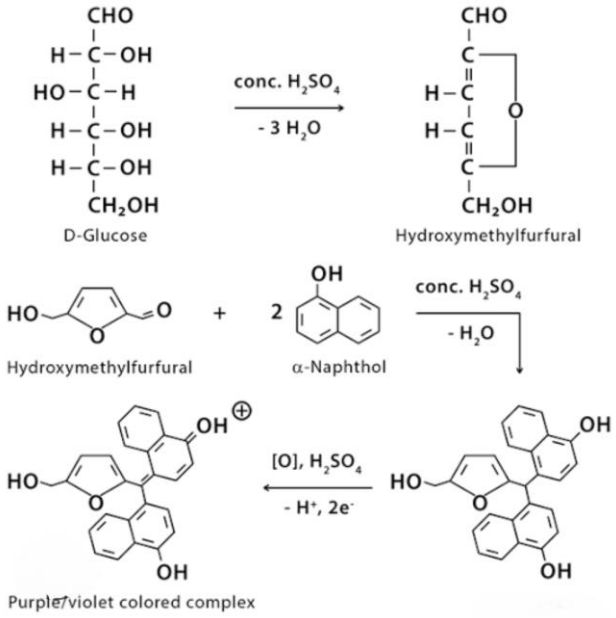
To 0.5ml filtrate add 2 to 3 drops of the dragendroff's reagent, if there is a formation of orange brown precipitatealkaloids is present . Potassium iodide + Bismuth nitrate is known as dragendroff's reagent.



2. Carbohydrates

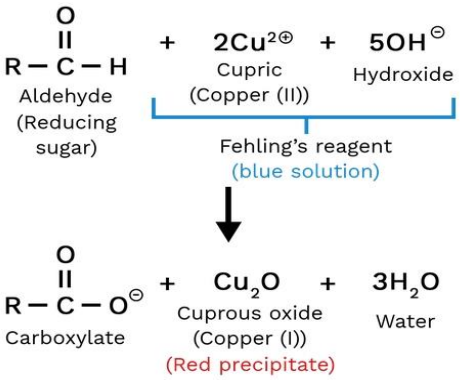
a. Molish's Test

To 2ml of sample add molish's reagent slowly and add concentrated H_2SO_4 along sides of the test tube, if there is a formation of violet ring then presence of Carbohydrates is confirmed.



b. Fehling's Test:

Mix 1ml of both Fehling's solution and then add 1ml of sample to it and then heat it for 10 minutes in water bath. Appearances of green, yellow or red solution presence of Carbohydrates is confirmed.



3. Glycosides

a. For Anthraquinone glycosides:

Borntrager's Test

3ml of extract mixed with dil. H_2SO_4 and boiled and filtered. To 2ml of filtrate add 2ml of chloroform. Shaken and separate the chloroform layer and add ammonia solution, formation of red or pink colour presence of Anthraquinone glycosides.

b. Cardiac glycosides

Keller-killiani test

To 2ml extract add 1ml glacial acetic acid and 1 drop of 5% ferric chloride solution then followed by con.H_2SO_4. Reddish brown colour appears at the junction of the two liquid layer and upper layers appears bluish green, presence of cardiac glycosides.

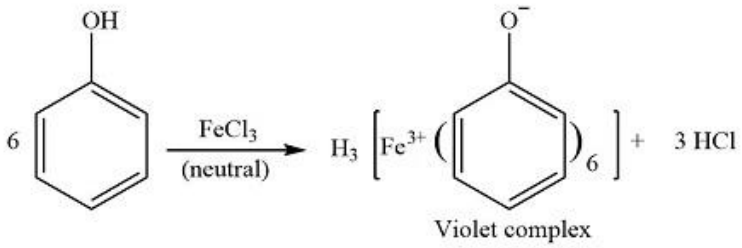
Legal's test

To the extract add 1ml of pyridine and sodium nitroprusside followed by addition of NaOH solution to make alkaline, formation of pink or red colour presence of cardiac glycosides.

4. Tannis and Phenolic compounds

a. $FeCl_3$ Test

To the extract add few drops of 5% ferric chloride solution, formation of deep blue or violet colour, presence of Phenolic compounds.

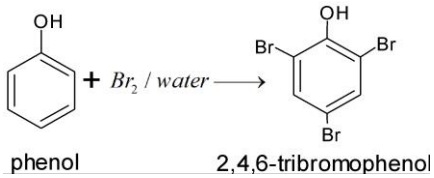


b. Gelatin Test

To the extract add 2ml of 1% solution of gelatin contain 10% NaCl, formation of white precipitate presence of tannis compunds.

c. Bromine water test :

Add bromine water to a extract of the drug, Condensed tannis gives buff coloured precipitate and hydrolysable tannis do not form any precipitate.



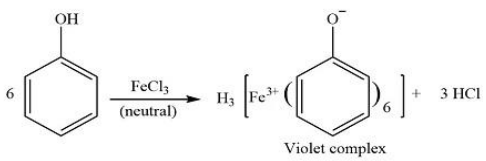
d. Phenazone Test

To 2ml of extract add 0.2g of sodium acid phosphate. Warm the solution, cool and filter. Add 2% phenazone solution to the filtrate. Tannis compunds are precipitated as bulky, coloured precipitate.

5. Flavonoids

a. $FeCl_3$ Test

To the extract add few drops of 5% ferric chloride solution, formation of deep blue or violet colour, presence of Phenolic compounds.

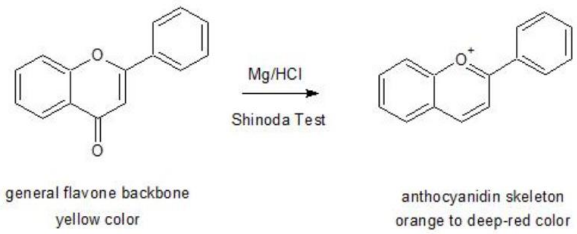


b. Lead acetate Test

To the extract add lead acetate solution, formation of yellow precipitate, indicates the presence of flavonoids.

c. Shinoda Test

To the extract add few magnesium turnings and con.HCl dropwise, the deep red colour indicates presence of flavonoids.



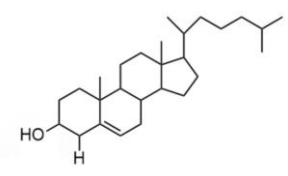
6. Steroids

a. Leibermann- Burchard Reaction

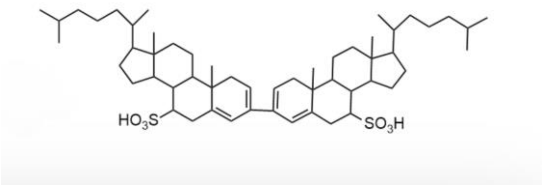
Mix 2ml of extract with chloroform and then add 1 to 2ml of acetic anhydride followed by 2 drops of con.H_2SO_4 from the sides of the test tube, the solution first red then blue and finally green colour indicates the presence of steroids.

b. Salkowski Reaction

To 2ml of extract add 2ml of chloroform and 2ml of con. sulphuric acid then shaken well, red colour in chloroform layer and greenish yellow fluorescence in acid layer indicates the presence of steroid



Cholesterol.



Bisulfonic acid of bi-cholestadiene

7. Terpenoids

a. Leibermann- Burchard Reaction

Mix 2ml of dry extract with chloroform and then add 1 to 2ml of acetic anhydride followed by 2 drops of con.H_2SO_4 from the sides of the test tube, formation of pink colour indicates the presence of Triterpenoids.

b. Salkowski Reaction

To 5ml of extract mixed with 2ml of chloroform and add 3ml of con. Sulphuric acid then shaken well, reddish brown colouration at the junction of two layers indicates the presence of Terpenoids.

8. **Protein and Amino acids**

a. Ninhydrin Test

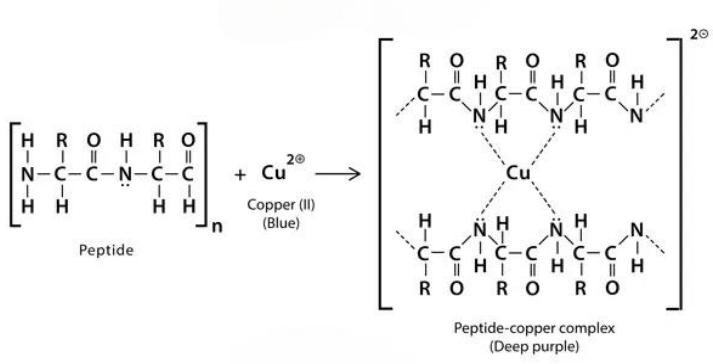
Heat 3ml of extract with 3 drops of 5% ninhydrin solution in boiling water bath for 10 minutes, purple or bluish colour indicates the presence of Amino acids.

b. Millon's Test

To 3ml of extract add 5ml of millon's reagent then warm it after the formation of white precipitate, white precipitate changes to brick red or dissolve to give red colouration. Presence of protein.

c. Biuret Test

To 3ml of extract add 4% NaOH and few drops of 1% copper sulphate solution, violet or pink colour indicates the presence of protein.



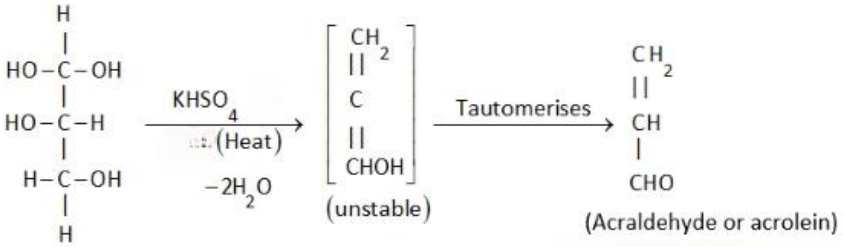
8. Fats and Oils:

a. Spot Test

Place small quantity of the extract in between two filter paper. Oil stain produced with any extract showed the presence of fixed oil and fats in the extract.

b. Acrolein Test

To 2ml of extract add 1ml of con.H2SO4 or 1g of KHSO4 and then heat it. Pungent odour indicates the presence of fat and oil.



UNIT-II

Extraction Techniques

General Methods of Extraction

The extraction of bioactive natural products from natural sources, such as plants, microorganisms, or marine organisms, involves a variety of techniques aimed at isolating compounds with therapeutic, nutritional, or cosmetic value. One of the most widely used methods is maceration, where finely ground plant material is soaked in an appropriate solvent (such as water, ethanol, or methanol) for a specified period, typically several days. The solvent helps dissolve the bioactive compounds, which are then separated from the plant material by filtration, and the solvent is subsequently evaporated to yield a crude extract. Percolation is another method, where a continuous flow of solvent is passed through the plant material, allowing for more efficient extraction. This method is particularly useful when large amounts of bioactive compounds are desired, as it reduces the extraction time.

More advanced methods like solvent extraction involve the use of different solvents, selected based on the polarity of the bioactive compounds, to optimize the extraction process. Steam distillation is a common technique used to isolate volatile compounds like essential oils, where steam passes through the plant material and the volatile components are condensed and collected separately. Another modern technique, supercritical fluid extraction (SFE), utilizes supercritical CO_2 as the extraction solvent. This method is advantageous for its efficiency and environmentally friendly nature, as it leaves little to no solvent residue in the final product. Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are also increasingly used for rapid and efficient extraction, often providing higher yields of bioactive compounds in shorter timeframes. These methods are essential in isolating a variety of compounds such as alkaloids, flavonoids, terpenes, and phenolic compounds, which are utilized in pharmaceutical, nutraceutical, and cosmetic industries for their beneficial properties.

Types of Extraction

Extraction:

Extraction is the method of removing active constituents from a solid or liquid by means of liquid solvent.

- The separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents.
- In this method the wanted components are dissolved by the use of selective solvents known as menstrum & undissolved part is a marc.
- After the extraction unwanted matter is removed.
- Extracts are prepared by using ethanol or other suitable solvent.

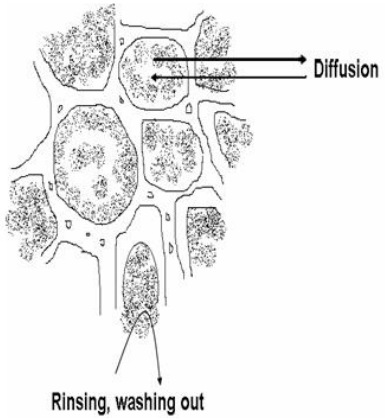
Extract : Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent.

Type of extracts

Dry extract (Tab, cap.) - E.g. belladonna extract

Soft (Ointment, suppository) - E.g. glycerrhiza extract.

Liquid : As tincture.

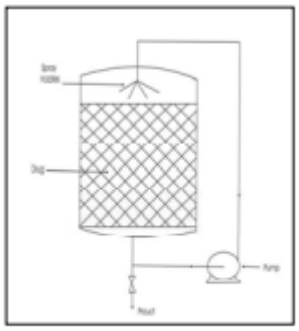


- Dissolution of extractive substances out of disintegrated cells.
- Dissolution of extractive substances out of intact plant cell by diffusion (requires steeping and swelling)
- Penetration of the solvent into the plant cells and swelling of the cells.
- Diffusion of the dissolved extractive substances out of the cell.

- Penetration of the solvent into the plant cells and swelling of the cells.
- Diffusion of the dissolved extractive substances out of the cell.

Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.



Circulatory extraction

Infusion Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

Types of Maceration

- Simple maceration: for organized and unorganized Crude drug

e.g. i) Tincture of Orange

ii) Tincture of Lemon

iii) Tincture of Squill

- Double maceration : Concentrated infusion of orange
- Triple maceration: The maceration process may be carried out with help of heat or stirring

  e.g. i) Concentrated infusion of Quassia ii) Concentrated infusion of Senna

Advantages

- The drug is extracted as many times as there are receivers – in this case, three. If more extraction stages are required, it is only necessary to have more receivers.

- The last treatment of the drug before it is discharged is with fresh solvent, giving maximum extraction.

- The solution is in contact with fresh drug before removal for evaporation, giving the highest possible concentration.

Factors affecting Maceration

Concentration gradient (C1-C2) is affected by several factors

1.Solid/solvent ratio: Yield decreases with constant quantity of solvent and increasing proportion of drug material.

2. Dissolution from disintegrated cells: Particle size

3. Steeping and swelling of plant material: Capillary dilation and increase in diffusion rate (Mucilage)

4. Diffusion from intact plant cell: Solvent must be able to solublilize substances

5. Temperature: increase solubility (diffusion coefficient), and decrease the viscosity

6. pH value: Influence the selectivity of extraction (qualitative and quantitative)

7. Interaction of dissolved constituents with insoluble support material of plant

8. Degree of lipophilicity

9. Effect of addition of surfactants, salts and co-solvents

Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents. e.g. Tea , Coffee

Decoction Process

Decoction is a method used to extract medicinal properties from tougher plant materials, such as roots, barks, and seeds. The process involves several key steps:

Preparation of Plant Material:

Begin by selecting the appropriate plant parts, which are often dried. The plant material is usually cut or crushed to increase surface area, enhancing extraction efficiency.

Water Addition:

Place the prepared plant material in a pot and add water. The amount of water can vary depending on the desired strength of the decoction.

Boiling:

Bring the mixture to a boil. Once boiling, reduce the heat and let it simmer. The simmering time typically ranges from 20 minutes to an hour, depending on the type and hardness of the material. The boiling process helps to break down cell walls and release soluble compounds.

Straining:

After simmering, remove the pot from heat and strain the mixture through a fine mesh or cheesecloth to separate the liquid extract from the solid plant material.

Storage:

The resulting decoction can be consumed immediately or stored in a refrigerator for a short period (usually 1–2 days). It's important to note that decoctions are best used fresh due to the potential degradation of compounds over time.

Advantages of Decoction

Effective Extraction:

Decoction is particularly effective for extracting compounds from hard, fibrous materials that require heat for solubility, such as minerals, tannins, and polysaccharides.

Concentration:

The boiling process reduces the volume of liquid, resulting in a more concentrated extract that can deliver stronger medicinal effects.

Versatility:

Decoctions can be used in various therapeutic applications, including herbal teas, medicinal syrups, and as a base for other herbal formulations.

Flavor Enhancement:

The cooking process often mellows the taste of herbs, making them more palatable compared to some tinctures or infusions.

Traditional Use:

Decoction is a well-established method in many traditional herbal systems, providing a culturally and historically rich approach to herbal medicine.

Limitations of Decoction

Heat Sensitivity:

Some delicate compounds, such as certain volatile oils and vitamins, can be degraded or lost during the boiling process. This limits the range of herbs suitable for decoction.

Time-Consuming:

The process requires time and attention, especially during the boiling and simmering stages, which may not be practical for everyone.

Short Shelf Life:

Decoctions do not have a long shelf life, often requiring refrigeration and use within a few days. This can be inconvenient for those seeking longer-term storage solutions.

Labor-Intensive:

Preparing a decoction can be more labor-intensive compared to other methods, such as maceration or infusion, which require less active involvement.

Inconsistent Dosage:

The concentration of active compounds can vary depending on the type of plant material, boiling time, and water quantity, making it challenging to ensure consistent dosing.

Percolation:

Percolation is an exhaustive extraction procedure by which all the soluble constituents are completely removed from a plant material by extracting the crude drug by fresh solvent.

The entire percolation process is explained by dividing it into following stages.

1. Comminution
2. Imbibition
3. Packing
4. Maceration
5. Percolation

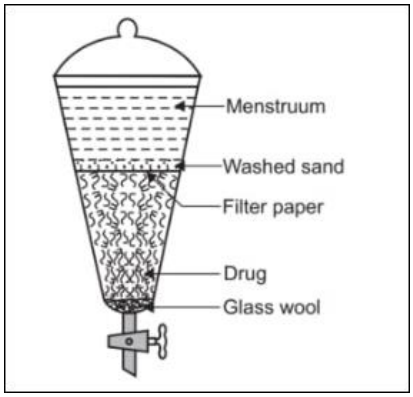
1. Comminution:

- ❖ It is the size reduction of drug usually from coarse powder
- ❖ To increase surface area
- ❖ To ensure complete removal of drug
- ❖ To slow down the movement of menstruum.
- ❖ For uniform packing.

2.Imbibation:

During imbibition the powdered drug is moistened with a suitable amount of menstruum and allowed to stand for 4 hours in a well closed container. During this period, the drug swells up by absorbing the menstruum. This moistening of drug is necessary for following objectives:

1. To avoid choking
2. To remove air
3. To avoid washing out of fine particles



3.Packing:

There are four stages. They are,

i. Sifting

ii. plugging

iii. Column packing

iv. Protective layer

v. Addition of menstruum.

i) Sifting:

Before packing imbibed drug in percolator, the moistened drug should drug should pass through sieve to break down any lump.

ii) Plugging:

To prevent exist of imbibed drug from the outlet is blocked but cotton or glass wool moistened with the solvent or menstruum.

iii) Column packing:

Imbibed moist drug added in percolator in little quantity each time and pressed lightly after each addition. Drug should be uniformly packed.

iv) Protective layer: A filter paper is placed on the packed drug column and layer of washed sand is placed on the filter paper. Protective layer prevents disturbance of the layer of the drug due to addition of menstruum.

v) Addition of menstruum*:*

Add sufficient quantity of menstruum to saturate the material when liquid start coming out of the outlet. Close the outlet and add a sufficient quantity of menstruum above the column of drug.

Maceration:

After packing sufficient menstruum is added and the top of the percolator is covered with lid. When the liquid begins to drip from the bottom of the percolator the tap fitted at its bottom is closed. Then the percolator is set aside for 24 hours.

- ❖ After 24 hours the lower orifice is opened and menstruum is collected with a controlled speed until 3\4 of menstruum is collected.
- ❖ Then more menstruum is added and collected from the lower orifice so that the marc does not become dry.
- ❖ The marc is pressed to extract which is combined with pervious liquid.
- ❖ Then it is allowed to stand and then it is filtered.

Types of percolation:

Percolation is divided into two types. They are

1. Reserve percolation
2. Modified percolation

1. Reserve percolation:

It is only used for the active constituents are heat stable

Similar to multiple maceration.

a) First percolation:

After the percolation process, the obtained percolate is reserved.

b) Second percolation:

The drug is again percolated with fresh menstruum till the drug get exhausted. The volume is mixed with menstruum obtained by pressing marc this mixture is then evaporated to a soft

extract till the volume is reduced to half. It is mixed with the first reserved percolate and the volume is adjusted by adding fresh percolate.

2. Modified percolation:

For preparation of concentrated extract if active constitute is heat sensitive or thermolabile.

Involves maceration and percolation for 3 to 4 times.

It is also called as percolation with interruption.

In simple percolation drug : menstruum ratio is 1 : 4.

In modified percolation drug contact with stationary menstruum for 3 to 4 times. Less menstruum is required and helps in extraction of more active constituents than the simple percolation method.

In modified percolation drug : menstruum ratio is 1 : 3.

a) First step:

The crude drug powder is imbibed with menstruum and kept for 4 hours.

The imbibed drug is kept for 24 hours in maceration and the percolate is filtered.

b) Second step:

Same drug is kept for 12 hours maceration and kept aside.

This process is repeated for the third step. The first, second and third percolate are mixed to get concentrated extract.

The marc is tested for exhaustion of active constituents. If still the constituent are present or not if it is present the process is continued.

Immersion:

Immersion extraction is a technique of often used in the extraction of compounds from various matrices, particularly in the context of food science, herbal medicine and environmental analysis. Here an overview of the method.

Principle:

Immersion extraction involves soaking a solid material in a solvent to dissolve the desired compounds, which are then separated from the matrix. The solvent penetrates the solid, extracting the target substances based on their solubility in the solvent.

Steps involved:

1.Preparation of the solid sample:

The solid material (e.g., plant material, food sample, soil) is prepared by drying, grinding, or cutting into smaller pieces to increase the surface area for extraction.

2.selection of solvent:

The solvent depends on the `nature of the compounds to be extracted. Common solvents include water, ethanol, methanol, acetone and hexane.

3.Immersion:

The prepared solid sample is immersed in the chosen solvent. This can be done at room temperature or with heating to enhance extraction efficiency.

4. Extraction time:

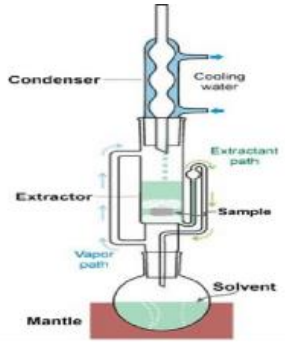
The duration of immersion can vary from a few minutes to serval hours, depending on the material and the desired compounds.

5.separation;

After the immersion period, the solid material is separation from the solvent,

usually by filtration, centrifugation steps may be carried out to isolate specific compounds.

Soxhlet apparatus:



Procedure:

❖ Add menstruum in flask and fix it in stand. Pack the extraction material or drug powder in a cylinder made up of filter paper and place it in a wide tube or body of Soxhlet apparatus.

❖ Fit the condenser on the top of the Soxhlet extractor.

❖ Connect the inlet and outlet with pump and start in flow cool water.

❖ Heat the content by heating element like water or sand bath.

❖ Menstruum starts boiling and vapour formed enter into condenser through side tube menstruum condenses in the condenser and hot liquid falls on the drug column present in the wide tube.

❖ When the wide tube gets filled with menstruum, the level of menstruum or extract also rises in syphon tube and then menstruum containing active constituents drains from the wide tube to the flask through this syphon tube.

❖ The active constituents are non-volatile in nature. The menstruum again vaporizes and then enter through the side tube and the cycle continuous.

❖ The same menstruum is used for the extraction of the drug and the active constituents present in the flask becomes concentrated

❖ The process is continued till all the drug is exhausted. Normally 15 cycles are repeated for complete exhaustion of the drug.

Limitation:

Only pure solvent or constant boiling mixture can used can be used for this process.

Some drug may block apparatus or makes the apparatus impermeable to the menstruum.

Examples; gums, resin, orange peel.

Advanced Techniques

Advanced extraction techniques are modern, highly efficient methods developed to isolate bioactive compounds from natural sources with improved yield, selectivity, and speed, while

minimizing solvent use and environmental impact. These techniques are essential in industries such as pharmaceuticals, nutraceuticals, cosmetics, and food science

Counter Current

The counter-current extraction (CCE) method is a highly efficient technique used in the separation and purification of bioactive compounds, especially in the pharmaceutical, chemical, and food industries. It involves two immiscible liquid phases, typically a solvent and a solution, flowing in opposite directions in a system designed for maximal interaction. The bioactive compounds are transferred from one phase to the other, driven by the difference in solubility, creating a high degree of separation. This method is particularly useful for compounds that are difficult to separate using conventional techniques.

Basic Principles of Counter-Current Extraction

In the counter-current extraction method, the two phases (solvent and solution) move in opposite directions through a series of stages, creating multiple opportunities for mass transfer of the solute (the compound to be extracted) from one phase to another. One phase usually contains the raw material (e.g., a plant extract), and the other phase is the solvent that selectively extracts the target compound.

The solute is repeatedly brought into contact with the solvent as the two phases pass through each other. This repeated contact maximizes the extraction efficiency by allowing the solute to move from the phase in which it has lower solubility to the phase in which it has higher solubility. Each stage of the process increases the concentration of the solute in the solvent phase, ultimately leading to high-purity extraction.

Steps Involved in Counter-Current Extraction

1. Preparation of the Two Phases:

- The first phase contains the feed material, typically a liquid or a dissolved bioactive compound in water, alcohol, or other solvents.

- The second phase is an immiscible solvent that has a greater affinity for the bioactive compound and will be used to extract it.

2. Introduction of Phases into the System:

- The two immiscible phases are introduced into the system, often a column or series of mixing and settling chambers, where they flow in opposite directions (counter-currently).

- The feed phase flows downward while the solvent phase moves upward, or vice versa.

3. Mixing and Extraction:

- In each stage, the phases are brought into intimate contact, either by mechanical mixing or agitation.

- The solute (bioactive compound) moves from the phase where it is less soluble into the phase where it is more soluble, driven by the solubility difference between the two phases. This creates a concentration gradient that enables efficient extraction.

- The process takes advantage of the differences in solubility, partition coefficient, and affinity of the compound for each phase.

4. Separation of Phases:

- After sufficient mixing, the phases are allowed to settle and separate based on their immiscibility.

- The solvent phase, now enriched with the bioactive compound, is collected from one outlet, while the depleted feed phase (raffinate) is removed from the other outlet.

5. Multi-Stage Extraction:

- The process is repeated over multiple stages, with the solvent phase being enriched with the target compound at each stage.

- In each stage, fresh solvent is introduced, or the same solvent is recycled in a continuous process to ensure maximal recovery of the bioactive compound.

6. Recovery of Bioactive Compound:

- Once the solvent phase is sufficiently enriched with the target compound, the solvent is removed, usually by evaporation or distillation, leaving behind the purified bioactive compound.

- The solvent can be recycled and reused in the system.

Key Features of Counter-Current Extraction

- Counter-Flow of Phases: The main feature of this method is the counter-current movement of the two phases, which maximizes the concentration gradient for mass transfer. Unlike co-current methods, where the phases move in the same direction, the counter-current setup enhances efficiency by continuously maintaining a large driving force for the solute transfer across each stage.

- Partition Coefficient: The effectiveness of counter-current extraction is determined by the partition coefficient, which defines how the solute distributes itself between the two immiscible phases. A high partition coefficient indicates that the solute prefers the solvent phase, leading to efficient extraction.

- Multistage System: In many cases, a single-stage extraction is not sufficient to achieve the desired separation. Therefore, the process is repeated in multiple stages (often automated or in cascades), each improving the concentration of the bioactive compound in the solvent.

Types of Counter-Current Extraction Systems

1. Continuous Counter-Current Extraction:

- In a continuous system, the feed and solvent are constantly introduced into the system, and the extraction takes place as the phases move through a series of stages. The solvent, now enriched with the target compound, is continuously withdrawn from the system, while the raffinate (spent phase) is removed separately.

- Continuous systems are preferred in large-scale industrial processes because they are more efficient and can handle large volumes of material.

2. Batch Counter-Current Extraction:

- In batch systems, the extraction process is performed in stages, and the phases are introduced and removed in batches rather than continuously. This method is generally used for smaller-scale operations or where precise control over each extraction stage is needed.

- Batch systems are often used in laboratory-scale extractions or in the early stages of process development.

Advantages of Counter-Current Extraction

1. High Efficiency:

- Counter-current extraction allows for continuous contact between the feed and solvent, ensuring a high degree of extraction efficiency. Since the phases are moving in opposite directions, each new portion of the solvent is in contact with the least extracted feed, maximizing the driving force for extraction.

2. Maximized Yield:

- The multistage nature of the process increases the yield of bioactive compounds, ensuring that even trace amounts of compounds are efficiently transferred to the solvent phase.

3. Selective Extraction:

- The use of immiscible solvents tailored to the solubility of specific bioactive compounds allows for highly selective extraction. This reduces the co-extraction of unwanted compounds and leads to a purer final product.

4. Adaptability:

- Counter-current extraction systems can be adapted to work with different solvents, solutes, and phases, making them suitable for a wide range of applications, including pharmaceuticals, nutraceuticals, and natural product extraction.

5. Scalability:

- Counter-current extraction can be easily scaled from laboratory settings to industrial production. Continuous systems, in particular, are well-suited for large-scale operations where high throughput is required.

Applications of Counter-Current Extraction

1. Pharmaceutical Industry:

In the pharmaceutical industry, counter-current extraction is used for the isolation and purification of active pharmaceutical ingredients (APIs) from natural sources like plants, microorganisms, and marine organisms. For example, the extraction of alkaloids, flavonoids, and terpenes from plants is efficiently carried out using this technique.

2. Food and Beverage Industry:

This method is also used in the food and beverage industry for the extraction of flavors, colors, and essential oils. It is particularly useful in the production of natural food additives and nutraceuticals.

3. Natural Product Chemistry:

In natural product chemistry, counter-current extraction is employed to isolate bioactive compounds from complex mixtures, such as plant extracts or fermentation broths. It is often used for extracting secondary metabolites, which have pharmaceutical or nutritional benefits.

4. Environmental Applications:

Counter-current extraction is applied in environmental processes, such as the removal of contaminants from wastewater or the extraction of valuable compounds from industrial waste streams.

Disadvantages of Counter-Current Extraction

1. Complex Setup:

- The equipment required for counter-current extraction can be complex, especially for continuous systems. This complexity increases the initial cost and maintenance of the system.

2. Energy-Intensive:

The need for repeated mixing, settling, and phase separation can make counter-current extraction energy-intensive, particularly in large-scale operations.

. Solvent Handling

The use of large volumes of solvent can pose challenges in terms of solvent recovery and environmental impact. Proper solvent recycling and disposal are critical to maintaining a

Steam Distillation

Steam Distillation: A Method for Extracting Bioactive Molecules

Steam distillation is one of the most commonly used methods for extracting volatile bioactive compounds, particularly from plant materials. This technique is based on the principle of co-distillation, where steam is used to carry volatile compounds away from the plant matrix, and subsequent condensation of the vapor yields the purified extract. Bioactive molecules like

essential oils, fragrances, and certain natural pharmaceuticals are often obtained through steam distillation. This essay will provide a detailed explanation of steam distillation, including its principles, process, applications, advantages, and limitations.

Principles of Steam Distillation

Steam distillation works on the principle that when a mixture of immiscible liquids, such as water and a volatile compound (e.g., essential oil), is heated, the total vapor pressure is the sum of the vapor pressures of the individual components. This means that the mixture will boil at a temperature lower than the boiling point of either component alone. As a result, bioactive molecules, especially those with high boiling points, can be distilled at lower temperatures than their standard boiling points when in the presence of water or steam.

This reduced boiling point is critical because it allows the extraction of thermally sensitive bioactive compounds without subjecting them to high temperatures, which might degrade or alter them. Steam distillation can be conducted at atmospheric or reduced pressure (vacuum steam distillation) to further lower the temperature of the process.

Process of Steam Distillation

The steam distillation process generally consists of four main steps: preparation, distillation, condensation, and separation.

1. Preparation:

- The plant material containing the bioactive compounds is collected and prepared. This may involve cleaning, drying, and grinding the plant matter to increase the surface area for extraction.

- The plant material is then placed in a distillation flask or chamber where it will be exposed to steam.

2. Distillation:

- Steam is generated in a separate boiler or introduced directly into the distillation chamber containing the plant material.

- As the steam passes through the plant matrix, it heats the plant material, causing the volatile bioactive compounds to vaporize.

- The volatile bioactive molecules are carried away by the steam, forming a mixture of steam and the vaporized compounds.

3. Condensation:

- The steam and vapor mixture is passed through a condenser, where it is cooled and condensed back into a liquid state.

- The condenser typically uses cold water to cool the vapor and allow the water and bioactive compounds to liquefy.

4. Separation:

- The condensed liquid typically forms two phases: a water phase and an organic phase. The organic phase contains the bioactive molecules (essential oils or other compounds), while the water phase may contain hydrosols (water-soluble aromatic components).

- Due to the immiscibility of the bioactive compounds with water, the two phases can be easily separated using a separatory funnel or decantation.

Applications of Steam Distillation

Steam distillation is widely used in industries ranging from perfumery and cosmetics to pharmaceuticals and food. Some key applications include:

1. Essential Oil Extraction:

- Essential oils are highly volatile bioactive compounds extracted from aromatic plants, including lavender, peppermint, eucalyptus, and rose. Steam distillation is one of the primary methods for extracting essential oils due to its ability to preserve the integrity of the volatile compounds.

- The extracted oils are used in aromatherapy, cosmetics, and as flavoring agents in the food industry.

2. Pharmaceuticals:

- Many plants contain bioactive compounds with medicinal properties, such as menthol, camphor, and eucalyptol, which can be extracted via steam distillation.

- These compounds are used in various pharmaceutical products for their antimicrobial, anti-inflammatory, and analgesic properties.

3. Fragrances:

- The perfume industry relies heavily on steam distillation to extract volatile aromatic compounds from flowers, leaves, and seeds. The extracted bioactive compounds are then blended to create perfumes and other fragrance products.

4. Food Industry:

- In the food industry, steam distillation is used to extract flavor compounds from spices, herbs, and citrus peels. The resulting extracts are used as natural flavoring agents in food and beverages.

5. Environmental Science:

- Steam distillation is also used in the extraction of environmental contaminants, such as volatile organic compounds (VOCs), from soil and water samples for analysis.

Advantages of Steam Distillation

Steam distillation offers several advantages, making it a preferred method for extracting bioactive molecules from natural sources:

1. Mild Operating Conditions:

- One of the most significant advantages of steam distillation is that it allows the extraction of thermally sensitive bioactive compounds at lower temperatures. This prevents the decomposition or degradation of heat-sensitive compounds, preserving their biological activity.

2. No Use of Organic Solvents:

- Unlike solvent extraction methods, steam distillation does not require organic solvents. This is particularly advantageous for the production of food-grade and pharmaceutical-grade extracts, where solvent residues can pose safety concerns.

3. High Purity of Extracts:

- The immiscibility of volatile bioactive molecules with water allows for easy separation of the desired compounds from the aqueous phase, leading to high-purity extracts.

4. Cost-Effective:

- Steam distillation is relatively simple and inexpensive compared to other extraction methods. It requires minimal specialized equipment and is suitable for large-scale industrial applications.

5. Scalability:

- The steam distillation process is easily scalable, making it suitable for both small-scale laboratory extractions and large-scale commercial operations.

6. Environmental Friendliness:

- Since steam distillation does not require harmful chemicals and relies primarily on water and heat, it is considered an environmentally friendly method. Additionally, the spent plant material can often be repurposed or composted, reducing waste.

Limitations of Steam Distillation

Despite its advantages, steam distillation has several limitations that must be considered, particularly when dealing with non-volatile or heat-sensitive compounds.

1. Not Suitable for Non-Volatile Compounds:

- Steam distillation is only effective for extracting volatile bioactive compounds. Non-volatile compounds, such as certain alkaloids, flavonoids, or large molecular weight bioactive molecules, cannot be extracted using this method.

2. Potential Decomposition of Heat-Sensitive Compounds:

- Although steam distillation operates at lower temperatures than the boiling points of most compounds, some sensitive bioactive molecules may still degrade or undergo chemical changes during the process, especially if high temperatures are used.

3. Limited Solubility of Some Bioactive Compounds:

- Some bioactive molecules may have limited solubility in the steam phase, which can result in incomplete extraction or loss of compounds.

4. High Water Consumption:

- Steam distillation requires significant amounts of water for the generation of steam and for cooling in the condenser. This can be a concern in areas with limited water resources or in large-scale operations where water usage must be minimized.

5. Energy-Intensive:

- The process of generating steam and maintaining

the temperature for long periods can be energy-intensive. This increases operational costs, particularly in continuous distillation processes where energy conservation is important.

Modifications and Variants of Steam Distillation

Several variants of steam distillation have been developed to address some of its limitations and improve its efficiency. These include:

1. Vacuum Steam Distillation:

- In vacuum steam distillation, the pressure in the distillation chamber is reduced, which lowers the boiling point of water and the bioactive compounds. This allows the extraction of heat-sensitive compounds at even lower temperatures, reducing the risk of thermal degradation.

2. Hydrodistillation:

- In hydrodistillation, the plant material is immersed directly in boiling water, and the steam generated from the boiling water carries the volatile compounds to the condenser. This method is often used for plants with high moisture content.

3. Cohobation:

- In cohobation, the aqueous phase (hydrosol) from the condensation is returned to the distillation chamber to increase the yield of essential oils. This technique is particularly useful when dealing with plants that produce small amounts of essential oil.

4. Fractional Steam Distillation:

- In fractional steam distillation, the distillation process is carried out in stages to separate different fractions of bioactive compounds based on their boiling points. This allows for the isolation of specific compounds from complex mixtures.

Supercritical Gases

Supercritical gas extraction

Supercritical fluid

A supercritical fluid can be defined as a form of matter in which the liquid and gaseous phases are indistinguishable. Supercritical fluids are having more densities comparable to liquids. As a result, these fluids have solvating power. Supercritical fluid exhibits physicochemical properties intermediate between those of liquids and gases. Both liquid-like and gas-like characteristics of supercritical fluids make them unique for chemical separation. In particular, supercritical fluid densities, diffusivities, and viscosities fall into ranges between those of liquids and gases.

Available supercritical fluid

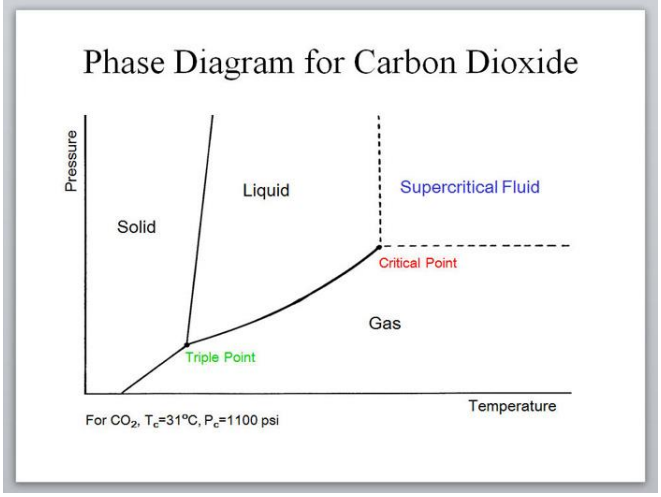
The most popular SFE solvent is carbon dioxide. It is inexpensive, non-flammable, relatively nontoxic, low critical temperature and commercially available even at high purity. The SFE solvent supercritical CO_2 have extraction conditions above the critical temperature of 31°C and critical pressure of 74 bar. Supercritical CO_2 is having density of around 200 bar pressure is close to that of hexane.

The solvation characteristics are also similar to hexane since it acts as a non-polar solvent. Around the supercritical region, CO_2 can dissolve triglycerides at concentrations up to 1% mass. Other SFE solvents used are nitrous oxide (laughing gas), nitrogen, propane, ammonia, fluoroform, freons, and water.

Super critical fluid extraction (SFE)

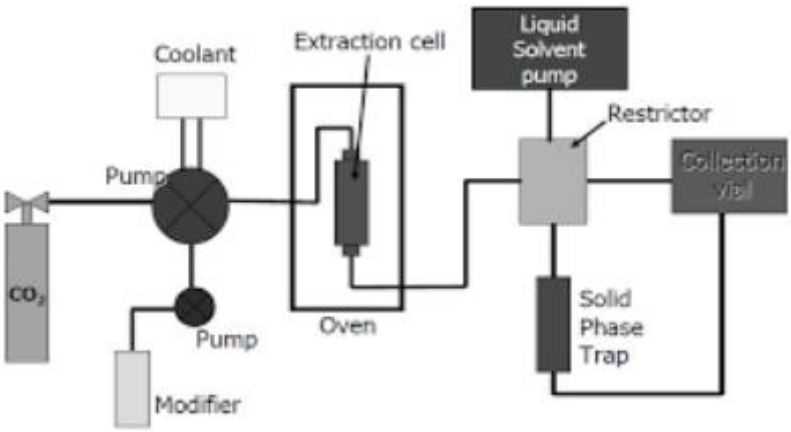
Supercritical Fluid Extraction (SFE) SFE can be defined as the process of segregating one component from the matrix by using supercritical fluids as the solvent. Extraction is usually done from a solid matrix, but also possible from liquids. SFE is useful as a sample preparation step (for analytical purposes) or to strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). In SFE, the

mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase solvating power. The process begins with CO_2 in vapour form. It is then compressed into a liquid before becoming supercritical. While supercritical, the extraction takes place. Supercritical Fluid Extraction (SFE) System extracts chemical compounds using supercritical carbon dioxide instead of an organic solvent. The supercritical fluid state occurs when a fluid is above its critical temperature (T_c) and critical pressure (P_c), when it is between the typical gas and liquid state Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it. The sample is placed in an extraction vessel and pressurized with CO_2 to dissolve the sample. Transferred to a fraction collector, the contents are depressurized and the CO_2 loses its solvating power causing the desired material to precipitate. The condensed CO_2 can be recycled. In SFE, the applications of supercritical carbon dioxide was having biggest interest, because it has a near ambient critical temperature (310C), thus biological materials can be processed at temperatures around 350C. The advantage here is that with a slight reduction in temperature or a slightly larger reduction in pressure can lead to precipitation of the entire solute. In addition, supercritical fluids can extract a product with minimal solvent residues. The solvation characteristics of supercritical CO_2 can be modified by the addition of an entrainer like ethanol.



Supercritical Fluid Extraction- Instrumentation

The instrumentation required to perform a successful SFE is commercially available. The process begins with a clean source of fluid, which in most cases is a high-pressure cylinder of CO2. A pump is used to increase the pressure of the fluid above its critical pressure.



The working extraction pressure is determined by the density required to dissolve the target analytes from the sample. The sample is contained in the extraction chamber, which is heated to the desired extraction temperature above the critical point. The pressurized fluid is brought to temperature by the chamber and allowed to flow through the sample matrix to extract the analytes. After the sample, the analyte fluid flows to a restrictor, this controls the flow rate of the fluid. The restrictor maintains the high pressure of the fluid in the chamber. At the restrictor, the supercritical fluid loses its solvating strength as its pressure drops to atmosphere. After the restrictor, the analytes can be collected for analysis.**Advantages of SFE**

· **Environmental safety**: SFE is a substitute to liquid extraction which uses organic solvents such as hexane or dichloromethane. There is always chance of solvent residue in the extract and matrix and there is always some level of environmental contamination from their use. Whereas carbon dioxide is easy to remove simply by reducing the pressure, leaving almost no trace, and it is also environmentally benign. The use of SFE with CO2 is also approved by the Soil Association for organic products. The CO2 used is largely a by-product of industrial processes or brewing, and its use in SFE does not cause any extra emissions.

· **Selectivity**: By changing the pressure and the temperature, the solvent strength of a supercritical fluid can be altered. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. By SFE, lipids can be removed using pure CO2 at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent.

· **Speed**: It is a fast process and completed in 10 to 60 minutes. It is a diffusion-based process, with the solvent required to diffuse into the matrix, and the extracted material to diffuse out of the matrix into the solvent.

· **Purity:** A supercritical fluid can be separated from an analyte by releasing pressure so that the product will be almost pure.

· **Recovery**: Recovery of analytes is simpler as compared to conventional techniques.

Applications:

SFE applications in the food, pharmaceutical, and fine chemical industries:

- Decaffeinating of coffee and tea
- Extraction of essential oils (vegetable and fish oils)
- Extraction of flavors from natural resources (nutraceuticals)
- Extraction of ingredients from spices and red peppers
- Extraction of fat from food products
- Fractionation of polymeric materials
- Extraction from natural products
- Photo–resist cleaning

Sonication

INTRODUCTION

Sonochemistry is the study of the chemical effects of ultrasound, which is sound with frequencies higher than the human audible range (>20KHz). The sound waves used in the sonication are usually generated by an ultrasonic probe which converts electrical energy into mechanical vibrations. These vibrations produce the high frequency sound waves that interact with a medium. This process also called Ultrasound Assisted Extraction (UAE)

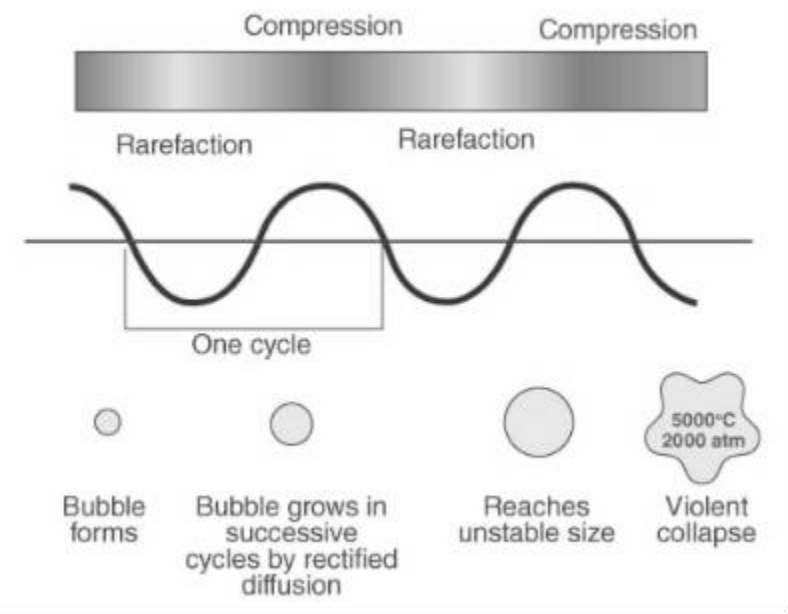
FREQUENCY RANGE

Low frequency: 20-40 KHz e.g. cleaning, degassing

Medium frequency: 40-80 KHz e.g. dispersion, emulsification

High frequency: 80-200 KHz e.g. cell distribution, nanomaterials synthesis

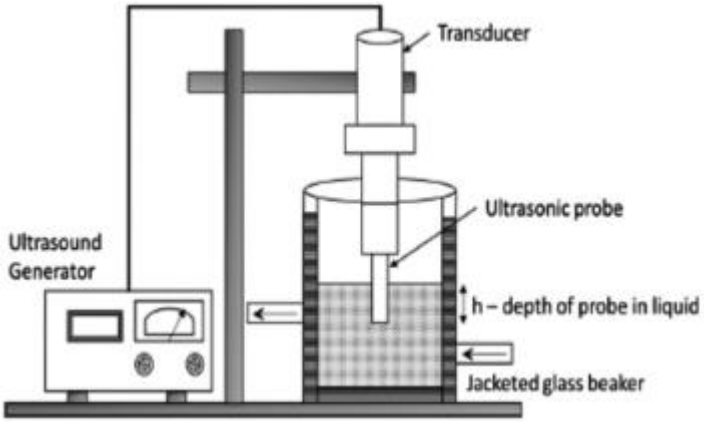
Very high frequency: 200-1000 KHz e.g. Advanced material processing



Sound waves

Principle of UAE

Ultrasonic energy waves transfer energy to the sonicated solvents (containing solid particles) by cavitation (generation of small vacuum bubbles or voids in the liquid medium) resulting in localized high temperature and pressure, this increased mechanical stress on the cell, thinning of cell membrane, cell distribution, increase permeability of the cell wall, facilitate solvent migration into the cell, mass transfer and the solubility of analyte in the solvent of improves.



Schematic diagram of UAE

ADVANTAGES

- Sonochemistry can reduce reaction times from days or hours to minutes.
- Sonochemistry can produce higher yields.
- Sonochemistry can operate at an average temperature close to ambient.
- Sonochemistry allows for precise control over the reaction location.
- Sonochemistry has many applications, including the synthesis of composites for energy storage, biomedical devices, and water sensors.

DISADVANTAGES

- Mechanical stress from ultrasound waves can damage equipment.
- Uniform ultrasound distribution is challenging at large scales.
- High-intensity ultrasound generates noise, posing health risks and requiring noise protection.

Micro Waves Assisted Extraction

Microwave assisted extraction (MAE) is a green approach to an analytical technique in which microwave radiation of frequency ranging from 0.915 to 2.45 GHz is used for the extraction of chemical compounds, especially from plant materials. This technique has become a new tool for organic synthesis and in the extraction of biological matrices for the preparation of analytical samples.

Microwave-assisted extraction is a process of using microwave energy to heat solvents in contact with a sample in order to separate analytes from the sample matrix into the solvent. This technique is used for the extraction of organic compounds from solid samples. Previously microwave ovens are used for the digestion of samples for metal analysis.

All microwaves, whether they are found in the home or the laboratory, operate at one frequency i.e. 2.45 GHz, even though in practice, the microwave region exists at frequencies of 100 GHz to 300 MHz(or wavelengths from 0.3mm to 1m). There are two types closed and open vessel MAE

SELECTION OF SOLVENT

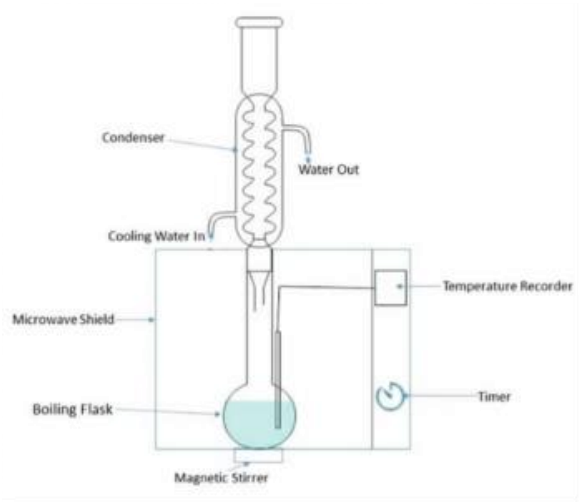
The selection of an organic solvent for microwave-assisted extraction (MAE) is essential, the solvent must be able to absorb microwave radiation and thereby becomes hot. The ability of an organic solvent to be useful for MAE can be assessed in terms of its dielectric constant, the larger the value of the dielectric constant, the better the organic solvent's ability to become hot.

Common organic solvents used in MAE are acetone, Acetonitrile, Dichloromethane, Hexane, methanol, etc.

PRINCIPLE

- Microwave radiation
- Moisture heated up
- Moisture evaporates
- Generation of tremendous pressure on cell wall
- Swelling of plant cell
- Rupture of the cell
- Leaching out the phytoconstituents

This phenomenon can be intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave.



Schematic diagram of MAE

MICROWAVE OVEN DESIGN

- **Magnetron -** For the generation of microwave energy
- **Wave guide -** For the propagation of microwave energy from the source of cavity
- **Cavity -** For placing the extraction vessel contains samples to be extracted
- **Circulator –** For reflecting and homogenization of radiation (for uniform radiation)

OPEN-VESSEL MAE:

- Extraction occurs in an open vessel or flask.
- Microwaves heat the solvent and sample directly.
- Volatile compounds can escape, which may lead to losses.
- Risk of overheating and boiling over.
- Typically used for small-scale extractions and method development.

CLOSED-VESSEL MAE:

- Extraction occurs in a sealed vessel or pressurized container.
- Microwaves heat the solvent and sample, but the vessel prevents volatile compounds from escaping.
- Higher pressure and temperature can be achieved, enhancing extraction efficiency.
- Reduced risk of overheating and boiling over.
- Typically used for larger-scale extractions and industrial applications.

ADVANTAGES

- Time saving as the extraction process can be completed from few seconds to 20 min
- Low solvent consumptions (few ml)
- Improves extraction yield

- Accuracy and precision
- Suitable for thermolabile phytoconstituents

DISADVANTAGES

- Risk of overheating and degradation of target compounds
- Equipment costs
- Safety concerns

Factors affecting the Choice of Extraction Process

The choice of extraction method for bioactive compounds is influenced by several key factors. These factors play a crucial role in determining the efficiency, selectivity, and overall success of the extraction process. Below are the primary factors that affect the selection of an appropriate extraction technique:

1. Nature of the Bioactive Compound

- **Polarity**: The polarity of the target compound greatly influences the choice of solvent and extraction method. Polar compounds (e.g., alkaloids, flavonoids) require polar solvents like water or ethanol, while non-polar compounds (e.g., lipids, essential oils) are best extracted using non-polar solvents like hexane or supercritical CO_2.
- **Volatility**: Volatile compounds like essential oils are best extracted using steam distillation or supercritical fluid extraction, whereas non-volatile compounds might require solvent extraction or Soxhlet extraction.
- **Thermal Stability**: Heat-sensitive bioactive molecules (e.g., proteins, vitamins, certain alkaloids) require gentle extraction methods such as cold pressing or low-temperature extraction to prevent degradation.

2. Plant Matrix or Source Material

- **Plant Material Composition**: The structure and composition of the plant material (e.g., leaves, roots, seeds, or bark) can affect the efficiency of the extraction process. For example, tough materials like roots may need to be pre-treated (e.g., grinding) to break down the cell walls for effective extraction.

- **Moisture Content**: High moisture content in the plant material may affect solvent extraction, necessitating drying or using a hydrodistillation method for essential oil extraction.

- **Particle Size**: Reducing the particle size (e.g., grinding) increases the surface area for the solvent to contact the bioactive compound, improving the extraction yield.

3. Selectivity of the Extraction Method

- **Specificity for Target Compounds**: Some methods are highly selective for certain bioactive compounds. For example, supercritical fluid extraction with CO_2 is ideal for non-polar compounds, while maceration or percolation might be used when broader extraction is acceptable. The goal is often to minimize the co-extraction of unwanted substances (e.g., chlorophyll, waxes).

- **Purity and Yield**: If high purity is required, methods like solvent extraction followed by purification techniques such as chromatography may be chosen. If yield is more important than purity, less selective methods like maceration might suffice.

4. Solvent Selection

- **Solvent Polarity**: The solubility of the bioactive compound in the solvent is crucial. Polar solvents (e.g., ethanol, methanol) are used for extracting polar compounds, while non-polar solvents (e.g., hexane) are chosen for lipophilic compounds.

- **Solvent Toxicity and Safety**: In food, pharmaceutical, and cosmetic industries, the solvent must be non-toxic or easily removable to avoid contamination of the final product. Water, ethanol, and supercritical CO_2 are preferred for their safety profiles, while organic solvents like hexane or methylene chloride may be used with caution.

- **Environmental Impact**: Green extraction techniques, such as supercritical fluid extraction or water-based extractions, are increasingly preferred due to concerns over the environmental impact of using organic solvents.

5. Cost and Feasibility

- **Cost of Equipment**: Some extraction techniques, like supercritical fluid extraction or microwave-assisted extraction, require expensive and specialized equipment, making them less accessible for small-scale or low-budget operations.

- **Operating Costs**: The overall cost of the extraction process includes energy consumption, solvent costs, labor, and time. Solvent extraction methods using inexpensive solvents like ethanol or methanol are more cost-effective than techniques requiring expensive gases or solvents.

- **Scalability**: Certain methods, such as Soxhlet extraction, are easy to scale up for industrial use, whereas techniques like ultrasonication or microwave-assisted extraction may require specialized setups that are harder to scale.

6. Time and Efficiency

- **Extraction Time**: Some methods, like maceration or Soxhlet extraction, may require long extraction times (hours or even days), while modern techniques like ultrasonic-assisted or microwave-assisted extraction can significantly reduce the processing time.

- **Efficiency**: Methods that provide high efficiency in a shorter time are often preferred in industrial settings. For instance, supercritical fluid extraction and microwave-assisted extraction offer high efficiency in terms of solvent use and time, which can reduce overall costs.

7. Temperature Sensitivity

- **Thermal Stability of Compounds**: Heat-sensitive compounds (e.g., certain vitamins, essential oils) can be degraded at high temperatures. In such cases, cold extraction methods like cold pressing, maceration at room temperature, or low-temperature supercritical CO_2 extraction are preferred.

- **Energy Consumption**: Methods that require high temperatures (e.g., Soxhlet extraction) consume more energy, making them less ideal for sensitive compounds and costly for large-scale production.

8. Regulatory Considerations

- **Food and Pharmaceutical Regulations**: In the pharmaceutical and food industries, extraction methods must comply with regulations regarding solvent use, purity, and final product safety. For example, water or ethanol-based extractions are generally preferred for food-grade or pharmaceutical products because they are considered safe.

- **Residual Solvent Content**: Regulatory limits on residual solvents (e.g., the FDA or EMA guidelines) influence the choice of solvents and methods. Non-toxic solvents like water and CO_2 are often chosen to meet regulatory standards.

9. Sustainability and Environmental Impact

- **Eco-Friendly Methods**: There is a growing emphasis on green chemistry, where the environmental impact of the extraction process is minimized. Solvent-free or low-energy extraction methods such as supercritical CO_2 extraction, water-based extractions, and enzymatic extractions are gaining popularity for their sustainability.

- **Waste Minimization**: The generation of solvent waste and the disposal of plant residues can impact the choice of extraction method. Techniques that allow solvent recycling or reduce solvent consumption are often preferred.

10. End-Use of the Extracted Product

- **Pharmaceutical vs. Cosmetic vs. Food Applications**: The intended application of the extracted bioactive compound plays a significant role in choosing the extraction method. For example, extracts for pharmaceutical use may require very high purity and specific activity, while food or cosmetic applications may tolerate lower purity or co-extraction of other components.

- **Formulation Requirements**: Some extraction methods yield concentrated or crude extracts, while others may provide a more purified product. The form of the final product (e.g., oils, powders, tinctures) determines the method used and the solvents involved.

UNIT-III

Drugs containing Terpenoids and volatile oils:

Terpenoids:

There are many different classes of naturally occurring compounds. Terpenoids also form a group of naturally occurring compounds majority of which occur in plants, a few of them have also been obtained from other sources. Terpenoids are volatile substances which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, citrus and eucalyptus.

The term 'terpene' was given to the compounds isolated from terpentine, a volatile liquid isolated from pine trees. The simpler mono and sesqui terpenes are chief constituent of the essential oils obtained from sap and tissues of certain plant and trees. The di and tri terpenoids are not steam volatile. They are obtained from plant and tree gums and resins. Tertraterpenoids form a separate group of compounds called 'Carotenoids'.

The term 'terpene' was originally employed to describe a mixture of isomeric hydrocarbons of the molecular formula $C_{10}H_{16}$ occurring in the essential oils obtained from sap and tissue of plants, and trees. But there is a tendency to use more general term 'terpenoids' which include hydrocarbons and their oxygenated derivatives. However the term terpene is being used these days by some authors to represent terpenoids.

By the modern definition: "Terpenoids are the hydrocarbons of plant origin of the general formula $(C_5H_8)n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives."

Classification

Most natural terpenoid hydrocarbon have the general formula $(C_5H_8)n$. They can be classified on the basis of value of n or number of carbon atoms present in the structure.

S.No.	Number of carbon atoms	Value of n	Class
1.	10	2	Monoterpenoids($C_{10}H_{16}$)
2.	15	3	Sesquiterpenoinds($C_{15}H_{24}$)
3.	20	4	Diterpenoids($C_{20}H_{32}$)
4.	25	5	Sesterpenoids($C_{25}H_{40}$)
5.	30	6	Troterpenoids($C_{30}H_{48}$)
6.	40	8	Tetraterpenoids($C_{40}H_{64}$)
7.	>40	>8	Polyterpenoids$(C_5H_8)n$

Each class can be further subdivided into subclasses according to the number of rings present in the structure.

i) Acyclic Terpenoids: They contain open structure.

ii) Monocyclic Terpenoids: They contain one ring in the structure.

iii) Bicyclic Terpenoids: They contain two rings in the structure.

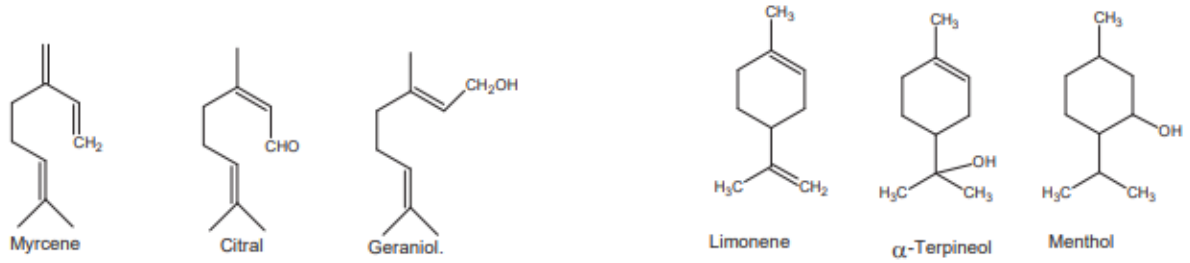
iv) Tricyclic Terpenoids: They contain three rings in the structure.

v) Tetracyclic Terpenoids: They contain four rings in the structure.

Some examples of mono, sesqui and di Terpenoids:

A) Mono Terpenoids:

i) Acyclic Monoterpenoids **ii) Monocyclic monoterpenoids**



Myrcene Citral Geraniol. Limonene α-Terpineol Menthol

iii)Bicyclic monoterpenoids: These are further divided into three classes.

a) Containing -6+3-membered rings

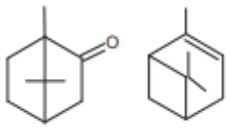
b) Containing -6+4- membered rings.

c) Contining -6+5-membered rings.

Thujane Carane Pinane Bornane (Camphane) non bornane (iso camphane)

Containing -6+3-membered rings -6+4-membered rings -6+5-membered rings

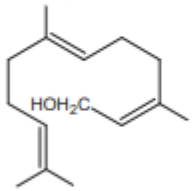
Some bicyclic monoterpenes are:



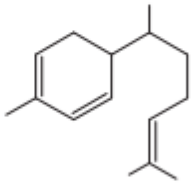
camphor α-pinene

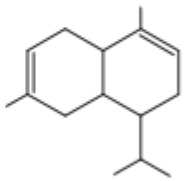
B) Sesquiterpenoids:

i) Acyclic sesquiterpenoids **ii) Monocyclic sesquiterpenoids** **iii) Bicyclic sesquiterpenoids.**



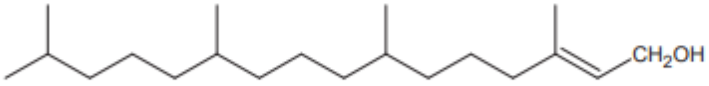
Farnesol


Zinziberene


Cadinene

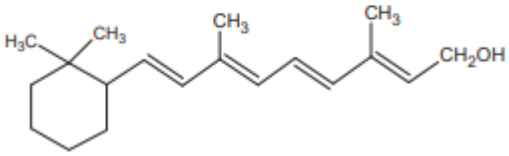
C) Diterpenoids:

i) Acyclic diterpenoids



Phytol

ii) Mono cyclic diterpenoids:

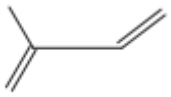


Vitamin A

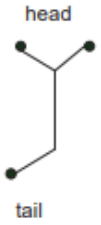
Isoprene rule

Thermal decomposition of terpenoids give isoprene as one of the product. Otto Wallach pointed out that terpenoids can be built up of isoprene unit.

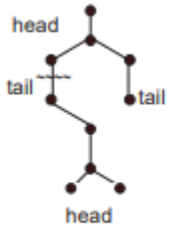
Isoprene rule stats that the terpenoid molecules are constructed from two or more isoprene unit.

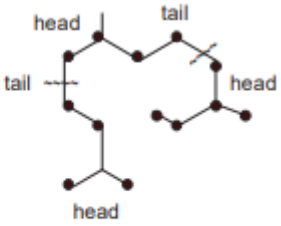

isoprene unit

Further Ingold suggested that isoprene units are joined in the terpenoid via ‘head to tail’ fashion. Special isoprene rule states that the terpenoid molecule are constructed of two or more isoprene units joined in a ‘head to tail’ fashion.

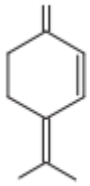


But this rule can only be used as guiding principle and not as a fixed rule. For example carotenoids are joined tail to tail at their central and there are also some terpenoids whose carbon content is not a multiple of five. In applying isoprene rule we look only for the skeletal unit of carbon. The carbon skeletons of open chain monotrpenoids and sesqui terpenoids are

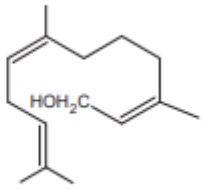




Examples.

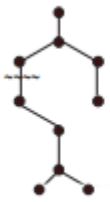


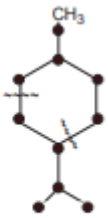
Myrcene
(monoterpene)



Farnesol (Sesquiterpene)

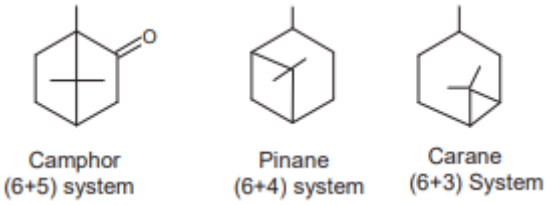
Ingold (1921) pointed that a gem alkyl group affects the stability of terpenoids. He summarized these results in the form of a rule called ‘gem dialkyl rule’ which may be stated as "Gem dialkyl group tends to render the cyclohexane ring unstable where as it stabilizes the three, four and five member rings.” This rule limits the number of possible structure in closing the open chain to ring structure.Thus the monoterpenoid open chain give rise to only one possibility for a monocyclic monoterpenoid i.e the p-cymene structure.





P-cymene structure

Bicyclic monoterpenodis contain a six member and a three member ring. Thus closure of the ten carbon open chain monoterpenoid gives three possible bicyclic structures.



Isolation and separation techniques

As a terpenoids are of wide occurrence, there is no general method their isolation. But mono and sesqui-terpenoids have common source that is essential oils. Their isolation is carried out in two steps:

- Isolation of essential oils from plant parts.
- Separation of terpenoids from essential oils.

Isolation of essential oils from plant parts:

The plant having essential oil usually have the highest concentration at some particular time. Therefore better yield of essential oil plant material have to be collected at this particular time. e.g. From jasmine at sunset.

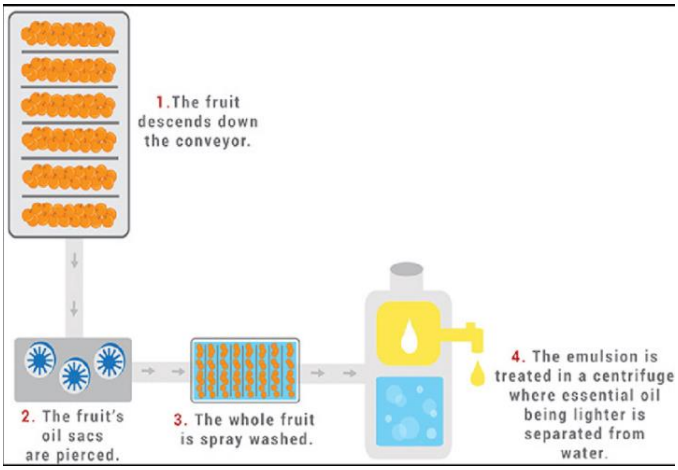
There are four methods of extractions of oils

a) Expression method
b) Steam distillation method
c) Extraction by means of volatile solvents
d) Adsorption in purified fats (enflurage)

a) **Expression method**

The plant material is crushed and the juice is screened to remove the large particles. The screened juice is centrifuged in a highspeed centrifugal machine when nearly half of essential oil is extracted. The other half of the oil is generally not extracted and such residue is used for inferior quality of oil.

Citrus, lemon and grass oils are extracted by this method.

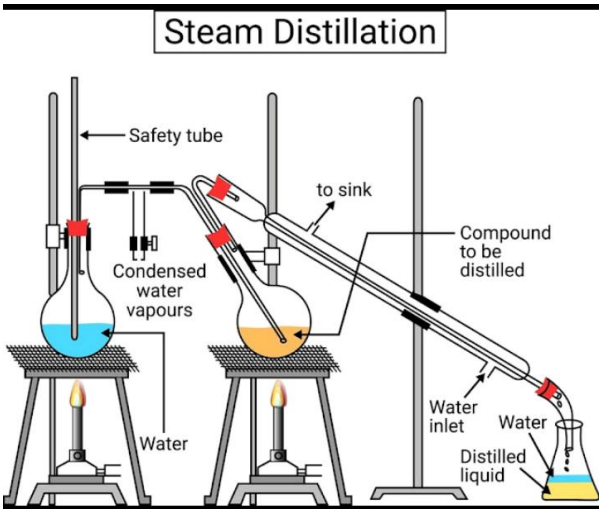


B) Steam distillation method

In this method macerated plant material is steam distilled to get essential oils into the distillate form are extracted by using pure organic volatile solvents, like light petroleum

Method should be used with a great care, since some essential oils are decomposed during distillation.

In such cases plant material is directly treated with light petrol at 50 degree and solvent is then removed by distillation under reduced pressure.



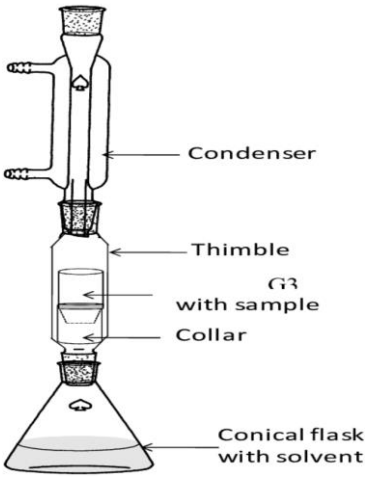
C) Extraction by means of volatile solvent

Solvents like hexane & ethanol is used to isolate essential oils.

It is used for the plant parts have low amount of essential oil.

Plant material are treated with solvent it produces a waxy aromatic compound called "concrete". Then it mixed alcohol the oil particles are released.

Then it passes through a condenser then it separated out. This oil is used in perfume industry or for aromatherapy purposes.



d)Adsorption in purified fats (enfleurage)

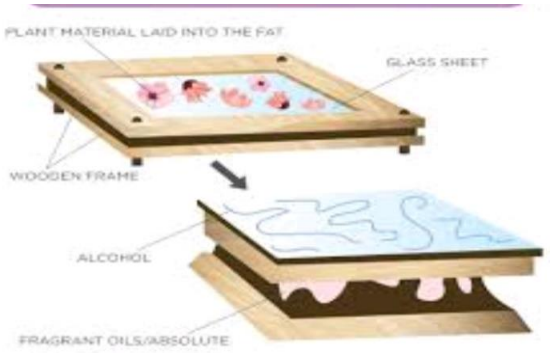
Purified fat is warmed at 50 degree on a glass plate. Surface of the fat is covered with flower petals kept the petals for several days; after that old petals are replaced with fresh petals.

These process is repeated until we will get the saturated fat then the saturated fat is digested with Ethanol; all the oil present in the fat is dissolved and small amount of fat is dissolved and small amount of fat will also be dissolved.

Fat can be removed by cooling the ethanolic extract at 20 degree(fat separates out) .

Now remaining Ethanolic part is fractionally distilled to remove. Ethanol at reduced pressure.

Now a days coconut charcoal is used in the place of fat due to greater stability and lighter absorptivity.



II. Separation of Terpenoids from essential oil

The various physical methods are as follows

I) Fractional distillation method:

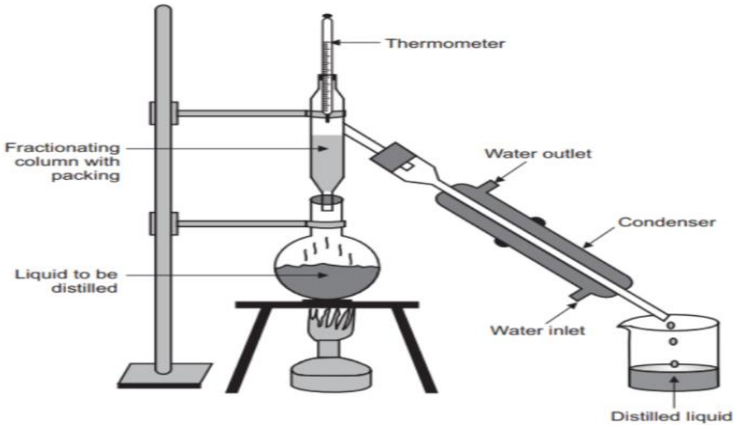
The various terpenoids present in essential oils are separated by Fractional distillation method.

The terpenoid- hydrocarbons distil over first followed by the oxygenated derivatives.

Distillation of the residue under reduced pressure yields the sesquiterpenoids and these are separated by fractional distillation.

On an industrial scale, especially designed stills are employed and an efficient condensing system is necessary to minimize loss of more volatile hydrocarbons.

Many times, the fractional distillation has to be carried out under reduce pressure and in presence of an inert gas.



ii) Chromatography:

Chromatography has been widely used both for isolation and separation of terpenoids.

In adsorption chromatography, the essential oil is made to flow through a particular adsorbent when the different types of terpenoids are adsorbed at different places on the adsorbent to from different chromatograms.

Then, the various chromatograms as eluted by different solvent system to get different elutes.

Each elute is then subjected separately to adsorption chromatography when different bands due to the various terpenoids present in elute are obtained which are then eluted to yield different terpenoids.

Vapour phase chromatography, partition chromatography and counter current separation method have been used for the separation of terpenoid.

General properties Camphor

Camphor:

Camphor is a waxy, colourless solid with a strong aroma. It is classified as a terpenoid and a cyclic ketone. It is found in the wood, barks of (**CINNAMON CAMPHORA**) a large evergreen tree found in East Asia; and in the kapur tree, a tall timber tree from South East Asia. It also occurs in some other related trees in the laurel family, notably *Ocotea usambarensis*. Rosemary leaves (*Rosmarinus officinalis*) contain 0.05 to 0.5% camphor, while camphorweed (*Heterotheca*) contains some 5%.A major source of camphor in Asia is camphor basil (the parent of African blue basil). Camphor can also be synthetically produced from oil of turpentine.

The compound is chiral, existing in two possible enantiomers as shown in the structural diagrams. The structure on the left is the naturally occurring (+)-camphor ((1*R*,4*R*)-bornan-2-one), while its mirror image shown on the right is the (−)-camphor ((1*S*,4*S*)-bornan-2-one). Camphor has few uses but is of historic significance as a compound that is readily purified from natural sources.

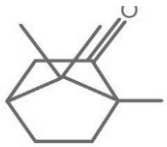
Camphor belongs to a group of organic compounds which are cyclic mono terpenoid ketones. Camphor is also termed as 2-Camphanone.

Production of Camphor:

- Camphor is naturally produced by trees and is processed for commercial use.
- Methylation with methyl iodide and a complicated reduction procedure produced camphor.
- Camphor can be produced from alpha-pinene. It is present in coniferous trees.

Structure of Camphor:

Camphor is made of carbon, hydrogen, and oxygen. The chemical formula of Camphor is $C_6H_{16}O$. It is a bicyclic monoterpene ketone. It is colorless in nature and it is a waxy colorless solid. It is highly flammable. It has a very strong smell. The smell of camphor is similar to that of a mothball. The IUPAC name of Camphor is 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one. It belongs to a category of organic compounds called terpenoid ketones.



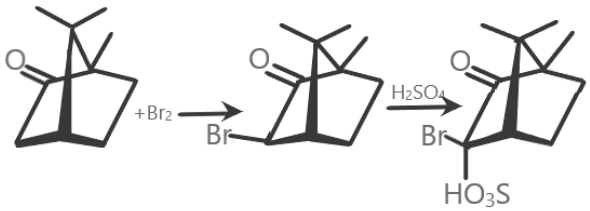
Extraction of Camphor Oil:

Physical Properties of Camphor:

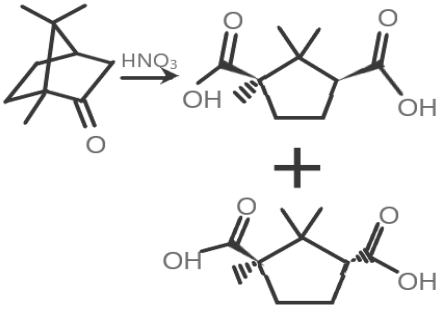
- The molecular formula of camphor is C6H16O.
- It is mostly found as a white waxy solid.
- The molecular weight of camphor is 154 amu.
- The density of camphor is 0.992
- Camphor has a pungent aromatic taste.
- It has a mothball-like smell.

Chemical Properties of Camphor:

- The boiling point of Camphor is around 177 0 C.
- The melting point of camphor is 2090 C.
- It is soluble in oils, and ethanol but not in Water.
- When Camphor reacts with Bromine in the presence of sulfuric acid, it undergoes bromination to produce (+)-9-bromocamphor and (-)-9-bromocamphor.



- When camphor undergoes oxidation it produces dicarboxylic acid known as camphoria.



Uses of Camphor

- It is an important ingredient of many creams or rubs whose aim is to reduce pain caused by insect bites, cold sores, minor burns, etc.
- It helps reduce fungal infections.
- It is sometimes used as a food additive to enhance the flavor of the food.
- It is used for some religious purposes.
- Camphor is used to reduce pains caused by arthritis.
- Camphor can be used as a cough suppressant.
- Camphor can be used as a muscle rub to reduce muscle cramps.

Pharmacological potential

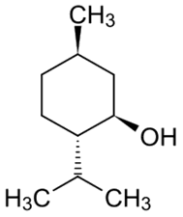
Osteoarthritis- A rub on cream containing camphor, glucosamine sulfate, Chondroitin Sulfate seems to reduce severity of symptoms of osteoarthritis by about half.

Toenail fungus- Early research shows applying camphor along with lemon eucalyptus oil. Menthol to toenail area helps to treat fungal infections.

Health Hazards of Camphor

- When Camphor comes in direct contact with the eyes it causes irritation.
- Inhaling of camphor acid can cause irritation in the nose and throat which leads to a cough.
- Continues Exposure to a high quantity of camphor causes Unconsciousness or even death.
- High Exposure to camphor may cause Kidney damage and has adverse effects on the nervous system.

Menthol:



Menthol is an organic compound, more specifically a mono terpenoid made synthetically or obtained from the oils of corn mint, peppermint, or other mints. It is a waxy, clear or white crystalline substance, which is solid at room temperature and melts slightly above. Menthol is a chemical naturally found in peppermint and other mint plants, but it can also be made in a lab. First added to tobacco in the 1920s and 1930s, menthol reduces the harshness of cigarette smoke and the irritation from nicotine.

Isolation Process:

- Take weighed quantity of coarse powder of leaves**.**

Extract oil by water or steam distillation.

- Separate the oil from water by using separating funnel and allow cooling.
- Crystals of menthol will separates out.
- Collect the crystals by centrifugation.
- Recrystallize menthol using acetone or other low boiling point solvent to which menthol having solubility.

General Properties of Menthol:

- Menthol occurs as white bold or fine crystalline granular substance with a peppermint like taste and odour.

- Menthol is slightly soluble in water but freely soluble in alcohol, chloroform, ether, glacial acetic acid, petroleum ether.
- It should be preserved in tight containers preferably at controlled room temperature.

Applications:

1. Cooling effect of menthol

Menthol is well known for its cooling effect; where it is inhaled, chewed, consumed. It have the ability to chemically activate the cold sensitive transient receptor potential cation channel.

2. Biological properties of menthol

Peppermint tea and peppermint essential oil have high content of menthol which are been used in traditional medicine to treat various conditions including infections, insect repellants.

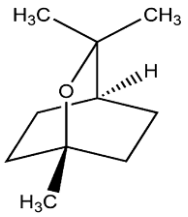
In vitro and in vivo studies have documented biological properties of menthol such as it is used as analgesic, anti-bacterial, anaesthetic actions.

3. Role in medicine and food industry

Menthol has found diverse applications in field ranging from pharmaceutical, cosmetic industries and in tobacco and food industry which been used as flavours which enhances preservative and cooling characteristics

Eucalyptol

Eucalyptol, (genus *Eucalyptus*), large genus of more than 660 species of shrubs and tall trees of the myrtle family (Myrtaceae), native to Australia, Tasmania, and nearby islands. In Australia the eucalypti are commonly known as gum trees or stringybark trees. Many species are cultivated widely throughout the temperate regions of the world as shade trees or in forestry plantations. Economically, eucalyptus trees constitute one of the most valuable groups within the order Myrtales.



Chemical Feature: Rich in the oxide 1,8 cineole syn eucalyptol and monoterpenes.

Extraction of Eucalyptol Oil

Eucalyptol can be extracted by means of microwave assisted or Ultrasound assisted extraction techniques.

- ✓ Collection of leaves
- ✓ Drying
- ✓ Crushing
- ✓ Pulverised form
- ✓ Steam distillation
- ✓ Eucalyptol oil can be extracted

Pharmacological Potential

Antibacterial Activity

50% ethanol extract of eucalyptol globulus leaves have anti-bacterial activity against oral pathogens. The leaves extract show excellent antibacterial activity against staphylococcus aureus streptococcus pneumonia from200 clinical specimens of patients with respiratory tract disorders.

Diuretic Activity

Plant part of eucalyptus oil is made to an emulsion made up shaking up equal part of oil and powerded gumaeabic with water used to treat urethral infection.

Wound Healing Activity

Intradermal administration of essential oils from leaves of eucalyptus and seeds of sesali indicum increases cutaneous capillary permeability when tested in Evans blue treated rabbit.so it is beneficial in wound healing activity.

Volatile Oils

Volatile oils, commonly known as essential oils, are concentrated plant extracts obtained through various methods of extraction. These oils capture the distinctive fragrance and therapeutic properties of the plant and are widely used in aromatherapy, cosmetics, and herbal medicine.

Methods of Preparation

1. Distillation

- Steam Distillation: The most common method, where steam passes through plant material, causing the volatile compounds to evaporate. The steam is then cooled to condense the vapor back into liquid, separating the essential oil from the water.

- Water Distillation: Similar to steam distillation but involves boiling the plant material directly in water. This method can be less efficient and may alter some oil properties.

- Dry Distillation: Plant materials are heated without water, causing them to release their essential oils. This method is less common and can be used for certain types of wood or resin.

2. Cold Pressing:

- This method is primarily used for citrus fruits. The outer peel is mechanically pressed to release the essential oils, which are then collected. Cold pressing preserves the oil's fragrance and therapeutic properties.

3. Solvent Extraction:

- In this method, plant materials are treated with a solvent (like hexane or ethanol) to dissolve the essential oils. The solvent is then evaporated, leaving behind a concentrated oil. This method is often used for delicate flowers that cannot withstand heat.

4. CO_2 Extraction:

- Supercritical CO2 extraction uses carbon dioxide at high pressure and low temperature to extract essential oils. This method is highly efficient and preserves the integrity of the oil, but it requires specialized equipment.

5. Enfleurage:

- An old technique mainly used for delicate flowers. Fresh flowers are placed in fat or oil, allowing them to release their fragrance. After several days, the flowers are replaced, and the fat is then processed to extract the essential oils. This method is labor-intensive and rarely used today.

Classifications of Volatile Oils

Essential oils can be classified based on various criteria:

1. Botanical Origin:

- Single Oils: Oils derived from one plant species (e.g., lavender oil, tea tree oil).

- Blend Oils: Mixtures of different essential oils, formulated for specific therapeutic effects (e.g., stress relief blends).

2. Chemical Composition:

- Terpene-Based Oils: Contain a high concentration of terpenes (e.g., limonene in citrus oils).

- Aldehyde-Based Oils: Characterized by aldehydes, often used for their antimicrobial properties (e.g., citronella oil).

- Phenol-Based Oils: Contain phenolic compounds, known for their antiseptic and anti-inflammatory properties (e.g., clove oil).

- Alcohol-Based Oils: Rich in alcohols that can be soothing and antiseptic (e.g., lavender oil).

3. Functional Properties:

- Antimicrobial Oils: Oils known for their ability to inhibit the growth of bacteria, viruses, or fungi (e.g., eucalyptus oil, tea tree oil).

- Relaxing Oils: Oils that promote relaxation and reduce stress (e.g., chamomile oil, bergamot oil).

- Stimulating Oils: Oils that invigorate or energize (e.g., peppermint oil, rosemary oil).

4. Plant Family:

- Many essential oils can be grouped according to their botanical family, such as:

  - Lamiaceae (Mint Family): Basil, peppermint, and rosemary.

  - Asteraceae (Daisy Family): Chamomile and geranium.

  - Rutaceae (Rue Family): Citrus oils like lemon and orange.

5. Extraction Method:

- Steam Distilled Oils: Most commonly produced oils.

- Cold-Pressed Oils: Primarily citrus oils.

- Solvent Extracted Oils: Often used for delicate floral scents.

Camphor oil

Camphor oil is a volatile essential oil extracted from the wood of the camphor tree (*Cinnamomum camphora*), as well as from other sources like the leaves. It is well-known for its distinctive aroma and numerous medicinal properties.

Structure

Camphor ($C_{10}H_{14}O$) is a bicyclic monoterpene ketone. Its structure features:

- **Bicyclic Framework**: Camphor consists of two fused rings.
- **Functional Groups**: It contains a carbonyl group (C=O) which classifies it as a ketone, contributing to its characteristic properties.

Method of Preparation

Camphor oil is typically prepared through the following methods:

Steam Distillation:

Source Material: Fresh leaves and branches of the camphor tree or the wood itself.

Process: The plant material is subjected to steam distillation. Steam passes through the plant material, causing the camphor to vaporize. The vapor is then condensed back into liquid form, where the essential oil is separated from water.

Solvent Extraction:

In some cases, a solvent can be used to extract camphor from the plant material, although this method is less common for camphor oil compared to steam distillation.

Uses

Camphor oil has a wide range of applications:

Medicinal Uses:

Topical Analgesic: It is commonly used in ointments and balms to relieve pain and reduce inflammation.

Antiseptic: Camphor oil has antimicrobial properties and can be used in some antiseptic formulations.

Respiratory Relief: It is used in cough syrups and chest rubs to alleviate cough and nasal congestion.

Aromatherapy:The strong scent of camphor oil is utilized in aromatherapy for its calming and revitalizing effects. It can help in reducing stress and anxiety.

Cosmetic Products: Camphor oil is often found in creams and lotions for its cooling effect and fragrance.

Insect Repellent: Its strong odor is effective in repelling insects, making it useful in some pest control formulations.

Traditional Medicine: In traditional practices, particularly in Ayurveda and traditional Chinese medicine, camphor is used for various ailments, including digestive issues and skin disorders.

Geranium oil

❖ Geranium oil mostly occurred in stems and leaves of Pelargonium roseum and it is belonging to the family of Geraniaceae.

❖ Geranium oil extracted through steam distillation method and it is used as fragrance, flavor and pharmaceutical industries,

❖ The common name of geranium oil is rose scented Pelargonium.

❖ Other species of geranium plant are Pelargonium Graveolens, Pelargonium Capitatum, Pelargonium Odoratissium linn.

❖ It is indigenous to Cape Province in South Africa and cultivated in various countries like Italy, France, Spain, Morocco, Algeria, Russia, India. In India it is found in south area like Nilgiris hills.

❖ Indian geranium oils obtained from other species and is known as Palmarosa oil.

❖ All varieties of geranium generally contain 0.08-0.4% of fragrant volatile oil.

❖ It contains two types of constituents namely, alcohols and esters.

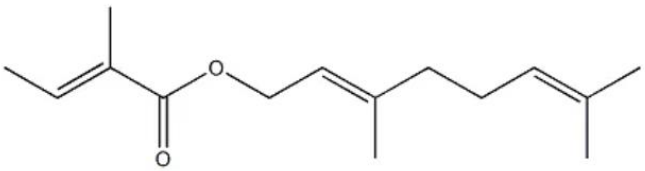
❖ The alcohols are geraniol and beta citronellol contribute about 60-70% while the esters namely geranyl tiglate, citronellyl formate, citronellyl acetate contribute about 20-30% of oil and several sesquiterpenes alcohols are also reported in the oil and are responsible for pleasant fragrance.

❖ Geranium is one of the important aromatic plants, yielding an essential oil which is highly priced for its profound and strong rose like odor.

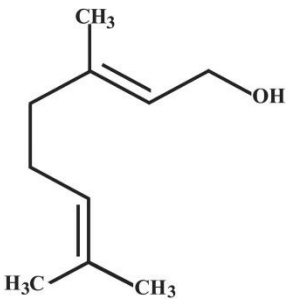
❖ Among the various parts of geranium plant, the leaves are strongly rose scented.

❖ The molecular formula of geranium oil is $C_{15}H_{24}O_2$.

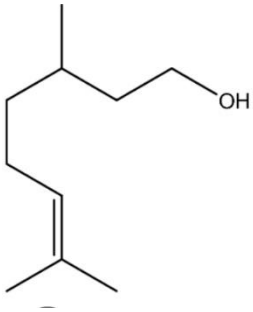
❖ The molecular weight of geranium oil is 236.349 and its boiling point is approximately 250° C



Structure of geranium oil



Geraniol



β-citronellol

Uses of Geranium Oil:

❖ Geranium oil used in creams, lotions, soaps and other products due to its astringent properties.

❖ It is used in alcoholic and non- alcoholic beverages, candies and diary products at 0.001% for flavoring.

❖ The oil is used in treatment of inflammation with its mild soothing effect.

❖ It is used as stimulant of the adrenal cortex and can be used to balance the production of androgens which occurs during menopause period.

❖ It is also used as good insect repellant.

❖ It is widely used for fragrance purpose in various perfumes, odorant for tooth and dusting powders, ointments etc.…

❖ Geranium oil historically been used in the treatment of dysentery, hemorrhoids, heavy menstrual flows.

❖ The French medicinal community currently used for treatment of diabetes, diarrhea, gall bladder problems, gastric ulcers, jaundice, liver problems, sterility and urinary stones.

❖ The leaves of geranium are also used in herbal teas.

❖ It can be added in shampoos and conditioners to promote healthy looking hair and scalp as well as to balance oil production.

❖ It has been studied for its potential anti-bacterial and anti-fungal effects, which may help in wound healing and preventing infections.

❖ It is also used to relief from shingles pain within minutes.

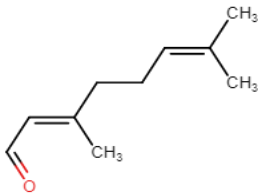
Citral

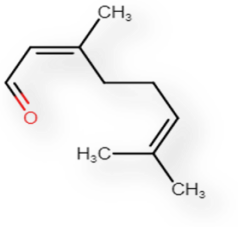
- Citral is an acyclic monoterpenoid.
- It is a major constituent of lemon grass oil in which it occurs to an extent of 60-80%.
- It is pale yellow liquid having strong lemon like odour, boiling point 224-228 ^{o}C and can be obtained by fractional distillationunder reduced pressure from Lemongrass oil.

Isomerism of citral

Two geometrical isomers occur in nature. The ***cis*-isomers** is known as **Citral-a** and ***trans*-isomers Citral-b**.

Ordinary citral obtained from lemongrass oil is, in fact, mixture of**Citral-a (90%)** and **Citral-b (10%)**.

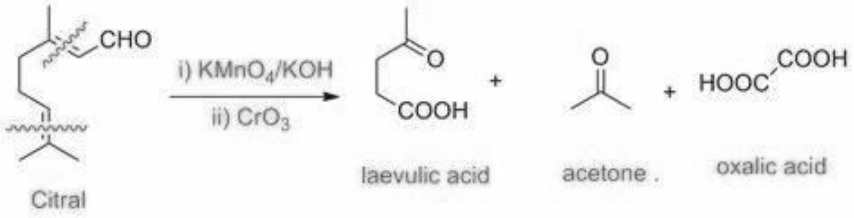




Neral Geranial

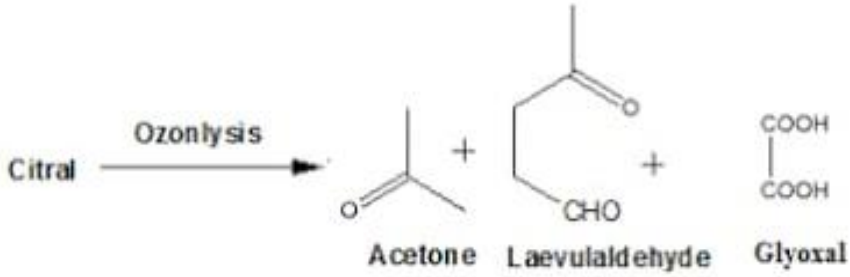
***Position of C=C bonds indicate*:**

Oxidation of citral with alkaline KMnO4, gives **Acetone**, **Levulinic acid** and **Oxalic acid**.



On ozonlysis;

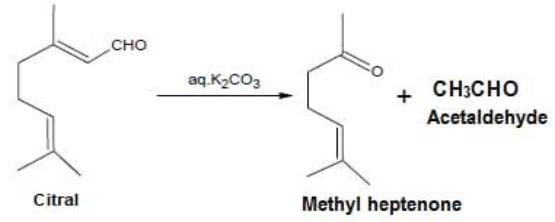
It gives acetone, laevulaldehyde.



Formation of above products shows that citral is an acyclic compound containing two double bonds. Corresponding saturated hydrocarbon of citral (mol. Formula $C_{10}H_{22}$) corresponds to the general formula $C_nH_{2n}+2$ for acyclic compounds, indicating that citral must be an acyclic compound.

Formation of p-cymene and product obtained from the ozonolysis reveals that citral is formed by the joining of two isoprene units in the head to tail fashion.

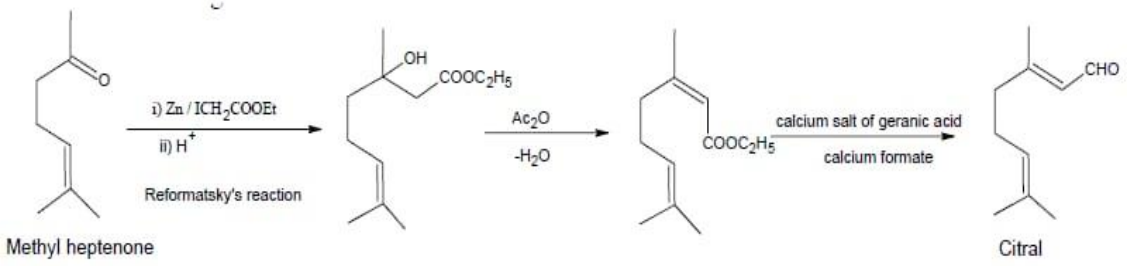
Citral on boiling with aqueous potassium carbonate yielded 6-methyl hept-5-ene-2-one (Methylheptenone) and acetaldehyde.
The formation of these can only be explained on the basis of proposed structure;



It appears that citral is product of aldol condensation of these two.

Barbier-Bouveault-Tiemann's synthesis:

In this synthesis methyl heptenone is converted to geranic ester by using Reformatsky's reaction. Geranic ester is then converted to citral by distilling a mixture of calcium salts of geranic and formic acids.



Uses:

It is extensively used in perfume and flavour industry and manufactures of vitamin A.

Citral can be obtained from myrcene. It is generally found in the peel of the orange. Because of their intense aroma and flavour are used in beverages, perfumes, cosmetics, and food. Essential oils that present citral shows antifungal, antimicrobial, antiparasitic features that make it a natural preservative.

Citral is widely used in aromatherapy due to its uplifting and invigorating properties. It is believed to have a calming effect on the mind and body, helping to reduce stress and anxiety. Citral is often used in essential oil blends for diffusers, massage oils, and room sprays.

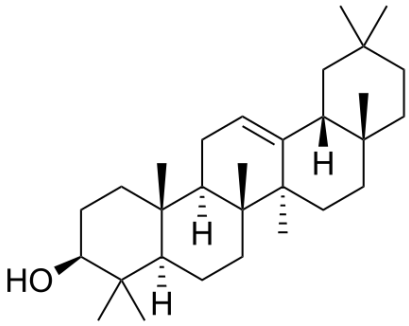
Citral is a volatile oil that is used in a variety of applications, including as a flavor and fragrance agent, and as a therapeutic agent. It is also used in the manufacture of cosmetics and other personal care products.

Recently it become as a drug for reducing blood pressure.

Pentacyclic triterpenoids:

Amyrines

1. The amyrines are three closely related natural chemical compounds of the triterpene class.
2. They are designated α-amyrines (ursane skeleton), β-amyrinesoleanane skeleton) and δ-amyrines.
3. Each is a pentacyclic triterpenol with the chemical formula $C_{30}H_{50}O$.
4. They are widely distributed in nature and have been isolated from a variety of plant sources such as epicuticular wax.
5. . In plant biosynthesis, α-amyrin is the precursor of ursolic acid and β-amyrin is the precursor of oleanolic acid
6. All three amyrines occur in the surface wax of tomato fruit. α-Amyrines is found in dandelion coffee**.**

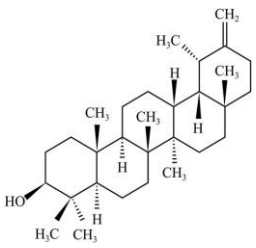


Pharmcological Applications:

1. **Anti-inflammatory:** Amyrines have been shown to possess anti-inflammatory properties, making them potential therapeutic agents for treating inflammatory diseases.
2. **Antimicrobial:** Amyrines exhibit antimicrobial activity against various microorganisms, including bacteria, fungi, and viruses.

3. **Antioxidant:** Amyrines have antioxidant properties, which can help protect against oxidative stress and cell damage.
4. **Anticancer:** Some studies suggest that amyrines may have anticancer properties, inhibiting the growth of cancer cells.
5. **Neuroprotective:** Amyrines may have neuroprotective effects, potentially helping to prevent or treat neurodegenerative diseases.
6. **Hepatoprotective:** Amyrines have been shown to protect the liver against damage and toxicity.
7. **Immunomodulatory:** Amyrines may have immunomodulatory effects, influencing the immune system's response.
8. **Antidiabetic:** Some research suggests that amyrines may have antidiabetic properties, helping to regulate blood sugar levels.
9. **Wound healing:** Amyrines may aid in wound healing by promoing tissue repair and regeneration.
10. **Cosmetic application :** Amyrines are used in some cosmetics and skincare products due to their antioxidant and anti-inflammatory properties.

Taraxasterol: Structure and pharmacological applications



Taraxasterol is a triterpenoid compound found in various plants, particularly in the dandelion (*Taraxacum officinale*). It has gained interest for its potential pharmacological applications and health benefits.

Structure

- **Chemical Formula**: $C_{33}H_{54}O$
- **Structure**: Taraxasterol has a pentacyclic structure characteristic of triterpenoids, which includes five interconnected rings. It features a hydroxyl group (-OH) at the C3 position, contributing to its biological activity.

Pharmacological Applications

Taraxasterol has been studied for several pharmacological properties, including:

Anti-Inflammatory Effects: Research indicates that taraxasterol can inhibit the production of pro-inflammatory cytokines, making it potentially useful in treating inflammatory conditions.

Antioxidant Activity: Taraxasterol exhibits strong antioxidant properties, helping to scavenge free radicals and reduce oxidative stress, which is linked to various chronic diseases.

Hepatoprotective Effects: Studies have shown that taraxasterol may help protect the liver from damage due to toxins and oxidative stress, suggesting its potential in treating liver diseases.

Antitumor Activity: Preliminary studies indicate that taraxasterol may inhibit the growth of certain cancer cell lines, showing potential as an anticancer agent. Its mechanism may involve inducing apoptosis (programmed cell death) in cancer cells.

Antimicrobial Properties: Taraxasterol has demonstrated activity against various bacteria and fungi, suggesting potential applications in combating infections.

Cardiovascular Benefits: Some studies suggest that taraxasterol may help improve lipid profiles and exert protective effects on the cardiovascular system.

Unit – IV

Drugs containing alkaloids

Alkaloids

Alkaloids are naturally occurring organic substances, predominantly found in plant sources including marine algae and rarely in animals (e.g. in the toxic secretions of fire ants, ladybugs and toads). They occur mostly in seed-bearing plants mainly in berries, bark, fruits, roots and leaves. Alkaloids often contain at least one nitrogen atom in heterocyclic ring. These are basic in nature and so referred the term alkaloid (alkali-like). Alkaloids possess remarkable physiological action on human and other animals. These are the active components of numerous medicinal plants or plant-derived drugs. Their structural diversity and different physiological activities are unique to any other group of natural products. Many drugs used by man for both medical and non medical purposes are produced in nature in the form of alkaloids e.g. atropine, strychnine, caffeine, nicotine, morphine, codeine, cocaine etc. Naturally occurring receptors for many alkaloids have also been identified in human and other animals, suggesting an evolutionary role for the alkaloids in physiological processes. Alkaloids are relatively stable compounds that accumulate as end products of different biosynthetic pathways, mostly starting from common amino acids such as lysine, ornithine, tyrosine, tryptophan, and others. These substances are usually colourless but several coloured alkaloids are also reported e.g. berberine is yellow, sanguinarine salt is copper-red and betanidin is red (Kokate et al., 2005). These are crystalline solids, having ring structure, definite melting points and bitter in taste. In plants they may exists in free state, in the form of salt or as N-oxides, rarely found in the form of glycosides (Biswas and Sharia, 1978; Tanahashi et al., 2000; Kashiwaba et al., 2000). In addition to the elements carbon, hydrogen and nitrogen, most alkaloids contain oxygen. A few such as coniine from hemlock and nicotine from tobacco are oxygen free. The free bases are sparingly soluble in water but readily soluble in organic solvents, however with their salts, the reverse is often the case, e.g. strychnine hydrochloride is more soluble in water than in organic solvents. Most of the alkaloids are optically active, generally due to the presence of tertiary nitrogen in their structures. This results the various isomeric forms having different physical, chemical and pharmacological properties e.g. (+)-tubocurarine isolated from Chondrodendron tomentosum (Bisset, 1992), have muscle relaxant activity, whereas its leavo isomer is less active.

Occurrence

Alkaloids are generally occur in all parts of the plant but some times accumulated only in particular organ, whereas at the same time other organs are free from alkaloids e.g. the edible tubers of potato plant are devoid of alkaloids, whereas the green parts contain the poisonous alkaloid solanine. The organ in which alkaloids accumulated is not always the site of their synthesis, e.g. in tobacco, nicotine is produced in the roots and translocated to the leaves where it accumulates (Harborne and Herbert, 1995). After the isolation of first alkaloid narcotine by French apothecary Derosne in 1803 and morphine by Hanoverian apothecary Serturner in 1806, more than ten thousand alkaloids have been discovered from different sources (Evans, 2006). Alkaloids are commonly found in the orders Centrospermae, Magnoliales, Ranunculales, Papaverales, Rosales, Rutales, Gentiales, Tubiflorae and Campanulales. True alkaloids are rarely occur in lower plants. Among the Pteridophytes and Gymnosperms, the bioactive alkaloids lycopodium, ephedra and taxus are well known. Lysergic acid and sulphur containing alkaloid gliotoxin are best known examples isolated from fungi. Nearly 300 alkaloids belonging to more than 24 classes are found in the skin of amphibians along with other toxins (Evans, 2006). The poisonous neurotoxic alkaloids were isolated from the skin of frogs belonging to genus Phyllobates. Daly, (1993) isolated various antimicrobial alkaloids from the skin of reptilian. Some indole and isoquinoline alkaloids were isolated from mammals including mammalian morphine.

Function of Alkaloids in Plants

The functions of alkaloids in plants are mostly unknown and their importance in plant metabolism has been debated. A single plant species may contain over one hundred different alkaloids and their concentration can vary from a small fraction to as much as 10% of the dry weight. Most alkaloids are very toxic and therefore, have the potential function in the chemical defence arsenal of plants against attack by herbivores and micro-organisms (Levin, 1976; Levin and York, 1978). For example, nicotine present in tobacco leaves inhibits the growth of tobacco hornworm larvae. Nicotine in pure form is also applied as an effective insecticide in greenhouses. In addition, alkaloids have been suggested to serve as a storage form of nitrogen or as protectants against damage by ultraviolet light. Some Phytochemists suggested that alkaloids are by-products of normal plant metabolism. It has been suggested that alkaloids may have a role in the defence of the plant against singlet oxygen ($1O_2$), which is damaging to all living organisms and is produced in plant tissues (Larson and Marley,

1984; Larson, 1988) in presence of light. It is also thought that alkaloids may provide a means of defence against insects and animals. Alkaloids may also be a reservoir (Wink and Michael, 1999) for molecules that plants often use.

Pharmaceutical Applications

Alkaloids showed quite diverse medicinal properties. Many of them possess local anesthetic properties, but their practical use is limited for clinical purpose. Morphine is one of the most known alkaloids which had been used and still is for medical purposes. This alkaloid is a powerful narcotic which is used for the relief of pain, but its usefulness is limited because of addictive properties.

Methyl ether derivative of morphine—codeine—naturally occurring next to morphine in the opium poppy, possesses an excellent analgesic activity and is shown to be relatively nonaddictive. These alkaloids act as respiratory or cardiac stimulants. Next, the alkaloid which is used as medication in many clinical applications is atropine. For example, injection with atropine is given to treat bradycardia (low heart rate).

Tubocurarine is an alkaloid, is an ingredient of poison curare, and is used in surgery as muscle relaxant. Alkaloids vincristine and vinblastine are used as chemotherapeutic agent in the treatment of many cancer types. Cocaine an alkaloid present in *Erythroxylum coca* is a potent local anesthetic. Ergonovine, an alkaloid from the fungus *Claviceps purpurea*, and the second alkaloid ephedrine isolated from *Ephedra* species both act as blood vessel constrictors. Also, ephedrine is used in bronchial asthma and to relieve discomfort of hay fever, sinusitis, and common colds.

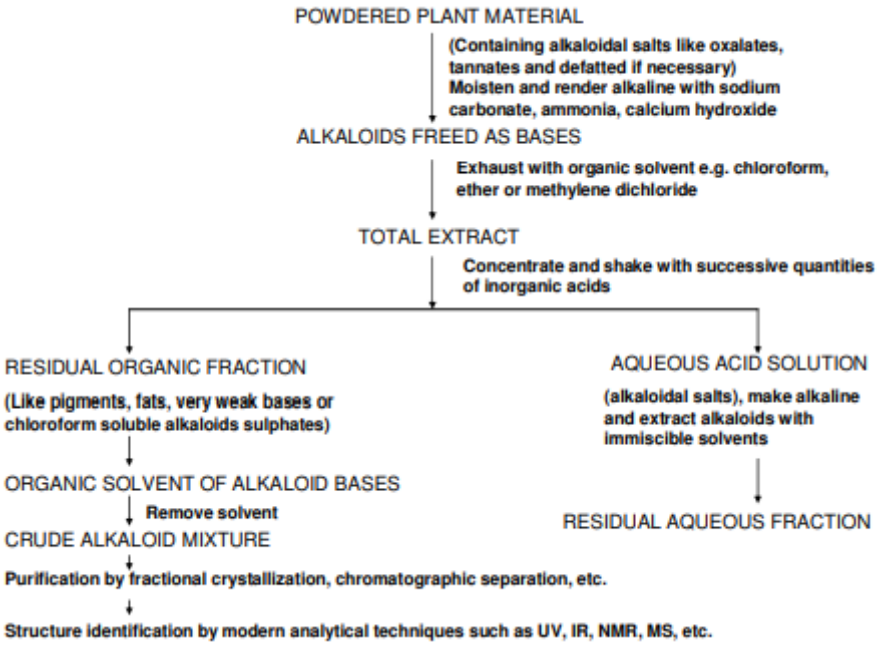
Quinine is a powerful antimalarial agent and more often is replaced by synthetic drugs, which are more effective and less toxic. Another alkaloid from *Cinchona* species is quinidine which has medical application as treatment of irregular rhythms of the heartbeat or arrhythmias.

Colchicine another alkaloid, present in plants of Liliaceae family, known for ages to treat acute gout attacks. Another clinically used alkaloid is lobeline isolated from *Lobelia inflata*, which has multiple mechanisms of action.

Isolation

The extraction of alkaloids is based on their basic character and solubility pattern. The general scheme for extraction is shown in Fig. 6. Extraction is usually served by one of the following general methods;

1. The plants are defatted with petroleum ether, especially in case of seeds and leaves to remove the fat soluble constituents and then with polar solvents. The extract is concentrated under reduced pressure and treated with alkali so that the free bases convert in their salts and separated with organic solvents. This process is known as Stas-Otto process. This method is frequently used in the extraction of ergotamine (Kokate et al., 2005) from ergot.
2. The powdered material is moistened with water and mixed with lime, which combines with acids, tannins and other phenolic substances and sets free the alkaloid salts. Extraction is then carried out with organic solvents such as ether or petroleum sprit. The concentrated organic liquid is then shaken with aqueous acid and allowed to separate. Alkaloid salts are now in aqueous liquid, while many impurities remain behind in the organic liquid.
3. The powdered material is extracted with polar solvents such as water or aqueous alcohol containing dilute acid. Pigments and other unwanted materials are removed by shaking with chloroform or other organic solvents. The free alkaloids are then precipitated by the addition of excess sodium bicarbonate or ammonia and then separated by filtration or by extraction with organic solvents.
4. The extract is treated with ammonia so as to convert the alkaloid salts into their free bases. Such liberated alkaloids in free base form are conveniently extracted with organic solvents like ether, benzene, chloroform etc. This method is not useful for the isolation of alkaloids of quaternary nitrogen.
5. The alkaloids present in the extract are converted into their reineckates by treating with $H[Cr(NH_3)_2(SCN)_4]$ (Reinecke's solution). The product is then dissolves in acetone and then passed this solution through an ion exchange column which afforded the alkaloids in a high state of purity.



Further purification of crude extract of alkaloids is done by following ways, which may, however vary for individual alkaloid.

1. Direct crystallization from solvent: It is a simple method of isolation in which the alkaloids crystallise directly by fractionation process and may not be useful in case of complex mixtures.

2. Steam distillation: This method is specially employed for volatile liquid alkaloids like coniine, sparteine and nicotine. However this method is not suitable for alkaloids of high molecular weights. In this method, the aqueous extract is made alkaline with caustic soda or sodium carbonate and then alkaloids are distilled off in steam.

3. Gradient pH technique: There is variation in the extent of basicity of various alkaloids of same plant. On the basis of this property, the crude alkaloid mixture is dissolved in 2% tartaric acid solution and extracted with benzene so that the first fraction contains neutral and/or very weakly basic alkaloids. The pH value of the aqueous solution is increased gradually by 0.5 increments to pH 9 and extraction is carried out at each pH with organic solvents. By this way, the alkaloids of different basicity are extracted and strongly basic alkaloids extracted at the end.

4. Chromatographic techniques: Chromatography is an ideal method for separation of a vast number of alkaloids. The separation of alkaloids carried out by using stationary and

mobile phase of different organic solvents. The different techniques of chromatography used for separation of individual alkaloid from complex mixture are as following;

a. Paper chromatography (PC): This technique is simplest and most widely used among other chromatographic techniques because of its applicability to isolation, identification and some times quantitative determination of all type of natural products. It is a partition as well as an absorption type technique, in which the mobile phase is either individual or mixture of organic solvents and the stationary phase is hydrophilic surface of paper. The choice of the solvent used to run a chromatogram depends upon the nature of alkaloid. Usually non polar solvents like benzene/ acetone/ water are commonly used for separation of non polar alkaloids and polar solvents like butanol/ acetic acid/ water used for separation of polar alkaloids. Both one and two dimensional ascending and descending, some times horizontal chromatographic techniques applied using Whatman filter paper sheet of various types i.e. No.1, 2, 3 etc. The paper sheet is washed with 5% 2N HCl to remove the impurities before starts the separation. The paper is visualised under UV light of long wavelength or spread with iodoplatinate reagent in the form of coloured spots. The Rf values and colour appearance of spots on paper sheet are useful in preliminary identification of alkaloids by this technique.

b. Thin layer chromatography (TLC): TLC is an important tool extensively used for identification, separation and determination of the purity of isolated alkaloid. Although TLC method is used in qualitative analysis but it having a great importance in quantitative analysis also (Oswald and Fluck, 1964; 1964). The TLC separation of alkaloids can be performed on silica gel, alumina, cellulose powder or kieselguhr. Silica gel is the most active stationary phase and good separation is achieved even when several milligrams substance applied. This method is also used for preparative separation of alkaloids (Tschesche et al., 1963). In TLC, the substances are identified preliminary on the basis of their mobility in suitable solvent system and moreover on the basis of their reaction with selective or specific detection reagents or on the basis of their absorption or fluorescence in UV light (Macek, 1972; Hais, 1970; Neher, 1970). Some typical solvent systems (Stahl, 1969) are as following;

C_6H_6/ EtOAc/ $(C_2H_5)_2NH$ (5:4:1) + 8% MeOH	C_6H_6/ EtOAc/ $(C_2H_5)_2NH$ (5:4:1, 7:2:1)
$CHCl_3$/ $(C_2H_5)_2CO$/ $(C_2H_5)_2NH$ (7:2:1) + 8% MeOH	$CHCl_3$/ $(C_2H_5)_2CO$/ $(C_2H_5)_2NH$ (7:2:1)
$HCON(CH_3)_2$/ $(C_2H_5)_2NH$/ EtOH/ EtOAc (1:1:6:12)	$CHCl_3$/ MeOH/ AcOH (6:1:3)
C_6H_{12}/ $(C_2H_5)_2NH$ (9:1 or 1:1)	C_6H_6/ $CHCl_3$/ $(C_2H_5)_2CO$ (14:3:3)
$CHCl_3$/ EtOH (9:1 or 8:2)	$CHCl_3$/ $(C_2H_5)_2O$/ H_2O (7:1:2)
Xylene/ BuOH/ MeOH/ $(C_2H_5)_2NH$ (5:5:7:3)	C_6H_{14}/ CCl_4/ $(C_2H_5)_2NH$ (5:4:1)

Developed TLC plate is sprayed by several visualizing reagents for detection of alkaloids. Many alkaloids can be seen on the chromatogram even in daylight and a large number yield typical fluorescence colours in UV light (365 nm). The reagents most commonly used for detecting alkaloids are Dragendorff reagent, acidified iodine- iodine solution, iodoplatinate, antimony- (III)chloride, cerium sulphate in sulphuric acid and cerium sulphate in phosphoric acid. Papaverrubine type alkaloids give a red spot with hydrogen chloride vapour, Rauwolfia alkaloids give colour with mixture of ferric chloride and perchloric acid and also with iodine vapour, phenylalkylamines are visualised with ninhydrin. Beside these reagents, cinnamaldehyde hydrochloric acid for indole alkaloids, sulphuric acid for purine bases and betaines and the van Urk reagent for ergot alkaloids are often used.

Preparative TLC: In this method, comparatively thicker layer of adsorbent are employed for preparative work and the separated bands of compounds are scraped from the plate and subjected to solvent extraction. This technique generates sufficient quantity of material for complete spectral analysis. Preparative TLC was performed for cryptopleurine and piperidylacetophenone (AlShamma et al., 1982) on 1 mm thick commercial plates precoated with Al2O3 GF-254 developing with ammoniated CHCl3 and determined the Rf values.

c. Column chromatography (CC): This method is very popular and used extensively for quantitative separation and purification of different natural products from crude plant extract or mixture. Column of different size and dimensions are used for separation. Choice of stationary phase (adsorbents) and mobile phase (organic/ inorganic solvents) is based on the nature of components present in the crude extract. A large number of adsorbents are available which can be used in the column chromatography, some being better for one type of components and some for others. For the separation of alkaloid, silica gel (Yinggang et al., 2003; Roumy et al., 2006) and neutral alumina (Hart et al., 1968) are extensively used. Beside these, some more adsorbents are kieselguhr, silicates (magnesium/calcium silicate), tricalcium phosphate, calcium sulphate, glass power, salts of heteropoly- tungstic, molybdic and tetraboric acids, ferric and chromic oxides, zinc carbonate/ ferrocyanide, charcoal, zirconium phosphate, hydrous zirconium oxide, lanthanum oxide, bentonites, cellulose powder (including acetylated, ion exchanger), starch, sucrose, mannitol, dextran gels (sephadex), polyamides etc.

Eluotropic solvents used in column chromatography, in order of increasing polarity are petroleum ether, n-hexane, cyclohexane, carbon tetrachloride, trichloroethylene, benzene,

dichloromethane, chloroform, diethyl ether, diethyl formamide, ethyl acetate, pyridine, n-propanol, ethanol, methanol, formamide and water. Polarity and composition of solvents is depending upon the nature of compound to be eluted i.e. non-polar compounds elutes with nonpolar solvents whereas polar compounds elutes with polar solvents. Polarity is changed by observing the mobility of the compounds to be elutes. Some solvent systems are commonly used for the separation of alkaloids are as follows;

Petroleum ether- chloroform (1-100%), n-hexane- chloroform (1-100%), benzenechloroform (1-100%), methanol- chloroform (1-100%), methanol- ethyl acetate (1-100%), pet. etherethyl acetate- diethylamide (5:1:0.3-20:1:0.3)

d. Gas liquid chromatography (GLC): Gas chromatography separates volatile substances by percolating a gas stream over a stationary phase. It can be applied to almost any type of compound which has reasonably volatility and stable under the chromatographic conditions. GLC is the most selective and versatile form of gas chromatography, since there exists a wide range of liquid phases usable upto 450^{o}C. This method is applicable for the examination of alkaloids of opium, tobacco, coniine and belladonna. Modification of the sample structure may be accomplished by derivatisation and the most widely applied general derivatisation is silylation. The compound containing labile functional groups viz. alcohols, amines, carboxylic acids and thiols are converted into corresponding silylamines, esters and thioesters. The common derivatising agents used in GLC are trimethylchlorosilane, hexamethyldisilazone and N, O-bis-trimethylacetamide.

e. High performance thin layer chromatography (HPTLC): This method is very useful in both qualitative and quantitative analysis. It is a major advancement of TLC, which requires shorter time, saving reagents and better resolution. The basic difference between conventional TLC and HPTLC is only in particle and pore size of the sorbents. The linear development method is most familiar technique in HPTLC. The analytical profiles for tropine alkaloids and several other natural products i.e. flavonoids and steroids have been developed using this technique. HPTLC is conveniently applied for compilation of profiles pertaining to varied range of bioactive constituents such as berberine, quinine, opium alkaloids, colchicines, chelidonine, sanguinarine, serpentine, raubasine, asarone, elemicine, eugenol, thymole coumarine, pulegon, flavones, diosgenin, silymarin, catechin, anthraquinone derivatives, gibberellins, antibiotics and number of other compounds of natural origin. Using external standards, the detection limits of bulaquine, chloroquine, and primaquine by HPTLC (Saxena

et al., 2003) on silica plates were 0.25, 0.59 and 0.53 µg respectively while the lower limits of quantification were 0.52, 1.21 and 1.07 µg respectively with detection wavelength at 254 nm. The separation was achieved on precoated Silica gel 60 F254 (20×20 cm, 0.2 mm) plates using n-hexane/ diethyl ether/ methanol/ diethylamine (37.5:37.5:25:0.5, v/v) as solvent system. HPTLC is a sophisticated and automated form of TLC.

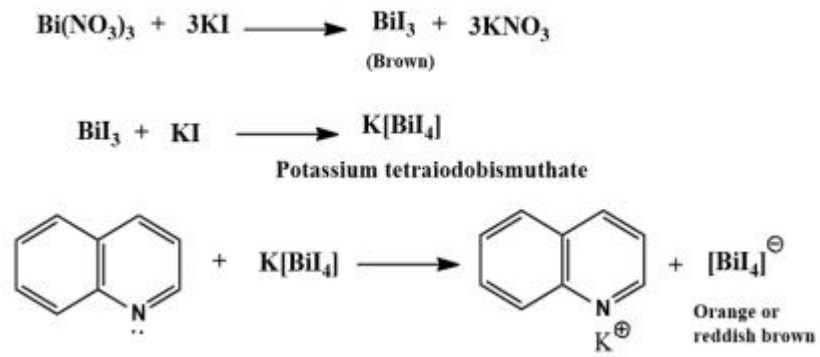
f. High performance liquid chromatography (HPLC): In HPLC method, the separation of one component from another is based upon their absorption of UV light and the functional group interaction with the analytical column substrate. The amount of caffeine in a sample of coffee may be determined to a high degree of accuracy using HPLC techniques. It has become the most versatile, safest, dependable, fastest and sensitive chromatographic technique for the quantitative and qualitative analysis of natural as well as synthetic compounds. The alkaloids like morphine, papaverine, codeine, emetine, antibiotics, ergot alkaloids etc. can be analysed by HPLC. Since the time of its invention in 1966 by Horvath and Lipsky, several natural products including several antibiotics (Cooper et al., 1973; Buhs et al., 1974; Wold et al., 1977) has been determined qualitatively as well as quantitatively successfully. Chloroquine, primaquine and bulaquine has resolved (Saxena et al., 2003) with the help of HPLC without no interference with retention times of 4.22, 5.72 and 17.26 min respectively by using RP C8 lichrospher 25 x 0.4 cm, 5µ column and the solvent was acetonitrile: sodium acetate buffer (pH 5.6) in ratio of 55:45 with detection wavelength 265 nm. The profile of berberine bridge enzyme catalyzed transformation of (S)-reticulin to (-)- scoulerine (Winkler et al., 2006) was carried out at four different time to determine the reaction progress. The series of common solvents used in this technique with relative decreasing order of polarity is acetic acid, ethylene glycol, glycerol, formamide, acetic anhydride, furfural, acetonitrile, methanol, ethanol, dimethyl formamide, propanol, iso-propanol, methyl ethyl ketone, butanol, cyclohexanol, pyridine, diethyl sulfoxide, methyl acetate, ethyl acetate, dioxane, tetrahydrofuran and chloroform.

Preliminary Qualitative Tests and General Properties

1)Dragendorff ’s test

Chemical Composition: Potassium bismuth iodide solution

Proposed reaction for Dragendroff's test

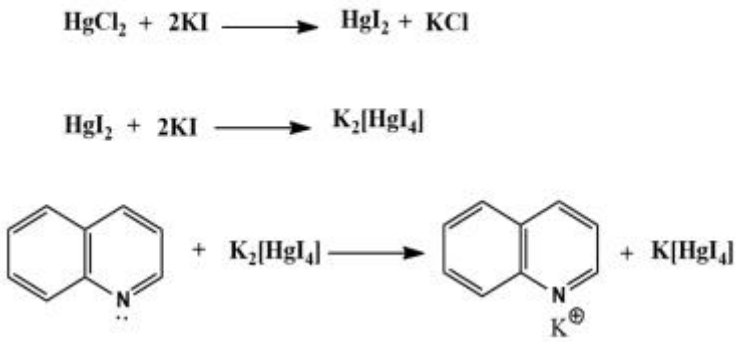


By adding 1 mL of Dragendorff"s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

2) Mayer's test.

Chemical Composition: Potassium mercuric iodide solution

Proposed reaction for Mayer test



Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

3) Hager's test.

Chemical Composition: Saturated solution of picric acid

Two milliliters of extract were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids.

Hager's reagent is a mixture of picric acid and sodium carbonate.

The reaction involves the formation of a colored precipitate when the reagent reacts with an alkaloid. The reaction is as follows:

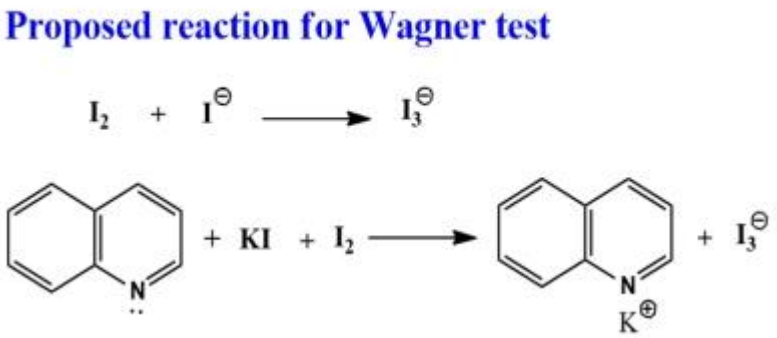
Alkaloid (R-NH_2) + Picric acid ($C_6H_3N_3O_7$) → Picrate salt $[C_6H_2N_3O_7\text{-R-}NH_3]^+$

In this reaction, the alkaloid molecule reacts with the picric acid molecule to form a picrate salt, which is the colored precipitate.

The R group represents the specific alkaloid molecule.

4) Wagner's reagent

Chemical Composition: Solution of iodine in potassium iodide



Color Obtained: Reddish-brown

5) Picrolonic acid:

Chemical Composition: Solution of picrolonic acid

Color Obtained: Yellow

The Picrolonic Acid test is a chemical test used to detect the presence of alkaloids. Here's the chemical reaction involved:

Picrolonic Acid ($C_{10}H_7NO_6$) + Alkaloid (R-NH_2) → Picrolonate Salt $[C_{10}H_6NO_6\text{-R-}NH_3]^+$

1. Protonation of the alkaloid:
R-NH_2 (alkaloid) + H^+ (proton) → R-NH_3+ (protonated alkaloid)

2. Reaction with Picrolonic Acid:

$R\text{-}NH_3$+ (protonated alkaloid) + $C_{10}H_7NO_6$ (Picrolonic Acid) → $C_{10}H_6NO_6\text{-}R\text{-}NH_3^+$ (picrolonate salt)

The resulting picrolonate salt is colored, indicating the presence of an alkaloid. The color of the salt varies depending on the specific alkaloid and reaction conditions.

6) Murexide test:

Chemical Composition: Potassium chlorate + HCl + NH_3

Color Obtained: Purple

Example: Caffeine

Reaction:

Alkaloid ($R\text{-}NH_2$) + Murexide ($C_8H_4N_6O_6$) + H^+ → Murexide-Alkaloid Complex (colored precipitate)

Mechanism:

1. Protonation of the alkaloid:

$R\text{-}NH_2$ (alkaloid) + H^+ (proton) → $R\text{-}NH_3^+$ (protonated alkaloid)

2. Formation of the Murexide-Alkaloid Complex:

$R\text{-}NH_3$+ (protonated alkaloid) + $C_8H_4N_6O_6$ (Murexide) → $C_8H_4N_6O_6\text{-}R\text{-}NH$

(Murexide-Alkaloid Complex)

The complex is stabilized by hydrogen bonding and electrostatic interactions between the alkaloid and Murexide molecules.

7) Mineral acids

Chemical Composition: Phosphotungstic acid, phosphomolybdic acid

Color Obtained: Yellow

Example: Colchicine

Reaction:

Alkaloid ($R\text{-}NH_2$) + Mineral Acid (HNO_3 or H_2SO_4) → Alkaloid Salt ($R\text{-}NH_3{+}X^-$) + Water

Mechanism:

1. Protonation of the alkaloid:

$R\text{-}NH_2$ (alkaloid) + H^+ (proton) → $R\text{-}NH_3^+$ (protonated alkaloid)

2. Formation of the alkaloid salt:

$R\text{-}NH_3^+$ (protonated alkaloid) + X^- (anion) → $R\text{-}NH_3{+}X^-$ (alkaloid salt)

The alkaloid salt is typically a crystalline solid, which can be isolated and identified

Common Mineral Acids Used:

- Nitric Acid (HNO_3)
- Sulfuric Acid (H_2SO_4)

8) Acidic p-methylaminobenzaldehyde

Chemical Composition: p-Methyl-aminobenzaldehyde and sulphuric acid

Color Obtained: Bluish-violet to red

Example: Indole

Reaction:

Alkaloid ($R\text{-}NH_2$) + p-Methylaminobenzaldehyde (C_8H_9NO) + HCl → Alkaloid-p-Methylaminobenzaldehyde Complex ($R\text{-}NH_3{+}C_8H_9NO{+}Cl^-$)

9) Nitric acid

Chemical Composition: Dilute Nitric Acid

Color Obtained: Orange-red

Example: Morphine

Alkaloid ($R\text{-}NH_2$) + HNO_3 (Nitric Acid) → Nitroalkaloid ($R\text{-}NO_2$) + H_2O

General Properties of Alkaloids:

Alkaloids are colourless, crystalline, non-volatile, solids; a few such as coniine and nicotine are liquids and a few even coloured, viz. berberine is yellow. The free bases (i.e. alkaloids themselves) are insoluble in water but soluble in most of the organic solvents. Most of the alkaloids are levorotatory (optically active), although a few are dextrorotatory (e.g. coniine), while a few are even optically inactive, viz. papaverine. Generally, the alkaloids are bitter in taste and have pronounced physiological activity.

Physical Properties of Alkaloids:

State:

- Most Alkaloids are crystalline solids.
- Few Alkaloids are amorphous solids. Eg: Emetine
- Some are liquids that are either Volatile (Eg: Nicotine) or Non-Volatile (Eg: Pilocarpine)

Color:

- The majority of alkaloids are odorless but some colored.

  Eg: Colchicine and Berberine are Yellow.

  Canadine is orange

  Betanedine is red.

Solubility:

- Both Alkaloidal bases and their Salts are soluble in Alcohol.
- Generally, the bases are soluble in organic solvents and insoluble in water.
- Bases soluble in Water: Caffeine, Ephedrine, Codeine, c[colchicine, Pilocarpine and Quaternery Ammonium Bases.
- Bases insoluble or sparingly soluble in certain organic solvents: Morphine in Ether, Theobromine and Theophylline in benzene.
- Salts are usually soluble in water and insoluble or sparingly soluble in organic solvents.
- Salt insoluble in Water: Quinine, monosulphate.

Chemical Properties of Alkaloids:

Alkaloids are amines, so their names usually end in "ine" (e.g., caffeine, nicotine, morphine, quinine). Most have complex chemical structures of multiple ring systems.

Oxygen:

- Most alkaloids contain Oxygen and are solid in nature. **Eg:** Atropine
- Some Alkaloids are free from Oxygen and are mostly liquids. **Eg:** Nicotine.

Stability:

Alkaloids are decomposed by heat, except Syrychonine and Caffeine (Sublimable).

Optical Activity:

- Alkaloids are optically active due to the presence of one or more asymmetric carbon atoms in their molecule.
- All are levorotatory.
- Optically active isomers show different physiological activities.
- Usually the I(-) isomer is more active than the d-isomer.
- **Eg**: I-ergotamine is 3-4 times more active than d-ergotamine.
- Exceptions: d-tubocurarine is more active that the corresponding I-isomer.

Medicinal Properties of alkaloids:

- The medicinal properties of alkaloids are quite diverse.
- Morphine is a powerful narcotic used for the relief of pain, though its addictive properties limit its usefulness.
- Codeine, the methyl ether derivative of morphine found in the opium poppy, is an excellent analgesic that is relatively nonaddictive.
- They can be used as, Analgestic & Narcotics. **Eg**: Morphine and Strychnine
- Used as CNS Stimulants. **Eg**: Caffeine & Strychine
- Used as Anticancers. **Eg**: Vincristine & Vinblastine

General methods of Structural Elucidation

Following are the general approach applied sequentially in the elucidation of structures of an Alkaloid —

[**1**] **Molecular formula Determination**: After a pure specimen has been obtained, it is subjected to qualitative analysis (invariably the alkaloids contain C, H & N; most alkaloids also contain O- as element). This is then followed by quantitative analysis and thus the empirical formula is obtained, determination of the molecular weight finally leads to the molecular formula. If the alkaloid is optically active, its specific rotation is also measured. For the simpler alkaloids of known molecular formula, we can also apply the principle of double bond equivalent, i.e. the number of double bond or/ and ring present in the structure. For the compound of molecular formula, $C_aH_bN_cO_d$, then —

$$DBE = (a+1) - \frac{b-c}{2}$$

[**2**] **Functional Group Analysis**: When an alkaloid contains oxygen, the functional nature of this element is determined: it may be present as – $OH (PhOH\ or\ ROH, -OCH_3$, $-OCOCH_3$ (acetoxy), $-OCOPh$ (bezoxyl), $-COOH$ or carbonyl group (ketone or aldehyde). Occasionally, lactone ring systems have also been detected / encountered, e.g. in narcotine hydrastine.

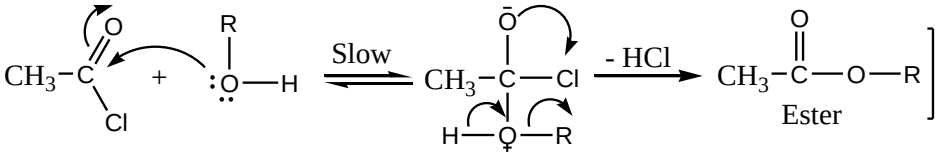
(***i***) **Hydroxyl Group**: The presence of – OH group may be ascertained by the action of acetic anhydride, acetyl chloride or benzoyl chloride on the alkaloid to give acetate, or benzoate respectively. These tests are applied carefully as 1^0- amines also respond to the above reagents and yields acetyl and benzoate derivatives.

$$ROH + (CH_3CO)_2O \longrightarrow ROOCCH_3 + CH_3COOH$$

$$ROH + CH_3COCl \longrightarrow ROOCCH_3 + HCl$$

$$ROH + C_6H_5COCl \longrightarrow ROOCC_6H_5 + HCl \ [IL\ Finar\ Vil\ 1, Page\ 256]$$

Mechanism:



When it has been ascertained that hydroxyl groups are present, then their number is also estimated either by Acetylation or by **Zerewitinoff's active hydrogen determination** method (active hydrogen atoms are those which is directly attached to $O, N \text{ or } S$ - atom)

In **Acetylation method**, the number of $-OH$ groups is determined by acetylating the alkaloid and hydrolysing the acetyl derivative with a known volume of $1N\ NaOH$ solution.

$$R - OH \xrightarrow{CH_3COCl} R - OCOCH_3 \xrightarrow{1N\ NaOH} ROH + CH_3COONa$$

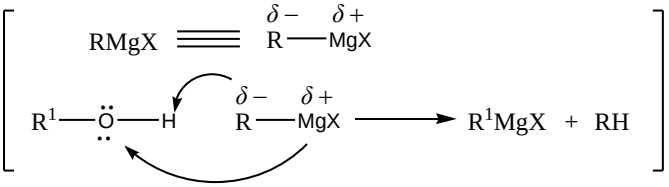
The excess of alkali is estimated by titration with a standard solution of HCl acid. The number of acetyl groups or hydroxyl groups can be calculated from the volume of alkali used for hydrolysis.

Zerewitinoff's method is the determination of active hydrogen atom (or reactive H – atom) by the double decomposition of the compound with CH_3MgI, whereby the alkyl group of CH_3MgI is converted to hydrocarbon (Methane). Since, the compound containing active hydrogen result in the quantitative yield of methane so can be used to determine the number of active hydrogen in the compound. The methane which is liberated is measured (by volume), one mole of methane being equivalent to one active hydrogen atom. For example —

$$CH_3MgI + ROH \longrightarrow CH_4 + ROMgI$$

$$CH_3MgI + RNH_2 \longrightarrow CH_4 + RNHMgI \xrightarrow{CH_3MgI} CH_4 + RN(MgI)_2$$

$$R^1R^2NH + CH_3MgI \longrightarrow CH_4 + R^1R^2NHMgI$$



Thus 1 OH Group = 1 $\diagdown$ NH $\diagup$ Group = 22.4 L of N_2 or H_2 at STP

The next problem is to decide whether the hydroxyl group is alcoholic or phenolic. The hydroxyl group is said to be **phenolic if** the alkaloid is —

(a) Soluble in $NaOH$,

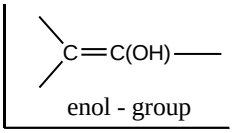
(b) Re-precipitated by CO_2 and

(c) Gives a colourization with neutral $FeCl_3$ solution.

$$PhOH + NaOH \xrightarrow{-H_2O} PhO^-Na^+ \xrightarrow{CO_2} PhO^-Na^+ + H_2CO_3 \longrightarrow PhOH + NaHCO_3$$

Insoluble Soluble Soluble Insoluble

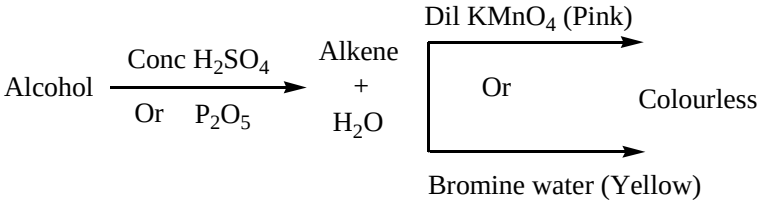
Phenol gives coloured water soluble precipitates (Complexes) with neutral $FeCl_3$ solution. This test is also given by the compounds containing "$enol$" group, and distinguishes between a phenol and alcohol.



$$C_6H_5OH + FeCl_3 \longrightarrow [Fe(OC_6H_5)_3]^{3-} + 3Cl^- + 6H^+$$

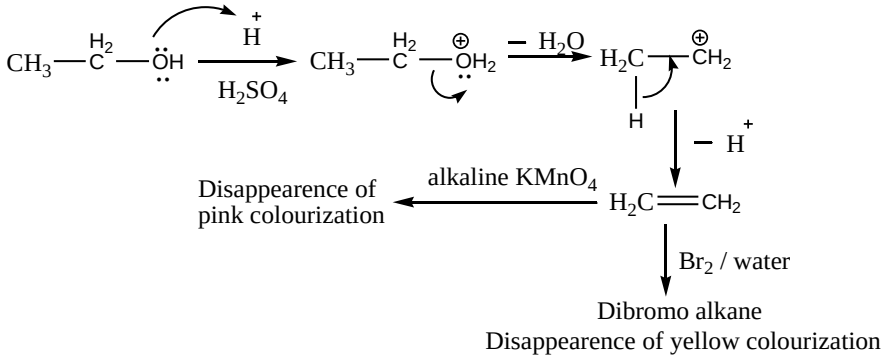
Violet colour

If the compound does not behave as phenol, the hydroxyl group may be assumed to be alcoholic and this assumption may be verified by the action of dehydrating agents (like $conc\ H_2SO_4, P_2O_5, etc.$)



$$CH_3 - CH_2 - OH \xrightarrow{H_2SO_4} CH_2 = CH_2 + H_2O$$

Mechanism:



However, to know the exact nature of the alcohol i.e. whether the alcoholic group is primary, secondary or tertiary is determined by the use of an oxidizing agent or by Victor Mayer tests as follows:

Methods of Distinguishing between the 3 - classes of Alcohols:

H_2CrO_4 *Or* CrO_3 oxidation
Yellow orange colour
↓ After oxidation
Green Colour

By Means of Oxidation: The nature of the oxidation products of an alcohol depends on whether alcohol is primary, secondary or tertiary.

Primary alcohol, on oxidation, 1st gives an aldehyde, and this on further oxidation gives an acid. Both the aldehyde and acid contains the same number of C- atoms as the original alcohol. For example —

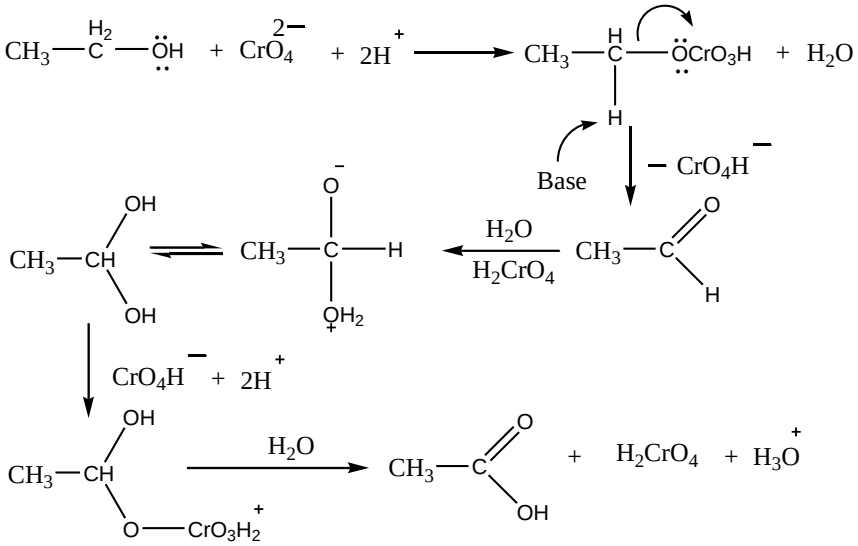
$$CH_3CH_2OH \xrightarrow[-H_2O]{[O]} CH_3CHO \xrightarrow{[O]} CH_3COOH$$

Mechanism with chromic acid:

$$H_2CrO_4 \leftrightharpoons CrO_4^{2-} + 2H^+$$

$$CrO_3 + H_2O \leftrightharpoons 2H^+ + CrO_4^{2-}$$

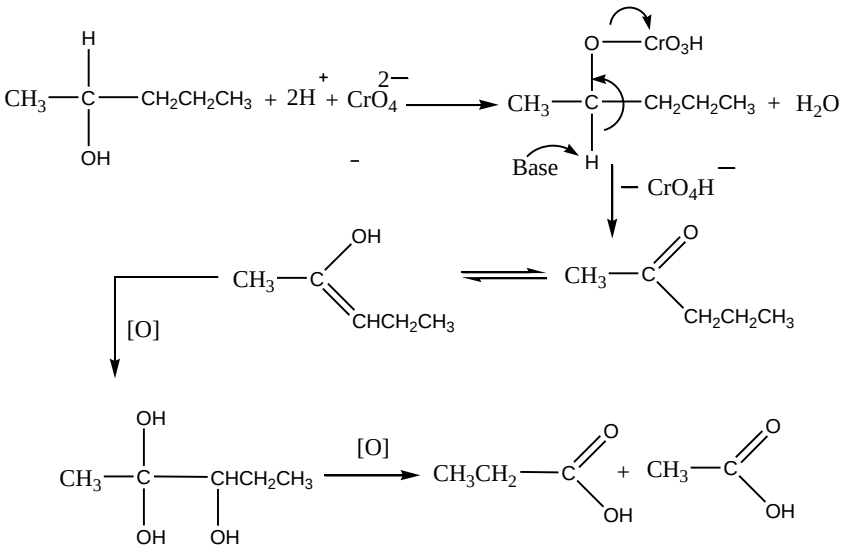
Now,



Secondary alcohol, on oxidation, 1st gives a ketone with the same number of C- atoms as the original alcohol. Ketones are fairly difficult to oxidise, but on prolonged action of the oxidizing agents produces a mixture of acids, each containing fewer carbon atoms than the original alcohol. For example —

$$CH_3CH(OH)CH_2CH_2CH_3 \xrightarrow[-H_2O]{[O]} CH_3-\overset{O}{\overset{\|}{C}}-CH_2CH_2CH_3 \xrightarrow{[O]} CH_3COOH + CH_3CH_2COOH$$

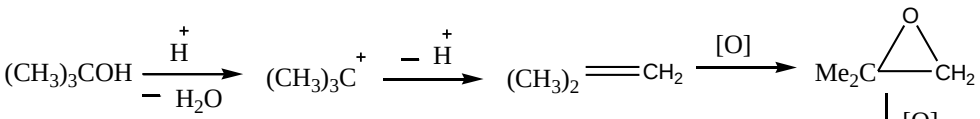
Mechanism:



Tertiary alcohols are resistant to oxidation in neutral or alkaline solution, but are readily oxidised by acidic oxidizing agents to a mixture of acids, each containing fewer carbon atoms than the original alcohol. For example —

$$(CH_3)_2C(OH)CH_2CH_3 \xrightarrow{[O]} (CH_3)_2COH + CH_3COOH$$

Similarly,



For details see oxidation - reduction chapter

(***ii***)

Carboxyl Group: The solubility of the alkaloid in $aq\ Na_2CO_3$ or NH_3 indicates the presence of carboxylic group. The formation of ester on treatment with an alcohol also indicates the presence of $-COOH$ group.

The number of carboxylic acid group may be determined volumetrically by titration against a standard solution of $Ba(OH)_2$, using phenolphthalein as an indicator or gravimetrically by Ag- salt method.

$$RCOOH \xrightarrow{aq} RCO_2^- + H^+;\quad Na_2CO_3 \xrightarrow{aq} NaHCO_3$$

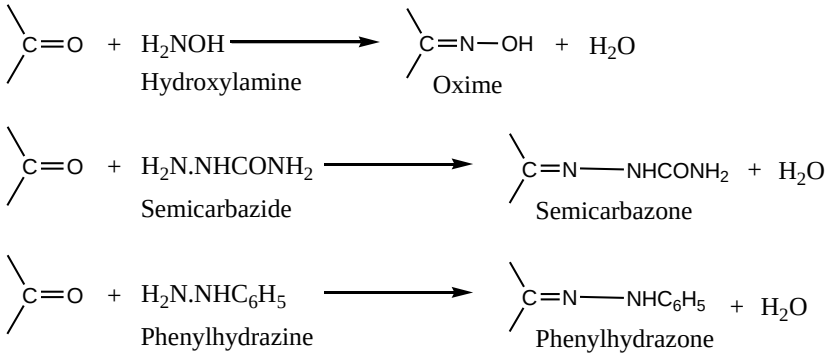
$$RCOOH + NaHCO_3 \longrightarrow RCO_2^-Na^+ + H_2O + CO_2$$

$$RCOOH + NH_3 \longrightarrow RCO_2^-NH_4^+$$

For the determination of number of $-COOH$ group, we actually determine the equivalent weight of the compound. Thus, we can determine the basicity of the acid i.e. the number of $-COOH$ group.

$$Basicity = \frac{Molecular\ weight\ of\ the\ acid}{Equivalent\ weight\ of\ the\ acid}$$

(***iii***) **Oxo Group**: The presence of an oxo group is readily ascertained by the formation of an oxime, semicarbazone and phenylhydrazone.



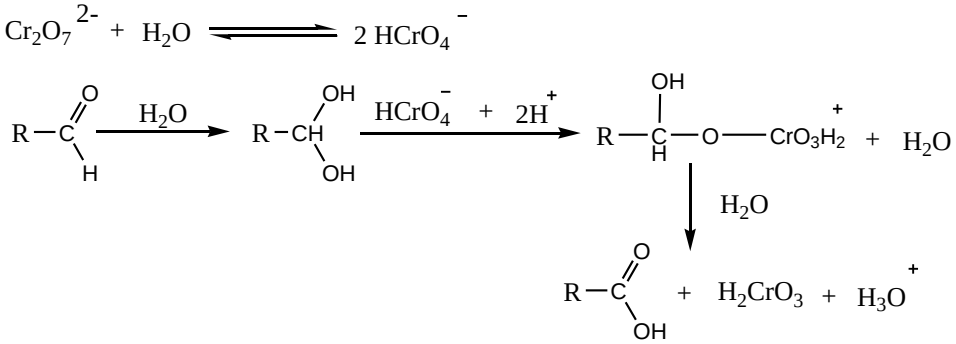
Distinction between Aldehyde & Ketone can be made on the basis of oxidation and reductions —

Aldehydes are very easily oxidized, and hence are powerful reducing agents. Aldehydes reduces Fehling solution (an alkaline solution containing a complex $Cu-tartrate$) to red cuprous oxide, and Tollen's reagent ($ammoniacal\ AgNO_3$ solution) to $metallic\ Ag$, etc.

$$RCHO + 2Ag(NH_3)OH \longrightarrow RCO_2NH_4 + 2Ag + 3NH_3$$

Tollen's reagent Silver mirror

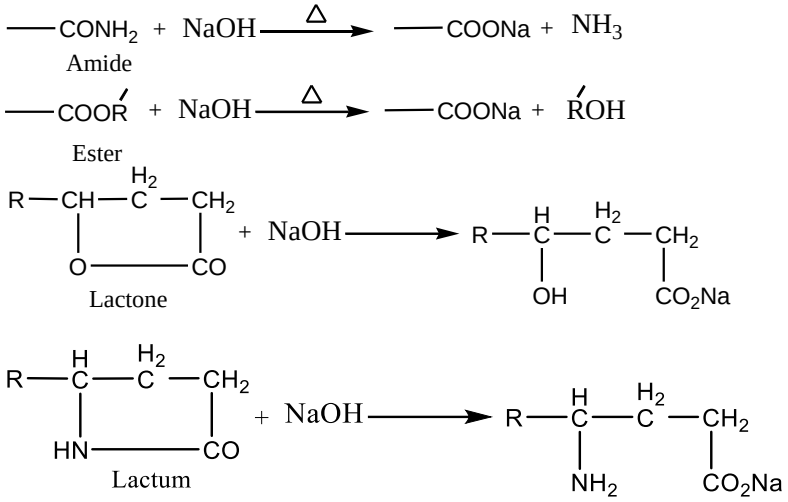
Aldehydes are readily oxidised by acid dichromate, permanganate, etc. The oxidation with dichromate is believed to proceed by a mechanism similar to that of primary & secondary alcohol.



Ketones are not easily oxidised, they do not reduce Fehling solution or Tollen's reagent.

The presence of an oxo group and distinction between an aldehyde and a ketone may be further confirmed by several physical methods such as IR, UV- Vis and NMR techniques.

($\boldsymbol{iv}$) **Esters, Amide, Lactone and Lactam Groups**: These groups can be detected and estimated by observing the products formed by the hydrolysis (acidic or alkaline) of the alkaloid,



($\boldsymbol{iv}$) **Methoxy Group ($-OCH_3$): Zeisel Method**

The presence of methoxy group and their number may be determined by Zeisel method. The alkaloid is heated with concentrated HI acid at its boiling point ($126℃$), the $-OCH_3$ groups are thereby converted to CH_3I, which is then absorbed by ethanolic $AgNO_3$ and AgI s weighted. From the weight of AgI, the number of $-OCH_3$ groups can be calculated.

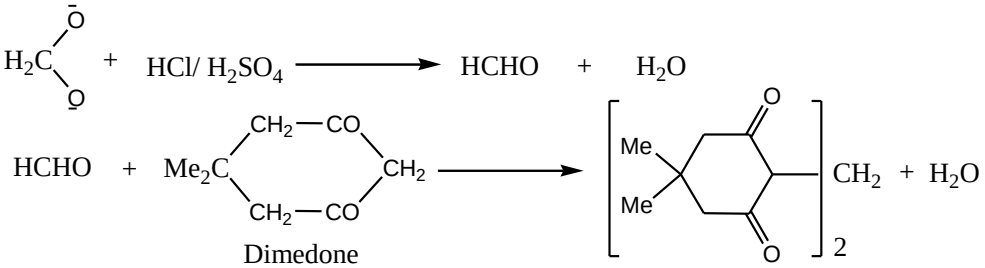
$$-O-Me+HI \longrightarrow -OH+MeI \xrightarrow{AgNO_3} AgI\ (ppt)$$

Estimated Gravimetrically

For example, **papaverine**, ($C_{20}H_{21}NO_4$), when treated with HI, consumes 4- moles of HI, producing 4- moles of AgI, and thus confirming the presence of $4-OCH_3$ groups.

$$C_{16}H_9N(OCH_3)_4+4HI \rightarrow C_{16}H_9N(OH)_4+-4CH_3I \xrightarrow{4AgNO_3} 4AgI\ (ppt)$$

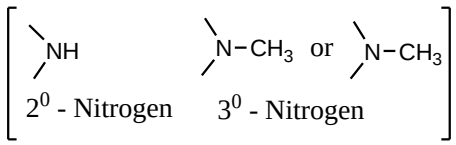
($\boldsymbol{vi}$) **Methylene-dioxyl Group** ($-OCH_2-O-$): The presence of this group is indicated by the formation of formaldehyde when the alkaloid is heated with HCl or H_2SO_4 acid. Formaldehyde obtained in this reaction is converted into ***dimedone*** derivative which can be estimated gravimetrically, thus giving the information about the number of the $-OCH_2-O-$ group.



Dimedone

(5, 5 - Dimethylcyclohexane - 1, 3 - dione) Dimedone derivative

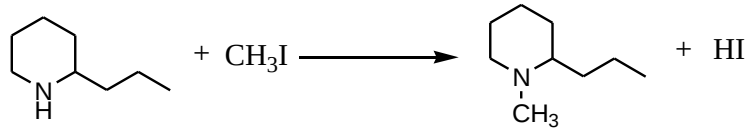
[**3**] **The Functional Nature of Nitrogen**: All alkaloids contain nitrogen as an element. But in the majority of the alkaloids, it is present as a part of a heterocyclic system. Therefore, it must be either secondary or tertiary. However, there are phenyl alkyl amine types of alkaloids (**adrenaline, ephedrine**, etc.) which do not contain N- as a part of heterocyclic ring, but in the form of a primary amine ($-NH_2$) group.



($\boldsymbol{i}$) The general reactions of the alkaloid with acetic anhydride, methyl iodide and nitrous acid often show the nature of the N – atom. For example, if all reactions are negative, then the N- atom is most probably tertiary. The difficulty here is that some alkaloids may undergo ring fission, the product being an N- ***acylated*** derivative.

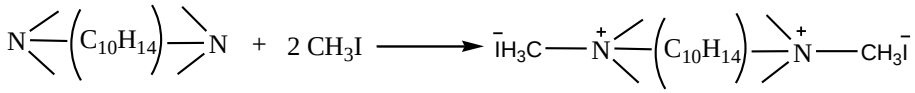
If the alkaloid reacts with one molecule of CH_3I to form N- methyl derivative, it means that a secondary N- atom is present. For example, **coniine**, $C_8H_{17}N$ reacts with 1 mol of CH_3I to form a $-N-CH_3$ derivative, indicating that coniine must contain a secondary N- atom.

$$C_8H_{16}NH + CH_3I \rightarrow C_8H_{16}N - CH_3 + HI$$

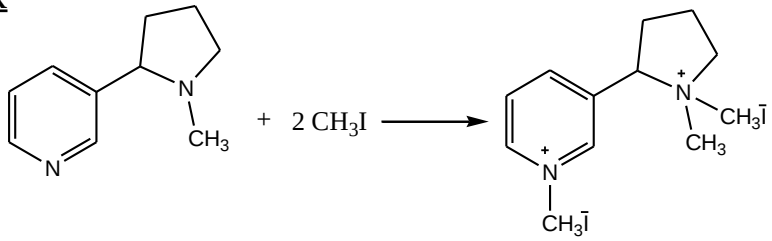


Coniine N - Methyl coniine

If the alkaloid reacts additively with two molecule of CH_3I to form a crystalline quaternary salt, this indicates that N- atom present in this alkaloid is tertiary. For example, **nicotine** reacts additively with two molecules of CH_3I, indicating that it contains both N- atoms as tertiary.

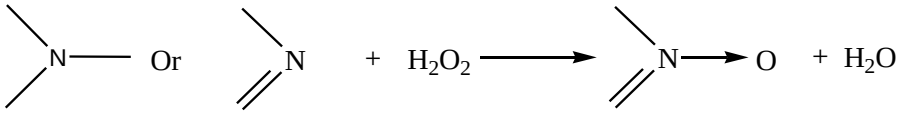


OR



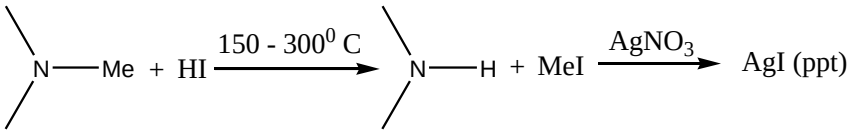
Nicotine N, N - Dimethyl nicotine

The presence of tertiary N- atom in an alkaloid can be detected by treating it with $30\%\ H_2O_2$, where by the tertiary N- atom is oxidized to amine oxide.



($\boldsymbol{ii}$) Distillation of an alkaloid with aqueous KOH usually leads to information regarding the nature and number of alkyl groups attached to N- atom. The formation of methyl amine, dimethyl amine or trimethyl amine indicates respectively the attachment of one, two or three methyl groups to an N- atom, the formation of ammonia shows the presence of an amine group in the alkaloid molecule.

($\boldsymbol{iii}$) **Herzig – Meyer Method**: The presence of N- methyl group and their number may be determined by means of Herzig-Meyer method — when the alkaloid is heated with HI acid at $150 - 300℃$ under pressure, $N - CH_3$ groups are converted into methyl iodide, which is then absorbed by ethanolic AgI and it is estimated gravimetrically.



Note: By the same method, $-OCH_3$ groups are estimated but the only difference is in the temperature, for $N - CH_3$ temperature is $150 - 300℃$, but for $-OCH_3$groups it is $126℃$.

($\boldsymbol{iv}$) The result of hydrolysis (by KOH) will show the presence of an amide, Lactum or betaine **(Betaine** → these are trialkyl derivatives of glycine, which exists as dipolar ions of formula $R_3N^+CH_2CO_2^-$. Betaine itself is a trimethyl derivative, and may be prepared by heating glycine with MeI in methanolic solution —

$$H_3N^+CH_2CO_2^- + 3MeI \longrightarrow Me_3N^+CH_2CO_2^- + 3HI$$

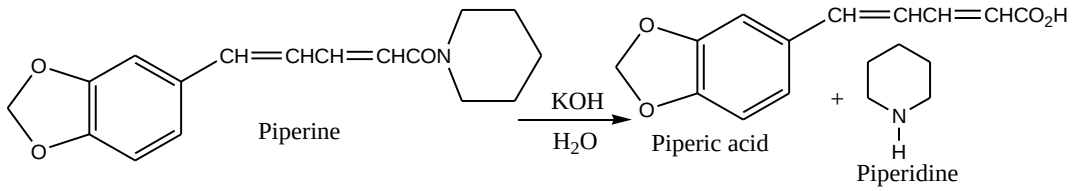
It is more conveniently prepared by warming an aqueous solution of chloroacetic acid with trimethyl amine —

$$(CH_3)_3N + ClCH_2COOH \longrightarrow Me_3N^+CH_2CO_2^- + 3HCl$$

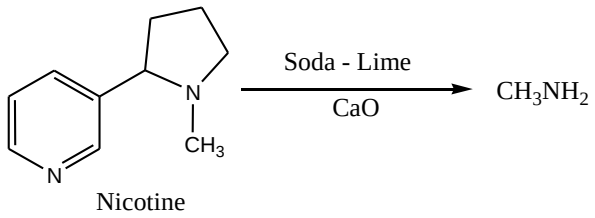
For example, the amide linkage if present shall be indicated by hydrolysis followed by the characterization of acid and amine moieties. For example, alkaline hydrolysis of **piperine** ($C_{17}H_{19}O_3N$), yields piperidine and piperic acid; since, piperidine is a base and piperic acid is a monobasic acid, therefore, piperine is a piperidine amide of piperic acid.

$$C_{17}H_{19}O_3N + H_2O \xrightarrow{KOH} C_5H_{11}N + C_{12}H_{10}O_4$$

Or



($\boldsymbol{v}$) The presence of N-methyl group is often detected by distillation of alkaloid with soda lime whereby methyl amine is obtained. For example, nicotine on heating with soda lime yields methyl amine, indicating that it must contain an N-methyl group.



[**4**] **Degradation of Alkaloids**: After knowing functional groups or elements present, to know their exact position of these groups we have to degradation of the alkaloid and identification of each fragment. Thus degradation is the most important part in the alkaloid chemistry to know the actual structure of the alkaloid. Most of the reactions used in such work are as follows —

(i) Hofmann's Exhaustive Methylation Method,

(ii) Emde's Degradation,

(iii) Von Braun's Method,

(iv) Reductive Degradation and $Zn-$ Dust Distillation,

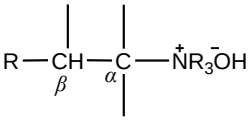
(v) Alkali Fusion,

(vi) Oxidation, and

(vii) Dehydrogenation

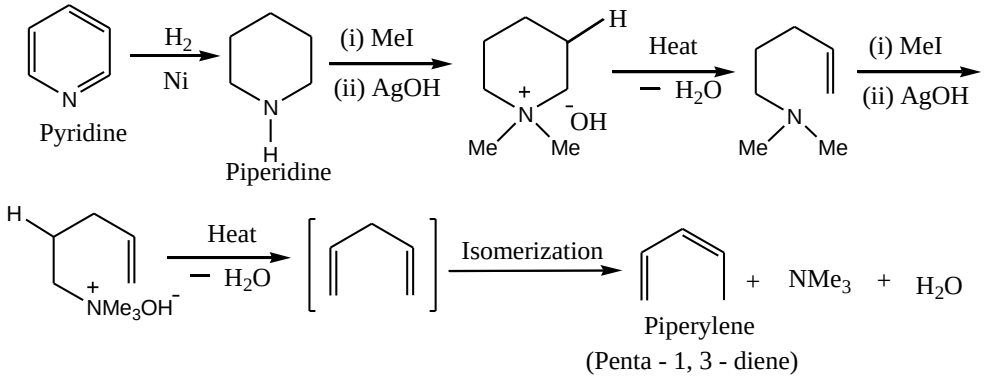
Hofmann's Exhaustive Methylation Method (1883):

This is the most important process in the alkaloid chemistry. In this process heterocyclic rings are opened with the elimination of nitrogen, and thereby the nature of the C- skeleton is thereby confirmed. The general procedure is to hydrogenate (by catalytic hydrogenation: $H_2/Ni, \Delta$) the heterocyclic ring (if this is unsaturated), convert this compound to the quaternary methyl ammonium hydroxide (reaction with 1) MeI, 2) $AgOH$) which is then heated ($200℃$). In this last step (heating step) a molecule of water is eliminated, **an *H*- atom in the *β*- position w.r.t. to the *N*- atom combining with the hydroxyl group**, and the ring is opened at the N- atom on the same side as the β-H- atom eliminated. The process is repeated on the product produced; this results in the complete removal of the N- atom from the molecule, leaving an unsaturated hydrocarbon, which, in general, isomerise to a conjugated diene. For example, **pyridine gives piperylene [**the **principle** of this method is that compound which contains the structural unit —

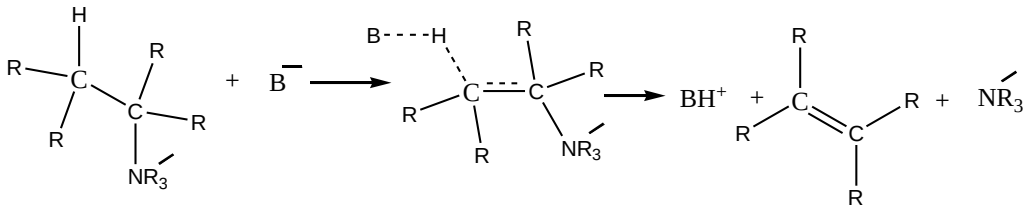


This eliminates a trialkyl amine on pyrolysis at $200℃$ or above to yield an olefin.

$R - CH_2 - CH_2 - N^+R_3OH^- \rightarrow R - CH = CH_2 + NR_3 + H_2O$]

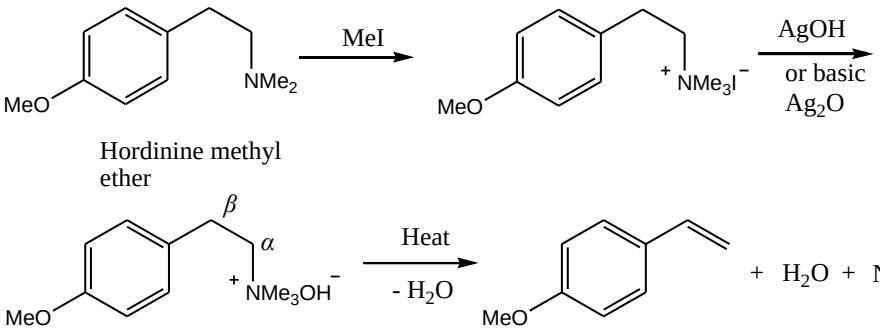


This reaction is generally proceeds by an E_2- mechanism in which the requisite β- hydrogen and quaternary N- group are present in the $trans$- $anti$-parallel configuration.

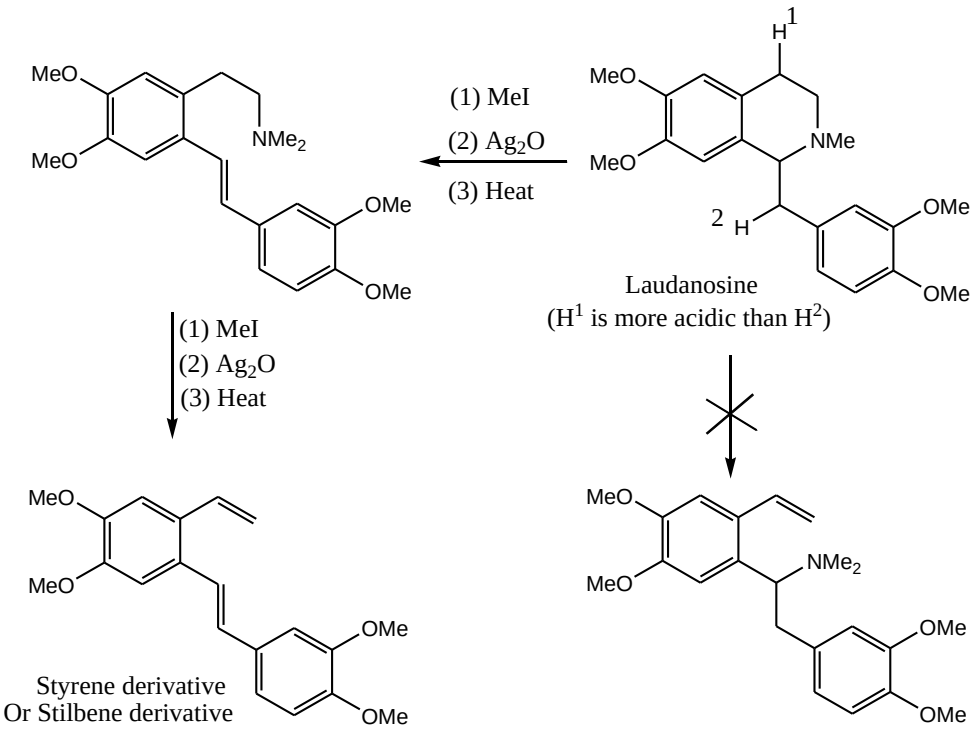


Although the general procedure for exhaustive Methylation is to heat the quaternary hydroxide at about $200°C$, in a number of cases the reaction may be carried out by refluxing an aqueous or ethanolic solution of KOH containing the methyl iodide or methyl sulphate as the base. This procedure is usually satisfactory for the bases which contain benzene ring in the β- position to the N- atom. This is explained on the basis that benzilic hydrogen has an increased acidity (and so is more readily removed) because of stabilization of the transition state by conjugation (with the benzene ring).

For example, **hordenine** methyl ether yields p- methoxy styrene



If the ring incorporates the N – atom, two complete sequences are to be carried out to eliminate it from the molecule. For example, **laudanosine** is converted into a styrene derivative (or **stilbine** derivative) as follows —



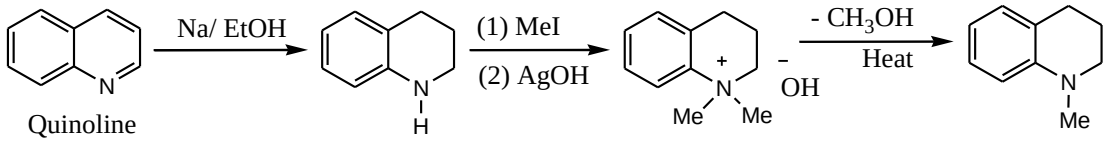
Hofmann's Exhaustive Methylation fails —

a) With unsaturated heterocyclic rings

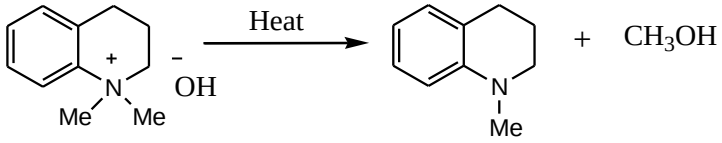
b) When there is no β-H- atom and

c) With tetrahydroquinoline.

For example, with quinoline (unsaturated heterocyclic ring) —



Even though compound containing a β-H- atom, the exhaustive methylation method may fail, for example, with tetrahydroquinoline —



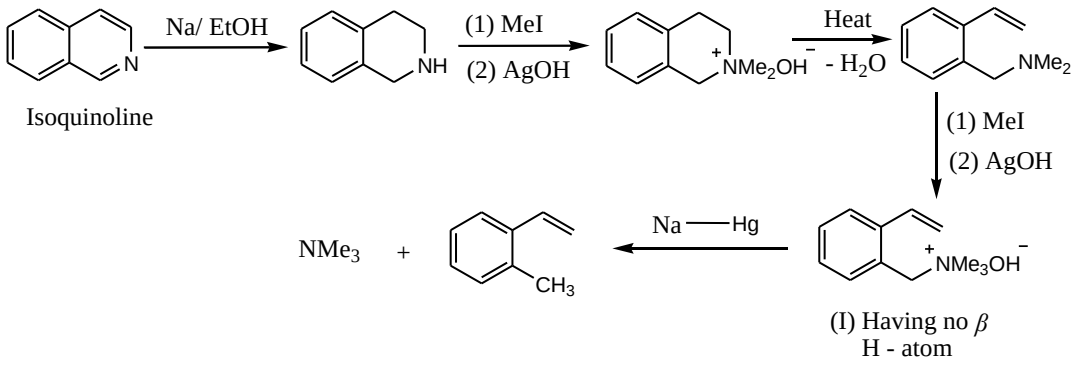
When more than one sequence is required for eliminating the nitrogen atom, the overall process is sometimes known as the exhaustive methylation and it is generally convenient in practice to hydrogenate the olefin formed after each elimination step, thus removing the possibility of double bond isomerism under the basic reaction condition.

Emde's Degradation:

When the base (alkaloid) does not contain a β-H- atom, Hofmann's exhaustive methylation is failed and we use **Emde degradation** method. In this method the final step involves the reductive cleavage of the quaternary ammonium salt (generally halide), by $Na—Hg$ in aqueous ethanol or with Na with **liquid ammonia**, or is catalytically hydrogenated.

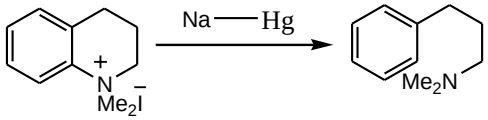
$$R - CH_2 - N^+R_3X^- \rightarrow R - CH_3 + R_3N^+HX^-$$

Emde method can be explained by considering the case of **isoquinoline**,

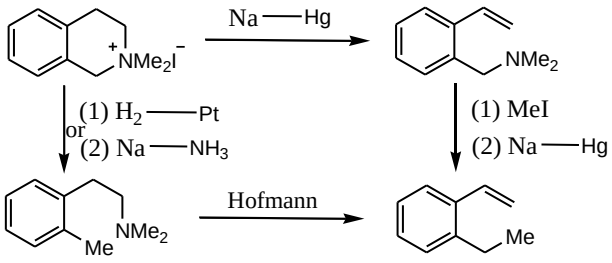


Hofmann's exhaustive methylation cannot be applied to the above compound (I), because it does not contain β-H- atom.

It has been seen earlier that exhaustive methylation fails with tetrahydroquinoline; however, the heterocyclic ring in this compound (tetrahydroquinoline) is opened by Emde degradation.



The Emde degradation on tetrahydroquinoline is also interesting —



Occasionally, successive Emde degradation have been employed as a means of eliminating a N- atom from an alkaloid but where possible the preferred sequence is **Hofmann followed by Emde** reaction.

Von Braun's Method: This method is of two types —

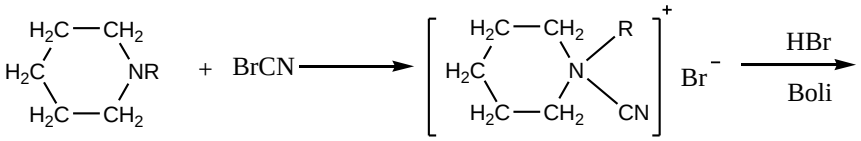
Type I: In the first method, the tertiary amine, which contains at least one alkyl substituent, is treated with $BrCN$ (cyanogens bromide). This results in cleavage of an alkyl-nitrogen bond to give an alkyl halide and a substituted cyanamide.

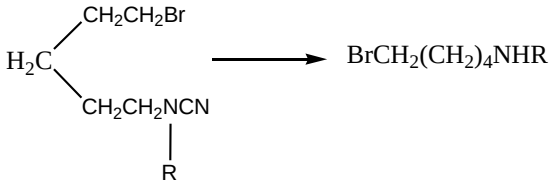
$$R_3N + CN - Br \rightarrow R - Br + R_2N - CN$$

Fission of unsymmetrical substituted amines generally takes place to yield the alkyl halide derived from the smallest alkyl substituent.

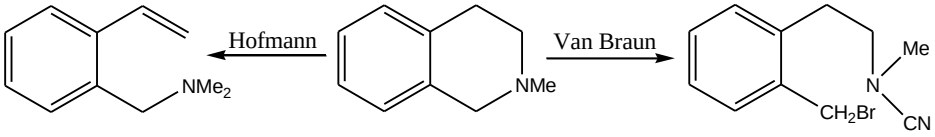
$$Et_2NMe + CN - Br \rightarrow Me - Br + Et_2N - CN$$

Now we will apply Von Braun's method to 3^0 - cyclic amines —

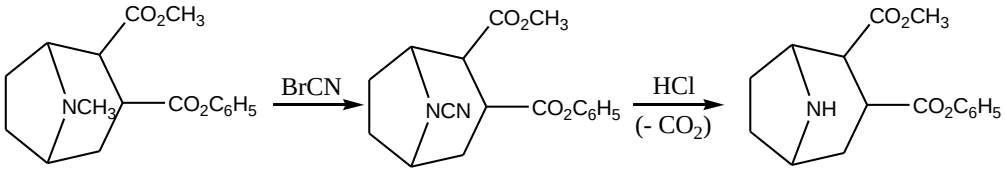




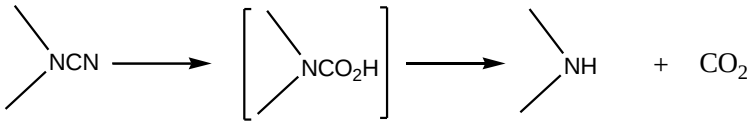
Von Braun's cyanogen method is often applicable to such compounds which do not responds to Hofmann's method. Furthermore, where both methods are applicable ring opening occurs at different points of the ring. For example —



In the above examples, ring is opened, however, in either cases **de-alkylation** takes place with the formation of the cyclic N- cyano derivative. For example, with **cocaine** —

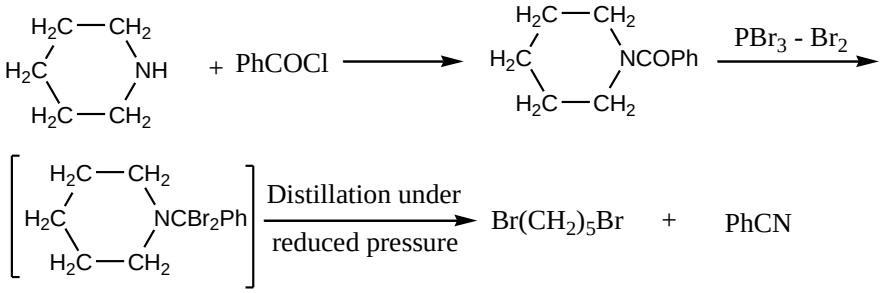


Hydrolysis of the cyano compound with hydrochloric acid brings about the following change:

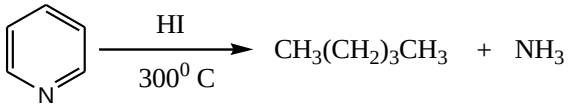


Thus, the final result is the removal of the N- methyl group without opening the ring.

Type II: **Von Braun's method for 2^0 – cyclic amines:** In this method cyclic amines are treated with benzoyl chloride in presence of $NaOH$ to yield benzoyl derivative, which on treatment with PBr_5 followed by distillation under reduced pressure yields an α, ω- dihalo compound with the elimination of benzonitrile. For example, with piperidine —

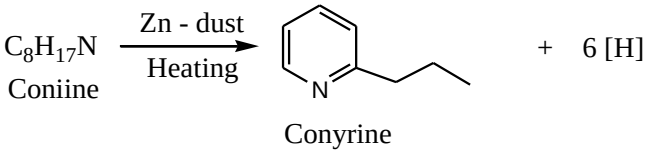


In a number of cases ring may be opened by heating with HI at $300°C$, for example —

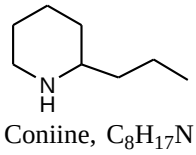


Reductive Degradation and Zn- Dust Distillation:

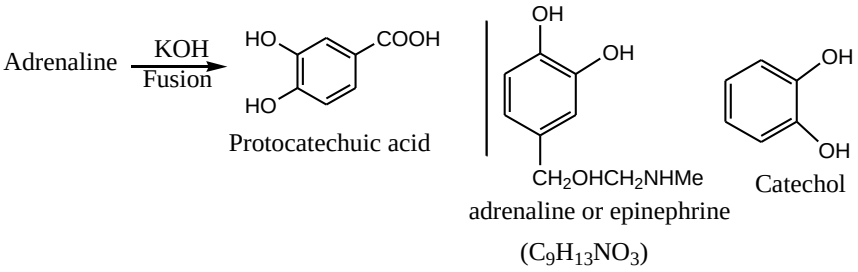
This usually gives the same compound or relatively smaller fragments, except that when the alkaloid contains oxygen. This is removed or dehydrogenated, for example —



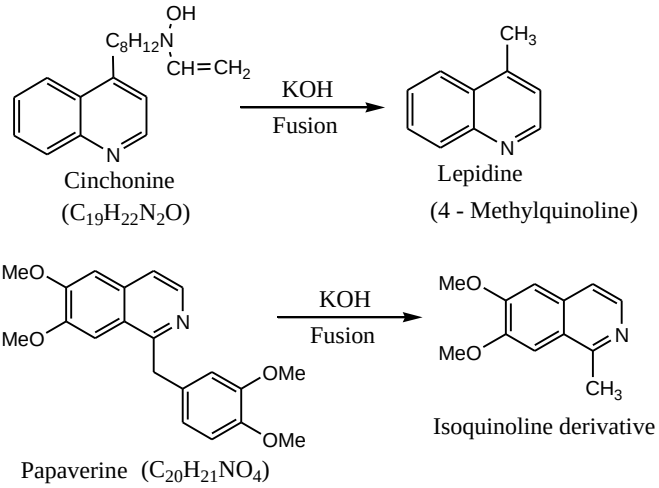
As **conyrine** is formed by the loss of six –H – atoms, it means that coniine must contain a piperidine ring.



Alkali Fusion: This is a very drastic method which is often employed to break down the complex alkaloid molecule into simpler fragments, the nature of which gives information on the type of nuclei present in the alkaloid molecule. For example, **adrenaline** when fused with solid KOH yields protocatechuic acid, indicating that adrenaline is a catechol derivative.



Similarly, **papaverine** *on* **fusion with alkali** yields an isoquinoline, indicating that papaverine must contain an isoquinoline unit. Also **cinchonine** when fused with alkali yields quinoline, showing the presence of quinoline nucleus in cinchonine.



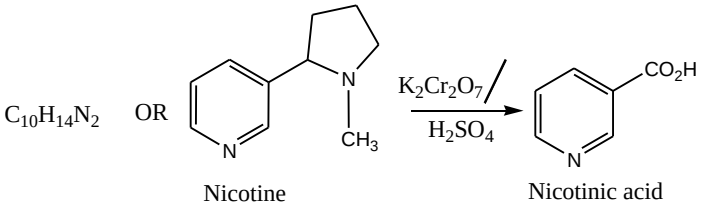
Oxidation: Oxidation is one of the most valuable means of determining the structure of alkaloids. By varying the "**strength**" of the oxidising agent, it is possible to obtain a variety of products —

1) For mild oxidation, usually $H_2O_2, O_3, I_2/\ EtOH$, or alkaline potassium ferricyanide, $K_3[Fe(CN)_6]$ are used.

2) Moderate oxidation may be carried out by means of acidic or alkaline $KMnO_4\ or\ CrO_3$ in ethanol.

3) Vigorous oxidation is usually carried out by $K_2Cr_2O_7\ in\ H_2SO_4$.

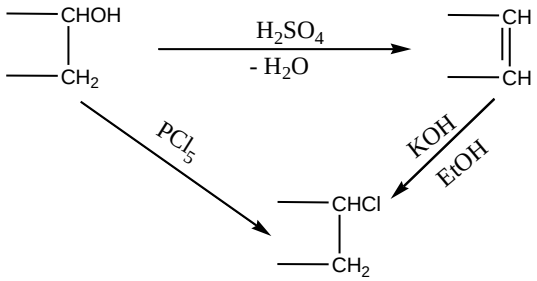
These reagents usually brake up an alkaloid into smaller fragments whose structures are either known or can be readily ascertained. For example, considering **nicotine** —



From the above reaction it can be concluded that nicotine contains a pyridine nucleus having a side chain at β- position.

The above classification of oxidizing agents is not rigid because the "strength" of an oxidizing agent depends to some extent on the nature of the alkaloid which is being oxidized.

In some cases where it can be done, better results are sometimes achieved by first dehydrating the compound and then oxidizing the unsaturated compound, thus obtained, oxidation is readily affected on a double bond. More recently, mercury acetate has been used to dehydrogenate certain alkaloids, thereby **introducing olefinic bonds**.



Dehydrogenation: When an alkaloid is distilled with a catalyst such as $S, Se, or\ Pd$, dehydrogenation takes place to form relatively simple and easily recognizable products which provide a clue to the gross fragments obtained during degradation, there occurs the elimination of peripheral groups such as hydroxyl or C- methyl.

[**1**] **Unsaturation**: The presence of unsaturation in an alkaloid may be ascertained by the addition of Br_2 or HX – acids or by the ability of hydroxylation with dilute alkaline $KMnO_4$. Reduction by means of $Na-Hg, Na-EtOH, Sn-HCl, HI$ acid *etc*. also may be used to show the presence of unsaturation. In some cases reduction may decompose the molecule. This often happens, when the catalytic reduction is employed (ring cleavage also occurs), and hence milder methods of reduction are desirable. Two particularly mild reducing agents are $LiAlH_4$ & $NaBH_4$. Na in liquid ammonia gives the Emde type of degradations.

[**2**] **Physical Methods**: Physical methods are now being used, in conjugation with chemical methods, to elucidate structure more easily. In alkaloid chemistry, the most important instrumental methods widely used are —

(i) UV – Visible Spectroscopy,
(ii) IR Spectroscopy,
(iii) NMR Spectroscopy,
(iv) Mass Spectroscopy,
(v) Optical Rotatory Dispersion (ORD),
(vi) Conformational Analysis, and
(vii) X – Ray Diffraction

[**3**] **Synthesis**: The structure of the alkaloid arrived at by the exclusive analytical evidence based on the foregoing methods is only tentative. The final confirmation of the structure must be done by the unambiguous synthesis.

Morphine

Molecular formula is $C_{17}H_{19}O_3N$. Nature of nitrogen atom: It adds on one molecule of CH_3I to form quaternary salt, indicating the presence of tertiary nitrogen atom. By Herzig-Meyer method, reveals the presence of NCH_3 group in morphine. Nature of oxygen atoms: Morphine is acetylated or benzoylated forming diacetyl or dibenzoyl derivative indicating that morphine contains two hydroxyl groups.

$$C_{17}H_{17}ON(OH)_2 \xrightarrow[2CH_3COCl]{\text{Acetylation}} \underset{\text{Diacetyl Morphine}}{C_{17}H_{17}ON(OCOCH_3)_2} + 2HCl$$

$$C_{17}H_{17}ON(OH)_2 \xrightarrow[2C_6H_5COCl]{\text{Benzoylation}} \underset{\text{Dibenzoyl Morphine}}{C_{17}H_{17}ON(OCOC_6H_5)_2} + 2HCl$$

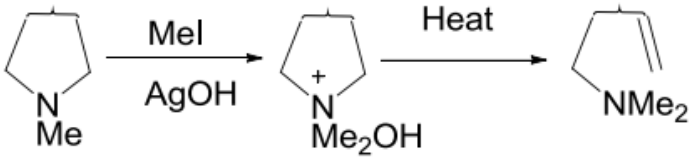
With Ferric Chloride, Morphine yields a characteristic violet colour which is soluble in NaOH to form monosodium salt which is reconverted to morphine indicating the hydroxyl group are phenolic in nature.

Morphine is treated with halogen acids, to form monohalogen derivative ie., one hydroxyl group is replaced by halogen acid. Hence, one of the hydroxyl group is alcoholic in nature. Morphine is heated with CH_3I in the presence of aqueous KOH, it is methylated to yield codeine, $C_{18}H_{21}O_3N$.

As codeine doesn't give colour with $FeCl_3$ and it is not soluble in NaOH it follows that phenolic OH in morphine is methylated. Futher, codeine on oxidation with chromic acid, it yields codeinone, a ketone indicating that the hydroxyl group in codeine is a secondary alcoholic in nature. Codeine is a monomethyl ether of morphine. The third oxygen atom is highly unreactive indicating its nature as an ether linkage.

Presence of ethylenic Bond: Codeine is reduced catalytically in the presence of palladium, suggesting both codeine and morphine contains one ethylenic bond. Presence of Benzene Nucleus: On bromination, morphine forms monobromo derivative with HBr, indicating the presence of benzene nucleus. Presence of cyclic tertiary base system: Codeine on exhaustive

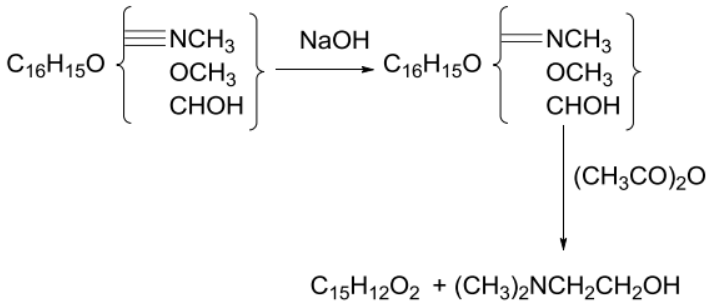
methylation yields α-codeimethine, contains one $–CH_2$ group more than codeine and the nitrogen remains intact indicating the presence of cyclic ^{t}-amine.



Presence of Phenathrene: Morphine on distillation with Zn dust it yields a phenathrene and a number of bases.

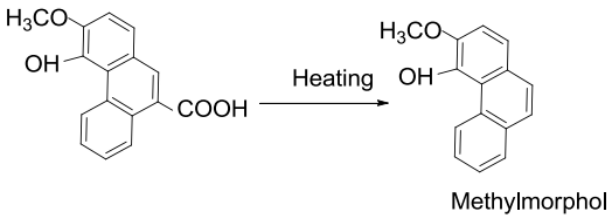
Codeine on treating with CH_3I it yields codeine methiodide, on boiling with NaOH yields methylmorphimethine on further boiling with acetic anhydride yields a mixture of methyl morphol and ethanoldimethyl amine.

Reactions:



a) Structure of Methyl morphol: Heating the compound $C_{15}H_{12}O_2$ with HCl at 180^o C yields methyl chloride and dihydroxy phenanthrene ie morphol is obtained. Diacetylmorphol on oxidation yields diacetyl phenanthraquinone indicating that the positions 9 and 10 are free. Diacetylphenathraquinone on oxidation with $KMnO_4$ yields phthalic acid indicating that the two hydroxyl groups are in the same ring. Methylmorphol is 4-hydroxy3-methoxyphenathrene.

Reactions:



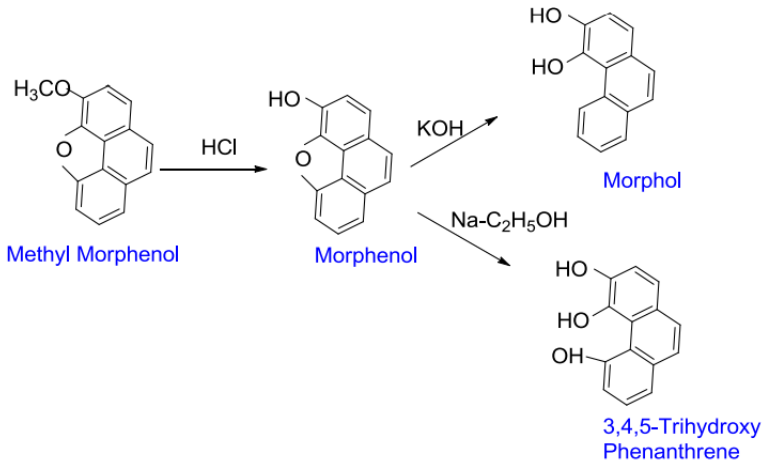
Methylmorphol

Presence of $-NCH_3$ group: The formation of ethanoldimethyl amine from methylmorphimethine revelas that both codeine and morphine contains a $N\text{-}CH_3$ group.

b) Structure of Morphenol: When β-methylmorphimethine is heated with water, it yields a mixture of trimethyl amine, ethylene and methyl morphenol.

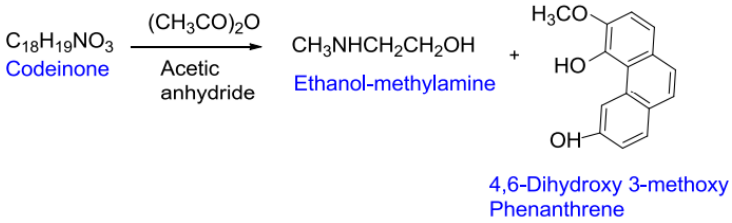
β–methylmorphimethine ⟶ Trimethyl amine + Ethylene + Methylmorphenol.

Methylmorphenol on demethylated with HCl, it yields morphenol, a compound with one phenolic hydroxyl group and an inert oxygen atom. When morphenol is fused with KOH, it yields 3,4,5-trihydroxyphenanthrene. Also morhenol on reduction with $Na\text{-}C_2H_5OH$ it yields morphol.



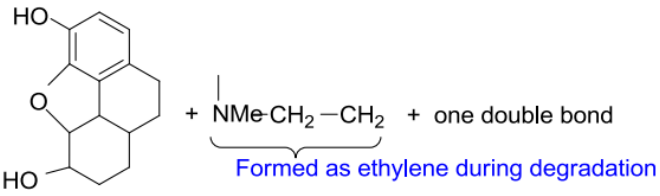
Thus, Morphenol contains an ether linkage at position 4 and 5 of the phenanthrene nucleus. The structure of morphenol and its production from codeine reveals that the two of the three oxygen atoms (i.e) One at C3, and the other ether linkage at C4 and C5 of the phenanthrene nucleus.

Position of third oxygen: Codeinone on heating with acetic anhydride yields ethanolmethyl amine and diacetyl derivative of 4,6-dihydroxy 3-methoxy phenanthrene.

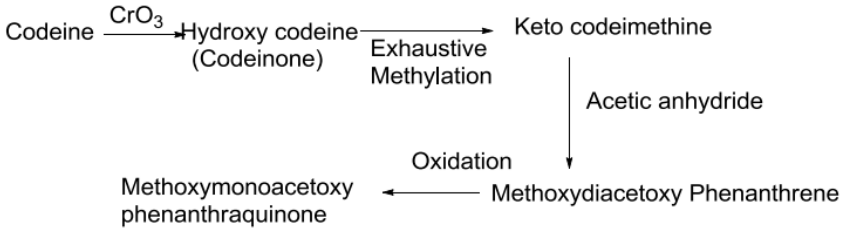


The hydroxyl group in the 6 position must be from the oxygen of the keto group in codeinone. Position of all three oxygen atoms in morphine are : One at C3- Phenolic; Other at C4 and C5 (Ether) and third (secondary alcohol) at C6 of the phenanthrene nucleus.

c) Structure of Morphine: Morphine forms monobromo derivative with bromine and monosodium salt with NaOH, indicating that morphine contains a benzenoid structure. On exhaustive methyaltion of codeimethines, ethylene and ethanol dimethyl amine is formed as the products,reveals the presence of N-CH_3 group. Also a double bond and a tertiary nitrogen has to be present in morphine. Hence, the partial structure of morphine is,

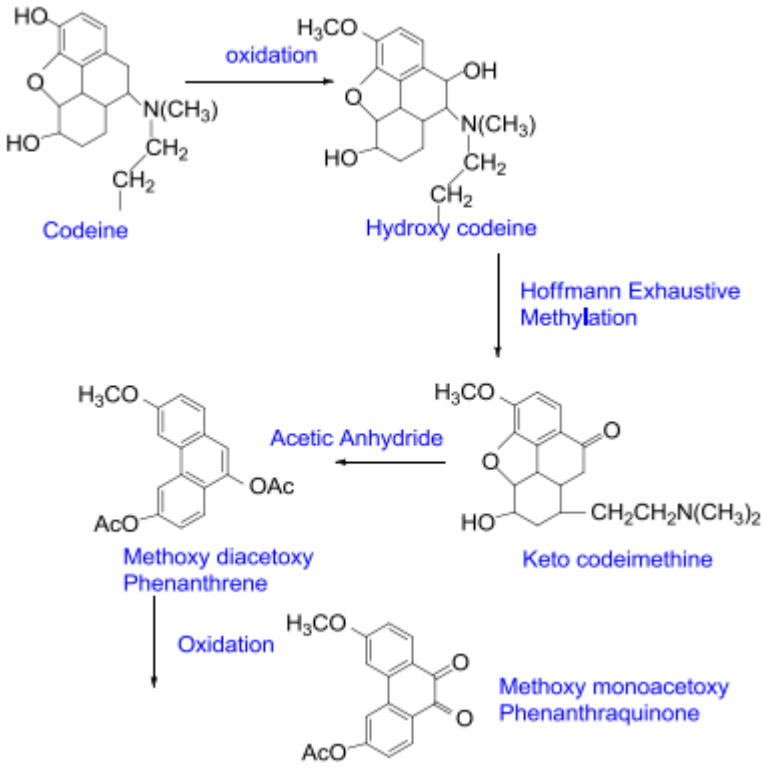


d) Point of linkage of CH_2-CH_2-N Me group:



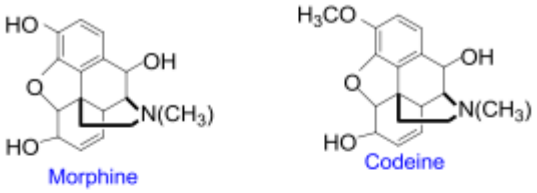
Loss of acetyl group reveals that one of the acetoxy groups must be present either at C9 or C10. The acetyl group is inserted via ketonic group which concludes that the new hydroxyl group in hydroxy codeine is present either at C9 or C10.

On the basis of steric consideration, the attachment at C9 is most probable. Hydroxyl group in hydroxycodeine is changed to keto group and a double bond is introduced between C9 and C10 during the fission of the nitrogen ring. Nitrogen must linked either to C9 or C10. The exact point of linkage of nitrogen is at C9, confirmed by its synthesis.

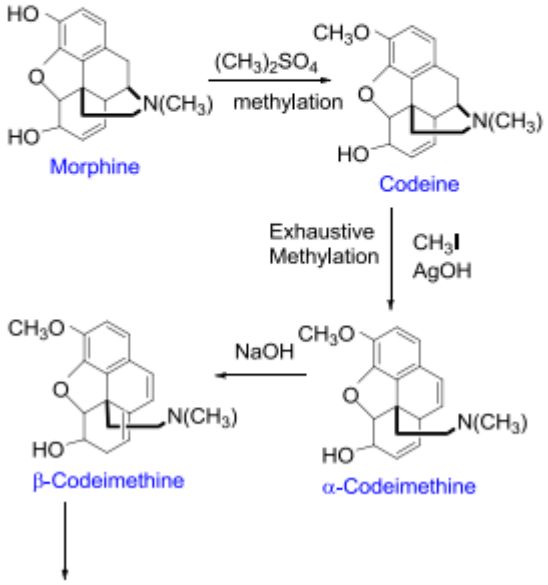


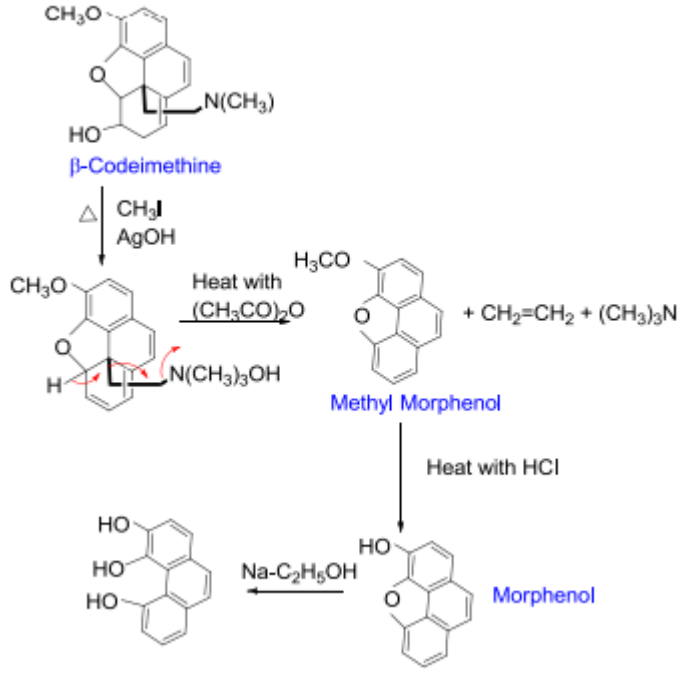
The carbon end of the side chain must be located at the angular position so that its extrusion from that position takes place during aromatisation at position C13 and C14. The former is explained as this structure explains the formation of thebaine to thebenine.

Position of double bond: Codeine on heating with PCl5 yields chlorocodide which on hydrolysis gives codeine, isocodenie, pseudocodeine and allopseudocodeine. The first two compound on oxidation gives the same ketone , revealing that they differ in the position of hydroxyl group at C6. The remaining two also yields same ketone, indicating that the hydroxyl group is at position C8. These changes can be explained only if a double bond is present at C7 and C8.

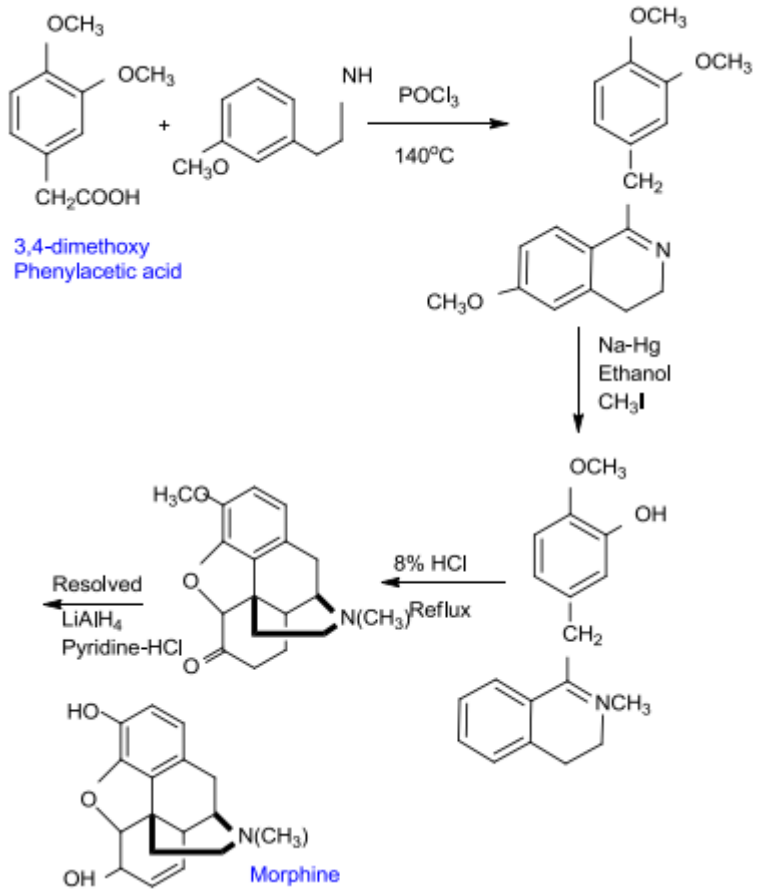

HO
OH
O
N(CH3)
HO
Morphine
H3CO
OH
O
N(CH3)
HO
Codeine

Reactions:


HO
O
N(CH3)
HO
Morphine
(CH3)2SO4
methylation
CH3O
O
N(CH3)
HO
Codeine
Exhaustive
Methylation
CH3I
AgOH
CH3O
O
N(CH3)
HO
β-Codeimethine
NaOH
CH3O
O
N(CH3)
HO
α-Codeimethine


CH3O
O
N(CH3)
HO
β-Codeimethine
△
CH3I
AgOH
CH3O
O
H
N(CH3)3OH
Heat with
(CH3CO)2O
H3CO
O
+ CH2=CH2 + (CH3)3N
Methyl Morphenol
Heat with HCl
HO
HO
HO
Na-C2H5OH
HO
O
Morphenol

Synthesis



Reserpine

Main constituent of Rauwolfia species. They are hypertensive and sedative reagents.

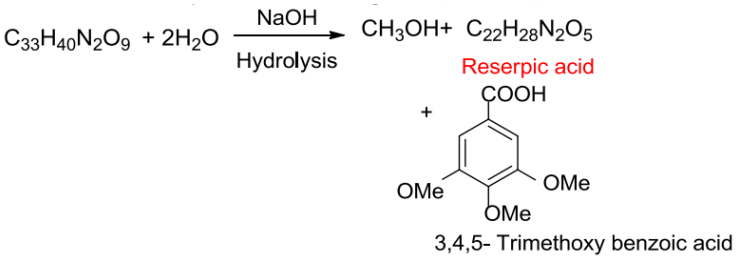
Constitution of Reserpine

Molecular formula is $C_{33}H_{40}N_2O_9$.

Presence of five methoxy groups: Resperpine on heating with HI, yields 5 molecules of CH_3I indicating the presence of 5 methoxy groups in resperpine.

Nature of N atom: It is a weak base indicating both the nitrogen is present in the ring. It doesn't have a hydroxyl group but forms monoacetyl derivative indicating one of the nitrogen as NH group. Other nitrogen is an 30 N.

Hydrolysis:When reserpine is hydrolysed with alkali solution it yields a mixture of methyl alcohol, 3,4,5 trimethoxy benzoic acid and reserpic acid ($C_{22}H_{28}N_2O_5$).



As reserpine doesnot contain –COOH or –OH groups, introduction of two -COOH groups and two alcoholic OH groups in its hydrolysis products reveals that reserpine is a diester. The ester linkage is confirmed by its reduction with LiAlH4 to reserpic alcohol, $C_{22}H_{30}N_2O_4$ and 3,4,5 trimethoxy benzyl alcohol.

a) Structure of Reserpic Acid:

Molecular Formula was found to be $C_{22}H_{28}N_2O_5$

Presence of one carboxyl group: Reserpic acid forms monosodium salt with NaOH indicates the presence of one carboxyl group.

Presence of one –OH group: Reserpic acid contains one alcoholic –OH group, is a secondary alcoholic group because reserpic acid oxidation yields a ketone.

Presence of two methoxy groups: By zeisel method, it is shown that reserpic acid contains two methoxy groups.

Nature of two nitrogen atoms: In reserpic acid, two nitrogen atoms are present in heterocyclic ring, one as secondary amino and the other as tertiary nitrogen atom. Thus reserpic acid contains two methoxy group, one COOH group and one alcoholic OH group.

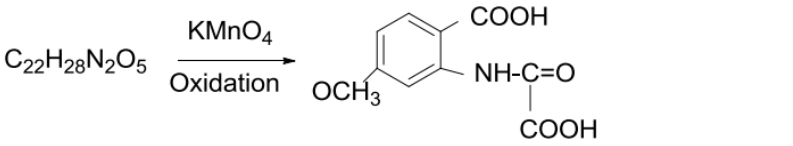
$$C_{19}H_{29}N_2 \quad \left\{ \begin{array}{l} 2\ OCH_3 \\ 1\ \text{-}COOH \\ 1\text{-}OH \end{array} \right.$$

Reduction of Reserpic acid: On reduction with $LiAlH_4$, it yields reserpic alcohol which has two methoxy, one –OH and one $–CH_2OH$ groups.

$$C_{15}H_{20}N_2 \quad \left\{ \begin{array}{l} 2\ OCH_3 \\ 1\ \text{-}CH_2OH \\ 1\text{-}OH \end{array} \right.$$

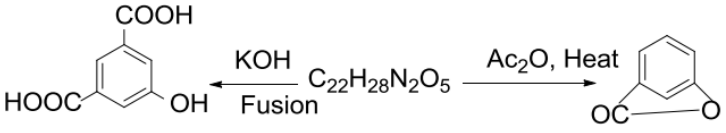
Oxidation of reserpic acid:

On oxidation with $KMnO_4$ it yields 4-methoxy N-oxalyl anthranilic acid as one of the oxidation products, confirming the presence of one indole nucleus in reserpic acid. Moreover, it reveals that one of the methoxy group is in m-position to NH group.



4--methoxy N-Oxalyl anthranilic acid.

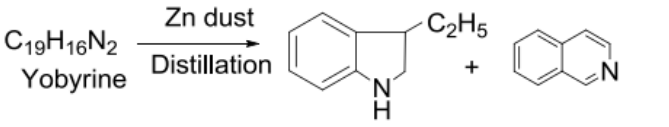
Fusion with KOH: Reserpic acid is fused with potash, to yield 5- hydroxyphthalic acid in which the hydroxyl group and –COOH group must be in m-position to each other. Reserpic acid on heating with acetic anhydride yields a γ-lactone.



γ-lactone

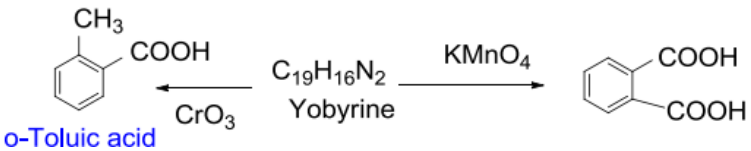
Dehydrogenation: when methyl reserpate is dehydrogenated with Se it yields a hydrocarbon with $C_{19}H_{16}N_2$. this hydrocarbon is obtained by dehydogenation of yohimbine with Se hence called as Yobyrine.

b) Structure of Yobyrine: When distilled with Zn dust, yobyrine yields 3-ethyl indole and isoquinoline



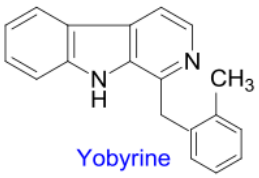
3-Ethyl Indole Isoquinoline

When yobyrine is oxidised with $KMnO_4$, it yields phthalic acid. On oxidation with CrO_3 it yields o-toluic acid.



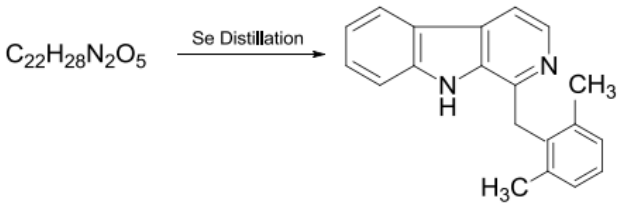
o-Toluic acid

Yobyrine gives condensation products with aldeydes indicating the presence of pyridine ring with a $–CH_2$ substituent adjacent to nitrogen atom. Thus the structure of yobyrine is



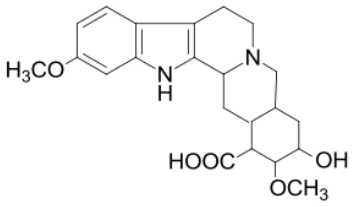
Yobyrine

Reserpic acid on dehydrogeation yields 11-hydroxy 16-methyl yobyrine indicating that –COOH group is present at C16. further, one of the methoxy group is present in the m-position to the NH group of the indole.



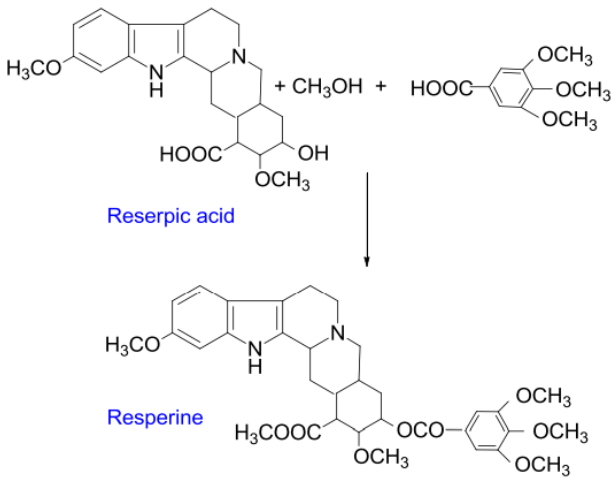
11-Hydroxy 16-methyl yobyrine

Further the COOH group and OH group are in m-position to each other. The COOH group is at C16. Hence the –OH group is at C18. The second methoxy group is t C17.



Reserpic acid

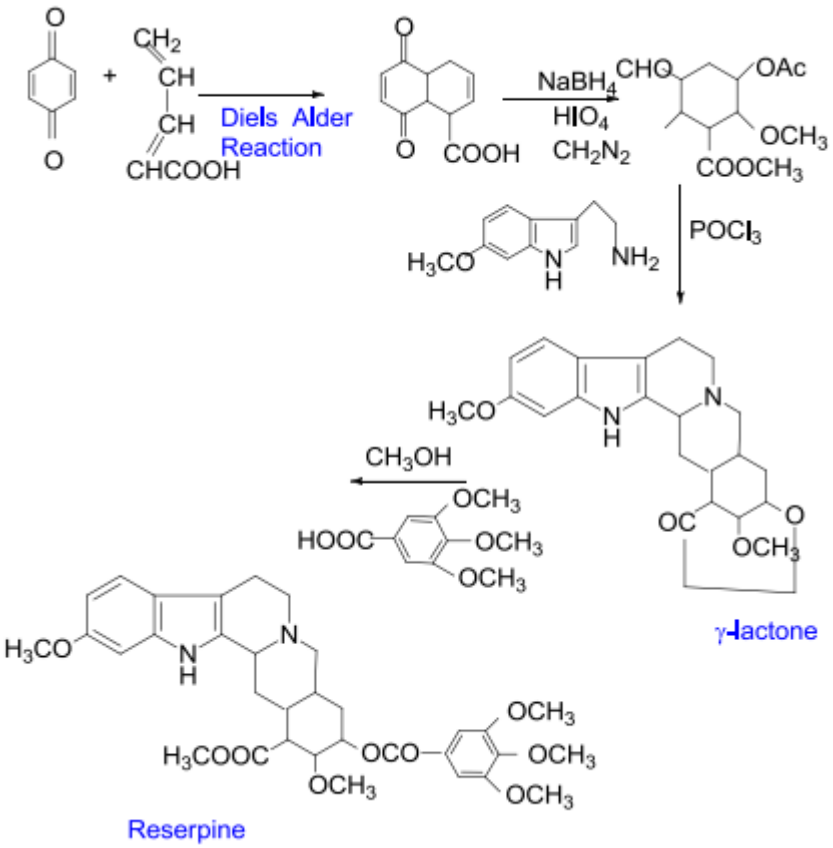
Structure of Reserpine



Reserpic acid

Resperine

Synthesis of Reserpine



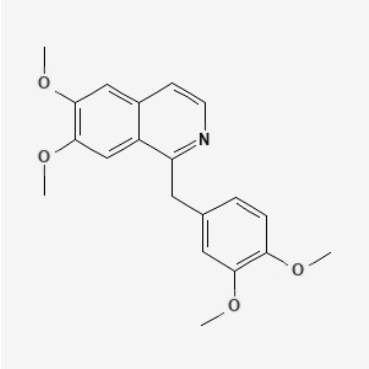
Papaverine

Papaverine is a benzylisoquinoline alkaloid that is isoquinoline substituted by methoxy groups at positions 6 and 7 and a 3,4-dimethoxybenzyl group at position

1. It has been isolated from Papaver somniferum. It has a role as a vasodilator agent and an antispasmodic drug. It is a benzylisoquinoline alkaloid, a member of isoquinolines and a dimethoxybenzene.

2. Papaverine is a natural product found in Papaver rhoeas, Papaver armeniacum, and other organisms with data available.

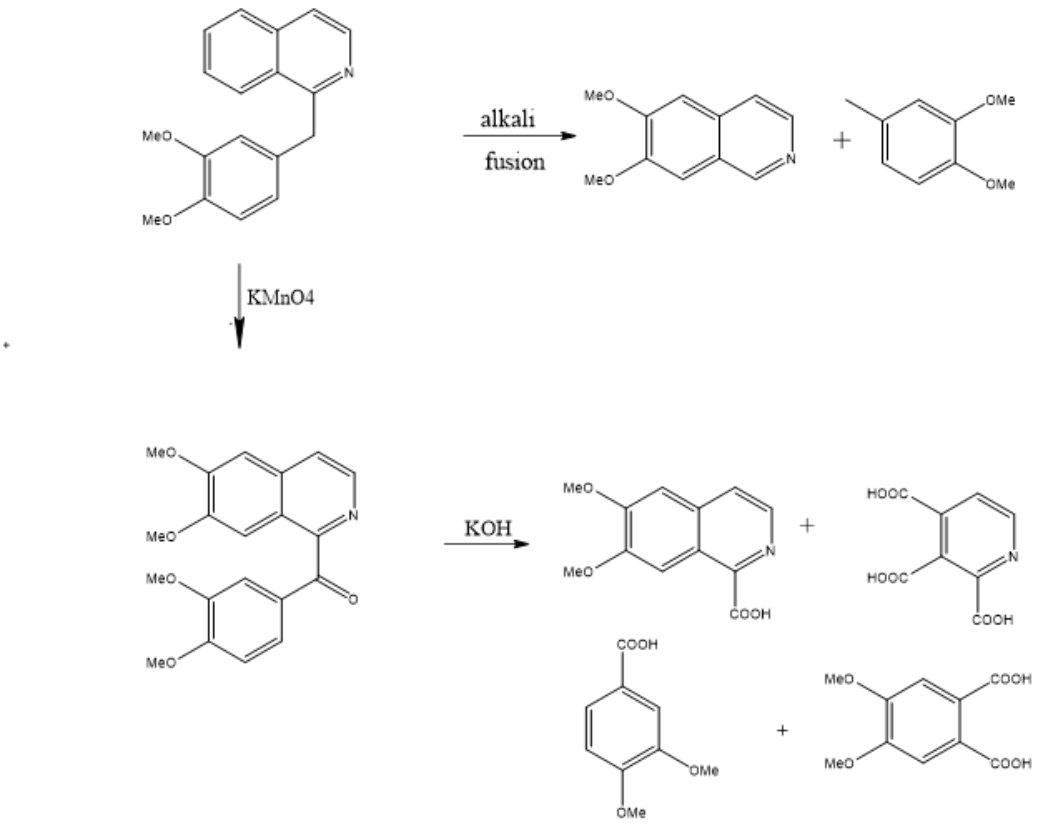
Structure



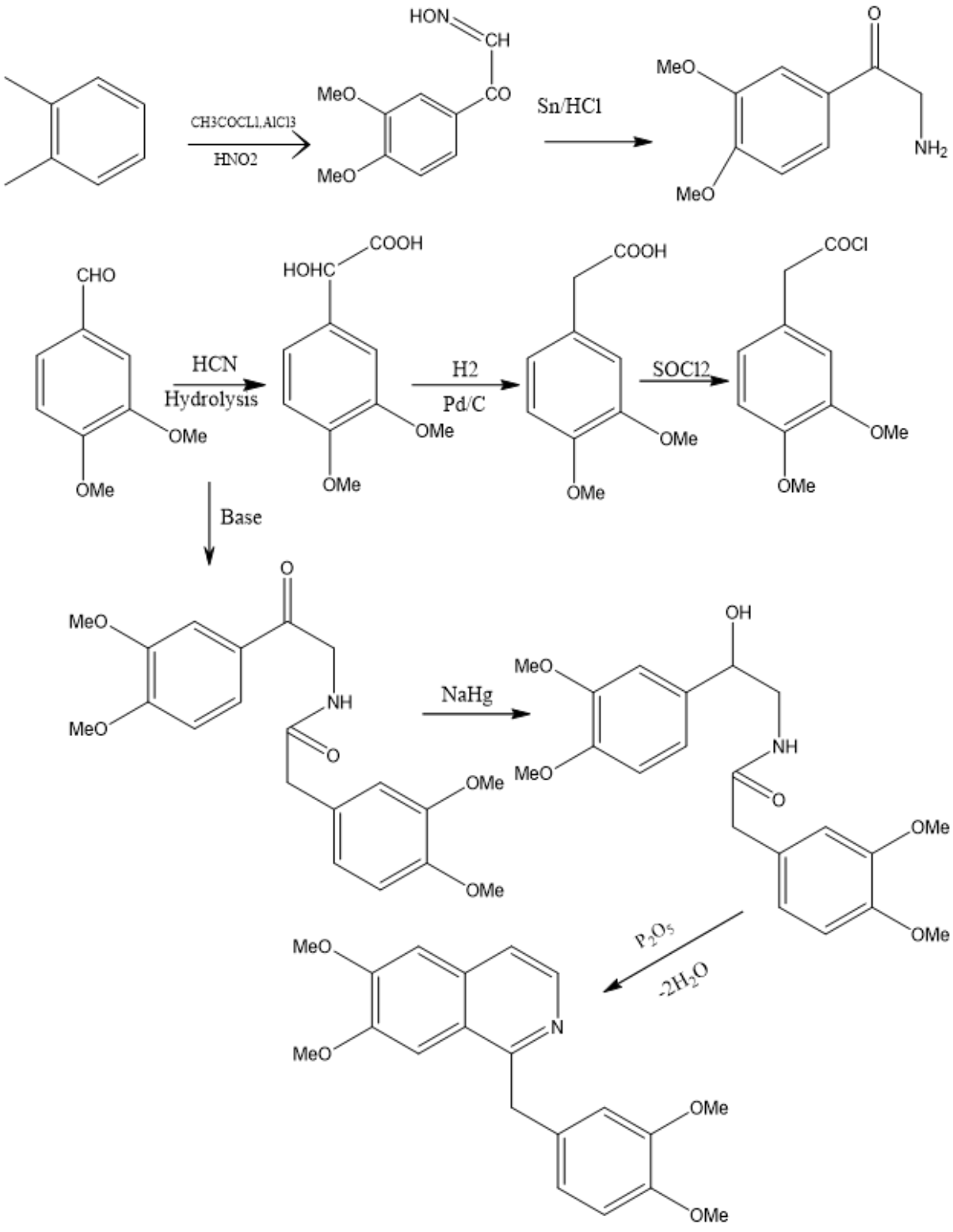
pH

Optimal pH for storage of papverine solutions: 2.0-2.8

Structural elucidation



Total Synthesis



Applications

Papaverine is a vasodilator. It relaxes the blood vessels which makes it easier for blood to pass through them. It is used to treat certain gallbladder or stomach.conditions that cause blood vessels to spasm. This medicine may also be used to relieve the spasms associated with urinary tract gallbladder or stomach.

Papaverine is used as an erectile dysfunction drug, alone or sometimes in combination. Papaverine, when injected in penile tissue, causes direct smooth muscle relaxation and consequent filling of the corpus cavernosum with blood resulting in erection. A topical gel is also available for ED treatment.

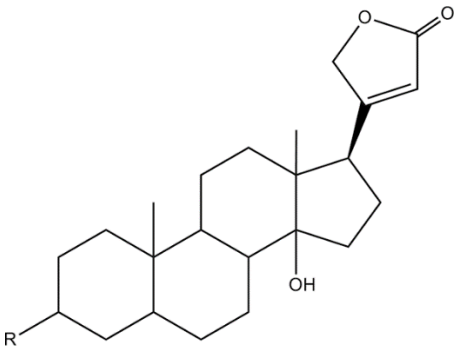
Unit – V

Plant Glycosides and Marine Drugs

Glycosides

Glycosides are certain molecules in which a sugar part is bound to some other part. Glycosides play numerous important roles in living organisms. Formally, a glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group and form glycosidic bonds via an O-glycosidic bond or an S-glycosidic bond; glycosides involving the latter are also called thioglycosides and the molecules containing an N-glycosidic bond are known as glycosylamines. The addition of sugar be bonded to a non-sugar for the molecule to qualify as a glycoside, The sugar group is then known as the glycone and the non-sugar group as the aglycone or genin part of the glycoside. The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide). The glycone and aglycone portions can be chemically separated by hydrolysis in the presence of acid. There are also numerous enzymes that can form and break glycosidic bonds. The most important cleavage enzymes are the glycoside hydrolases, and the most important synthetic enzymes in nature are glycosyltransferases. Mutant enzymes termed glycosynthases have been developed that can form glycosidic bonds. There are a great many ways to chemically synthesize glycosidic bonds.

Basic Ring System



	R
A	=O
B	OH
C	O Glc

Classification

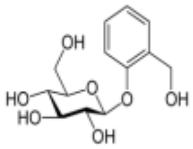
1. By glycone: If the glycone group of a glycoside is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide; etc. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted.

2. By type of glycosidic bond: Depending on whether the glycosidic bond lies "above" or "below" the plane of the cyclic sugar molecule, glycosides are classified as α-glycosides or β-glycosides. Some enzymes such as α-amylase can only hydrolyzed α-linkages; others, such as emulsin, can only affect β-linkages. Stereochemically these considered as a theoretical aspect, because the plants contain only β- glycosides

3. By aglycone:

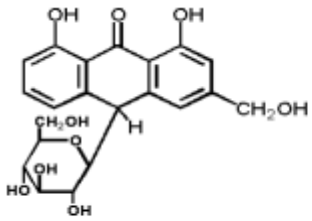
i. Steroidal glycosides or cardiac glycosides: Here the aglycone part is a steroidal nucleus. These glycosides are found in the plant genera Digitalis, Scilla, and Strophanthus. They are used in the treatment of heart diseases e.g. congestive heart failure and arrhythmia.

ii. Alcoholic glycosides: An example of an alcoholic glycoside is salicin which is found in the genus salix. Salicin is converted in the body into salicylic acid, which is closely related to aspirin and has analgesic, antipyretic and antiinflammatory effects.

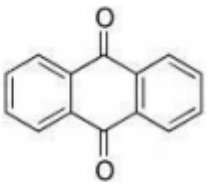


[Salicin ($C_{13}H_{18}O_7$) is an alcoholic β-glycoside that contains D-glucose**]**

iii. Anthraquinone glycosides: These glycosides contain an aglycone group that is a derivative of anthraquinone. They are present in senna, rhubarb and aloes; they have a laxative effect. E.g. Barbaloin.

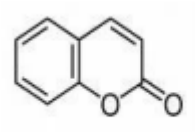


[Barbaloin]

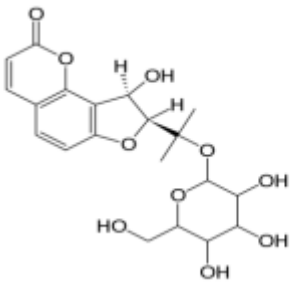


[Anthraquinone (9, 10-dioxoanthracene)]

iv. Coumarin glycosides: Here the aglycone is coumarin. An example is apterin which is reported to dilate the coronary arteries as well as block channels those obtained from dried leaves of Psoralia corylifolia have Main glycosides psoralin and corylifolin.

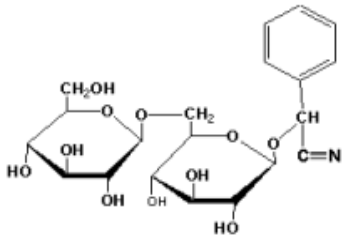


[Coumarin (benzopyrone)]



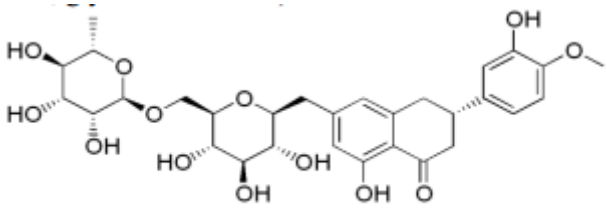
[Apterin]

v. Cyanogenic glycosides: In this case, the aglycone contains a cyanide group and the glycoside can release the poisonous hydrogen cyanide if acted upon by some enzyme. An example of these is amygdalin from almonds.



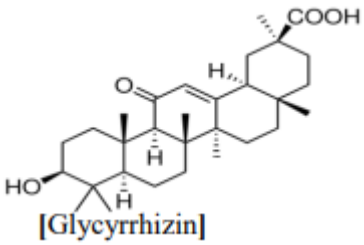
[Amygdalin]

vi. Flavonoids glycosides: Here the aglycone is a flavonoid. This is a large group of flavonoid glycosides. E.g. - Hesperidin (aglycone: Hesperetin, glycone: Rutinose).



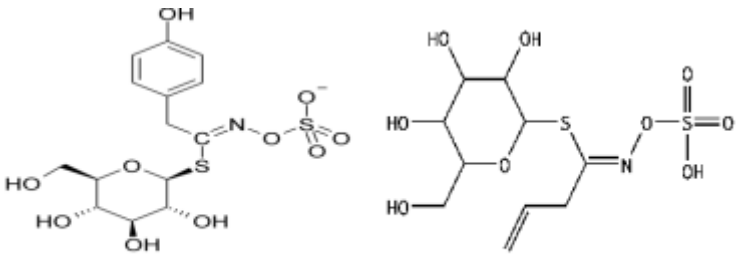
[Hesperidin]

vii. Soponins: These compounds give a permanent froth when shaken with water. They also cause hemolysis of red blood cells. Soponins glycosides are found in liquorice (Glycyrrhizin). Their medicinal value is due to their expectorant effect.



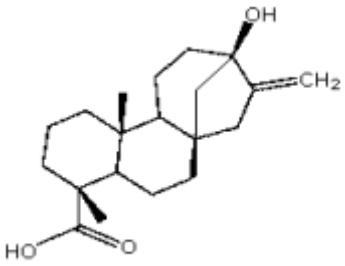
[Glycyrrhizin]

viii. Thioglycosides: As the name implies, these compounds contain sulfur. E.g.- sinigrin, found in black mustard, and sinalbin, found in white mustard.



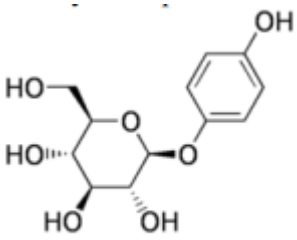
[Sinalbin] [Sinigrin]

ix. Steviol glycosides: These Steviol is sweet glycosides found in the stevia plant Stevia rebaudiana bertoni have 40- 300 times the sweetness of sucrose. These glycosides have steviol as the aglycone part. Glucose or rhamnose glucose combinations are bound to the ends of the aglycone to form the different compounds.



steviol

x. Phenolic glycosides: Here the aglycone is a simple Phenolic structure. E.g. - arbutin found in the Common Bearberry Arctostaphylos uva-ursi. It has a urinary antiseptic effect.



[Arbutin]

Isolation

The isolation and purification of constituents present in the extract depends upon the physical and chemical characteristics of the compound to be separated. The physical techniques employed for this purpose are:

i) Fractional Crystallization

ii) Fractional Distillation

iii) Fractional Liberation

iv) Paper Chromatography

v) Thin Layer Chromatography

vi) Column Chromatography

vii) High Performance Thin Layer Chromatography (HPTLC)

viii) High Performance Liquid Chromatography (HPLC)

i) Fractional Crystallization:

Phytopharmaceuticals crystallize out at the point of supersaturation in the solvent in which they are soluble. Slow concentration, slow evaporation, refrigeration are the processes that are involved in the crystallization of products.

ii) Fractional Distillation

Fractional distillation is useful for the separation of volatile oils and hydrocyanic acid from plant materials. The components like citral, citronellal and eucalyptol are separated by fractional distillation.

iii) Fractional Liberation

Some groups of compounds having the tendency of precipitation and comes out the solvent. For example, a mixture of alkaloids gets liberated when they treated with excess of alkali.

iv) Paper Chromatography

It is based on principle of the partition chromatography in which the components of the extract get distributed between two liquid phases. One phase is stationary liquid which is usually water held in the fibres of Whatman filter paper and other is a mobile phase. It is used for the study of flavonoids, glycosides, alkaloids, carbohydrates, amino acids and proteins.

v) Thin Layer Chromatography

It is used for qualitative screening of different plant extracts. The stationary phase is generally silica gel G supported over glass or aluminium plates and mobile phase is different solvents or mixture of solvents. This technique is used for isolation of compounds on small scale in the laboratories.

vi) Column Chromatography It comprises of the open column in which stationary phase is packed in the column and different solvents with different polarity are used for the separators

of mixture of components. This is useful for the purification of natural products, biochemicals, vitamins, hormones etc.

vii) High Performance Thin Layer Chromatography (HPTLC) This is the advanced technique of TLC. In HPTLC, samples to be chromatographed are applied to the self coated plates in the form of a band without damage to the layer. HPTLC is highly used in the study of natural products, herbal cosmetics, and it is also effective in the analysis of pesticides, and used in biochemical research.

viii) High Performance Liquid Chromatography (HPLC) This technique is mainly applicable in the microanalysis of chemical constituents. This is basically a form of column chromatography where 243 Pharmacognosy and Phytopharmaceuticals packing material is of uniform particle size and regular shape. The columns are finely packed and pressure up to 5000 lb in^{-1} can be employed to achieve acceptable flow rates.

Properties

Glycosides possess a range of physical and chemical properties, influenced by both the glycone and the aglycone components. Common properties include:

1. Solubility:

- Glycosides are generally soluble in water due to the presence of the hydrophilic sugar moiety.
- Their solubility in organic solvents varies depending on the nature of the aglycone.

2. Stability:

Glycosides are relatively stable under normal conditions but can be hydrolyzed by acids, bases, or enzymes (glycosidases) to yield the sugar and aglycone.

3. Optical Activity:

Many glycosides exhibit optical activity due to the chiral centers in the sugar moiety.

4. Taste:

Glycosides can have various tastes, including bitter (e.g., cardiac glycosides), sweet (e.g., glycyrrhizin), and tasteless.

5. Biological Activity:

Glycosides often have significant biological and pharmacological activities, such as cardiac glycosides used in heart disease treatment and saponins used for their detergent properties.

Qualitative Analysis

The 100 mg of extract is dissolved in 5 ml of distilled water and filtered.

1. Molish's test: Two drops of alcoholic solution of α–naphthol are added to 2 ml of filtrate and 1 ml of concentrated sulpuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.
2. Fehling's test: An equal volume of Fehling solution A and B are added to and equal volume of filtrate and it should boil in a water bath. The formation of red precipitate indicates the presence of sugar.
3. Barfoed's test: An equal volumes of filtrate and Barfoed's reagent are mixed and heat in a water bath. A red precipitate confirms the presence of sugar.
4. Benedict's test: A mixture of plant extract and the Benedict reagent is heated on water bath for 2 minutes and a characteristic colored precipitate indicates the presence of sugar. For detection of glycosides, the plant extract is hydrolyzed with concentrated hydrochloric acid and the filtrate should be subjected to following tests.
5. Borntrager's test: A 2 ml of filtrate is mixed with 3 ml of chloroform and 10% ammonia is added to that. A pink color solution indicates the presence of glycosides.
6. Legal's test: The plant extract is dissolved in pyridine and sodium nitroprusside is added to that. Then the solution is made alkaline using 10% sodium hydroxide and pink color solution proves the presence of glycoside.

Pharmacological activity of Senna glycosides

1. Chemical Composition

Senna glycosides primarily consist of sennosides, which are glycosidic compounds derived from anthraquinones. The most notable ones include sennoside A and sennoside B. These compounds are not directly active but are converted into active forms by gut bacteria.

2. Mechanism of Action

- Stimulation of Intestinal Motility: Senna glycosides stimulate the enteric nervous system, enhancing peristalsis, which is the wave-like muscle contractions that move food through the intestines.

- Increased Fluid Secretion: They promote secretion of mucus and electrolytes, increasing the water content in the intestines, leading to softer stools.
- Effects on Gut Microbiota: Some studies suggest that senna may influence the composition of gut microbiota, although the clinical significance of this is still under investigation.

3. Therapeutic Uses

- Constipation: Senna is widely used for the short-term treatment of constipation, particularly in situations where rapid relief is needed (e.g., preparation for medical examinations).
- Bowel Preparation: It can be part of bowel prep regimens before surgeries or diagnostic procedures.

4. Dosage and Administration

Senna is available in various forms, including tablets, capsules, and teas. Typical dosages can vary, but it is generally recommended to start with the lowest effective dose to minimize side effects.

5. Safety and Side Effects

- Short-term Use: Generally considered safe for short-term use, but prolonged use is discouraged due to the risk of developing tolerance and dependence.
- Side Effects: Common side effects include abdominal cramps, diarrhea, and nausea. Chronic use can lead to more severe issues such as electrolyte imbalances (particularly hypokalemia) and potential colonic atony.
- Contraindications: Not recommended for individuals with bowel obstructions, abdominal pain of unknown origin, or inflammatory bowel diseases (e.g., Crohn's disease, ulcerative colitis).

6. Drug Interactions

Senna may interact with other medications, particularly those affecting electrolyte balance (e.g., diuretics), and may enhance the effects of other laxatives. It’s crucial to consult a healthcare provider before combining senna with other treatments.

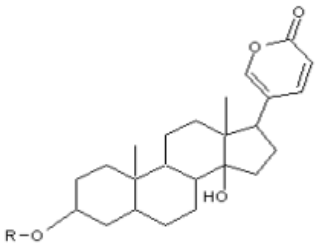
Cardiac glycosides

Increasing the force of contraction of the heart (positive inotropic activity) is very important for most heart failure patients. There are several mechanisms by which this could be achieved. Cardiac steroids are perhaps the most useful and Phosphodiesterase inhibitors, such as amrinone and milrinone, have also been explored and so are direct adenylate cyclase stimulants, such as forskolin. These drugs all act by affecting the availability of intracellular Ca^{+2} for myocardial contraction or increasing the sensitivity of myocardial contractile proteins. The cardiac glycosides are an important class of naturally occurring drugs whose actions include both beneficial and toxic effects on the heart. Plants containing cardiac steroids have been used as poisons and as cardiotonic agents which are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation and flutter.

Congestive Heart Failure: Heart diseases can be primarily grouped into three major disorders: cardiac failure, ischemia and cardiac arrhythmia. Cardiac failure can be described as the inability of the heart to pump blood effectively at a rate that meets the needs of the metabolizing tissues. This occurs when the muscles that perform contraction and force the blood out of heart are performing weakly. Thus cardiac failures primarily arise from the reduced contractility of heart muscles, especially the ventricles. Reduced contraction of heart leads to reduced heart output but new blood keeps coming in resulting in the increase in heart blood volume. The heart feels congested. Hence the term congestive heart failure. Congested heart leads to lowered blood pressure and poor renal blood flow. This results in the development of edema in the lower extremities and the lung (pulmonary edema) as well as renal failure.

Cardiac glycosides are divided into two main types:

1. Bufadienolides are C24 steroids



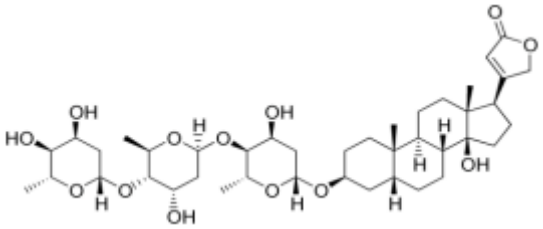
- The primary cardiac glycoside present in Helleborus is the bufadienole, hellebrin.
- Hellebrigenin, the aglycone of hellebrin is more potent than the glycoside itself

2. Cardenolides (most prevalent) are C23 steroids.

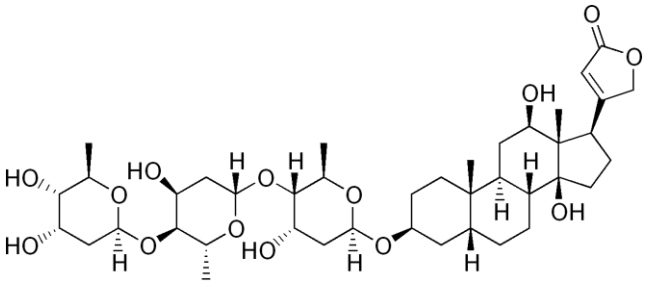
- Cardenolides have a hormonal nature as substances. Their effects are on the heart and kidney.
- Strong, bitter and disagreeable taste.
- Cardiotonic = affect contractions of the heart muscle.
- Break down in fermentation by enzymatic action.
- Treatment: atropine and activated charcoal, lidocaine for H. viridis
- Assumed mechanism of action: inhibition of the Na^+, K^+, -ATPase resulting in increased intracellular sodium and subsequent intracellular calcium leading to enhanced muscle contraction in cardiac tissue.
- Cardenolide structure: Cardenolides are C23-steroids with methyl groups at C-10 and C-13 and a five-membered lactone at C-17. They are aglycone constituents of cardiac glycosides and must have at least one double bond in the molecule.
- The class includes cardadienolides and cardatrienolides.
- Members include:
  - Digitoxin
  - Acetyldigoxins
  - Acetyldigitoxins
  - Digoxigenin
  - Digitoxigenin
  - Strophanthins
  - Digoxin
  - Cymarine
  - Acetyldigoxins

Digoxin & Digitoxin

Digitoxin is a cardiac glycoside. It has similar structure and effects to digoxin (though the effects are longer-lasting). Unlike digoxin (which is eliminated from the body via the kidneys), it is eliminated via the liver, so could be used in patients with poor or erratic kidney function. However, it is now rarely used.



Digitoxin



Digoxin

Toxicity: Digitoxin exhibits similar toxic effects to the more-commonly used digoxin, namely: anorexia, nausea, vomiting, diarrhoea, confusion, visual disturbances, and cardiac arrhythmias.

Actions:

The main pharmacological effects of digoxin are on the heart. Extracardiac effects are responsible for many of the adverse effects. Its main cardiac effects are

- A decrease of conduction of electrical impulses through the AV node, making it a commonly used antiarrhythmic agent in controlling the heart rate during atrial fibrillation or atrial flutter
- An increase of force of contraction via inhibition of the Na+ /K+ ATPase pump.

Mechanism of action

Digoxin binds to a site on the extracellular aspect of the α-subunit of the Na+ /K+ ATPase pump in the membranes of heart cells (myocytes). This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. The proposed mechanism is the following: inhibition of the Na+/K+ pump leads to increased intracellular Na+ levels, which in turn slow down the extrusion of Ca2+ by the Na+ /Ca2+ exchange pump that relies on the high Na+ gradient. This effect causes an increase in the

length of Phase 4 and Phase 0 of the cardiac action potential, which when combined with the effects of Digoxin on the parasympathetic nervous system, lead to a decrease in heart rate.

Increased amounts of Ca2+ are then stored in the sarcoplasmic reticulum and released by each action potential, which is unchanged by digoxin. This leads to increased contractility of the heart. This is a different mechanism from that of catecholamines.

Digoxin also increases vagal activity via its action on the central nervous system, thus decreasing the conduction of electrical impulses through the AV node. This is important for its clinical use in different arrhythmias.

Clinical use:

i. The most common indications for digoxin are probably atrial fibrillation and atrial flutter with rapid ventricular response. The arrhythmia itself is not affected, but the pumping function of the heart improves owing to improved filling.

ii. The use of digoxin in heart problems during sinus rhythm. In theory the increased force of contraction should lead to improved pumping function of the heart. Digoxin is no longer the first choice for congestive heart failure, but can still be useful in patients who remain symptomatic despite proper diuretic and ACE inhibitor treatment. It was proven to be ineffective at decreasing morbidity and mortality in congestive heart failure.

Dose:

1. Digoxin is usually given by mouth, but can also be given by IV injection in urgent situations (the IV injection should be slow, heart rhythm should be monitored).
2. The half-life is about 36 hours; digoxin is given once daily, usually in 125 μg or 250 μg dosing. Adverse effects: The occurrence of adverse drug reactions is common, owing to its narrow therapeutic index. Adverse effects are concentration-dependent which are also more common in patients with low potassium levels (hypokalemia), since digoxin normally competes with K+ ions for the same binding site on the Na+ /K+ ATPase pump.

Common adverse effects:

1. (≥1% of patients) include: loss of appetite, nausea, vomiting, diarrhea, blurred vision, visual disturbances (yellow-green halos), confusion, drowsiness, dizziness, nightmares,

agitation, and/or depression. Less frequent adverse effects (0.1%–1%) include: acute psychosis, delirium, amnesia, shortened QRS complex, atrial or ventricular extrasystoles, paroxysmal atrial tachycardia with AV block, ventricular tachycardia or fibrillation, heart block.

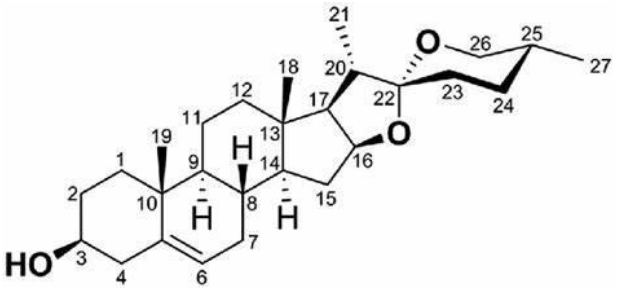
2. But rarely seen adverse effect of digoxin is a disturbance of colour vision (mostly yellow and green colour) called xanthopsia.

3. Digoxin has potentially dangerous interaction with verapamil, amiodarone, erythromycin, epinephrine antimalarial medication and Hydroxychloroquine (as would be injected with a local anesthetic).

Steroidal saponins glycosides

Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic. They are mainly produced by plants but also by lower marine animals and some bacteria, but get their name from the soapwort plant (Saponaria), the root of which was used historically as a soap (Latin sapo means soap). The combination of hydrophobic or fat-soluble sapogenin and hydrophilic or water-soluble sugar part enhances the foaming ability of saponins.

Diosgenin



Diosgenin (DG, 25R-spirost-en-3β-ol) is a C27 spiroketal steroid sapogenin belonging to a family of spirostanol steroidal compounds. Its molecular formula is $C_{27}H_{42}O_3$ with a relative molecular mass of 414.62. DG is a white needle crystal or light amorphous powder with a proven thermal and chemical stability under various physical conditions. DG is relatively stable against temperature and light exposure. However, DG is destabilized when it is exposed to hydrochloric acid. DG is strongly hydrophobic (with Log P = 5.7), and it is insoluble in water. The solubility of DG is around 0.7 ng/mL in aqueous medium. However, it

is highly soluble in most nonpolar organic solvents (such as chloroform, dichloroethane, propanol, ethyl acetate, and propylacetate) and in partially polar solvents (such as acetone, methanol, and anhydrous ethanol).

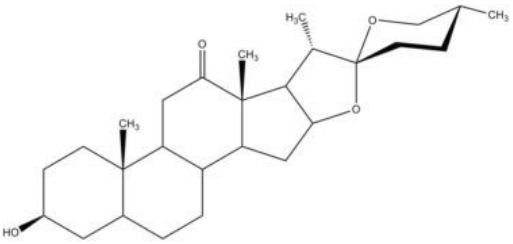
Sources

Primary sources of diosgenin (DG) include the Dioscorea species, Heterosmilax species, and Trigonella foenum-graecum, although DG and related steroidal sapogenins can be commercially obtained from tubers of various Dioscorea species. DG is present in high levels in tubers of various wild yams (D. villosa Linn). A total of 137 types of Dioscorea species contain DG. Of them, 41 contain DG at more than 1%. The seeds of fenugreek (T. foenum graecum Linn) and the rhizomes of D. zingiberensis are also important sources of DG. In addition, Trillium govanianum and Costus speciosus contain around 2.5% and more than 2.12% of DG, respectively. DG is mainly generated by the hydrolysis of steroidal saponins in the presence of a strong acid, base, or enzyme catalyst. Currently, microbial transformation is a promising method for the production of DG because of its environmentally friendly, highly specific, and mild reaction conditions at a low cost.

Biosynthesis

DG is biosynthesized from cholesterol via the isoprenoid pathway in several plant species. The biosynthesis of DG starts with acetyl CoA. It involves several steps to generate squalene that cyclizes to yield lanosterol. Lanosterol is further catalyzed to cholesterol by various enzymes. Cholesterol is sequentially converted to glucoside furostanols and spirostanols. These glycosides are eventually converted to spirostanols after the elimination of the glucose molecules at C26, resulting in ring closure during the catalysis of glucosidases. DG aglycone may convert to glycoside forms with mono-, di-, or trisaccharides known as saponins (Figure 1, Compounds 2 and 3; Compound 3 is also called dioscin). The attachment of a carbohydrate moiety improves both the solubility and potency of DG.

Hecogenin



Hecogenin is a naturally occurring steroid sapogenin derived from various plants, particularly those in the Agave family, such as Agave sisalana. It has garnered interest due to its potential pharmacological properties and applications. Here's an overview of hecogenin:

1. **Chemical Structure**

- **Type**: Hecogenin is a steroidal sapogenin, characterized by a steroid backbone. Its molecular formula is C27H42O4.
- **Structure**: The compound features a steroidal core with specific functional groups that contribute to its biological activity.

2. **Pharmacological Activities**

- **Anti-inflammatory Effects**: Hecogenin has been studied for its anti-inflammatory properties, which may make it beneficial in conditions characterized by inflammation.
- **Antimicrobial Properties**: Some studies suggest that hecogenin exhibits antimicrobial activity against various pathogens, though more research is needed in this area.
- **Potential in Cancer Therapy**: Preliminary research indicates that hecogenin may have anticancer properties, with potential mechanisms involving apoptosis (programmed cell death) in cancer cells.
- **Cholesterol-Lowering Effects**: Hecogenin may help reduce cholesterol levels, contributing to cardiovascular health.

3. **Applications in Medicine**

- **Pharmaceutical Use**: Due to its biological activities, hecogenin is of interest in drug development, particularly for anti-inflammatory and anticancer agents.
- **Cosmetic Use**: Its potential skin benefits have led to interest in hecogenin for use in cosmetics and topical formulations.

4. **Mechanism of Action**

The precise mechanisms of action of hecogenin are still being investigated. Some proposed mechanisms include:

- **Inhibition of inflammatory mediators**: Hecogenin may modulate pathways that lead to the production of pro-inflammatory cytokines.
- **Interaction with cell signaling pathways**: It may influence various signaling pathways involved in cell growth and apoptosis.

5. **Safety and Toxicity**

- **Toxicity Studies**: Research on the safety profile of hecogenin is limited, and while it appears to have a favorable safety margin, more extensive studies are needed to fully assess its toxicity and side effects.

Plant pigments

Plant pigments is a term used to refer to coloured molecules naturally present in plant parts that not only impart colour to these parts but also help the plant perform some important metabolic activities like photosynthesis and promote plant growth. These pigments are formed by biosynthesis in plants. All plant pigments are organic, i.e. are made up of carbon chains. The colour of the pigment depends on the ratio of absorption to scattering of light. For example, taking carotenoids, the most commonly found plant pigment, the molecules absorb the green and blue part of the visible spectrum and scatter the red light thus appearing red in colour to our eyes.

Occurrence

The most noticeable and widespread pigments of plants are chlorophylls. These are cyclic tetrapyrrole pigments chelated with magnesium, and they share structural features with the haem and bile pigments of animals. The carotenoids also associated with photosynthesis and additionally providing bright colours to flowers and fruits. Carotenoids are terpenoid pigments present in all photosynthetic plants and they also occur in photosynthetic bacteria such as Erwinia and Rhodobacter. Annual production of carotenoids by plants, algae and dinoflagellates has been estimated at 100 million tons. The flavonoids are phenylpropanoid compounds of widespread occurrence. There are several major classes of flavonoids;

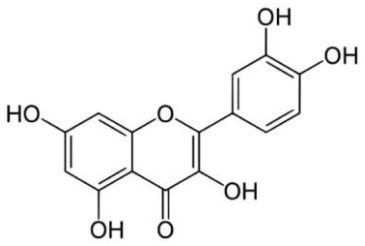
however, only a few of these provide pigments to plants, in particular the anthocyanins and proanthocyanidins (condensed tannins). The betalains are nitrogenous pigments that are the most taxonomically restricted of the major plant pigment groups, being found only in a few families of the order Caryophyllales and some fungi. Their occurrence is mutually exclusive to that of the anthocyanins.

General Methods of Structure Determination

- **Spectroscopic methods:** provide quick crude identification of pigments in an extract or mixture. Multiple spectroscopic methods are available at **Lifeasible**:
  - **Absorbance spectroscopy**: based on differential absorbance spectra of different plant pigments, this is the simplest way to identify and quantify major pigments in a given sample.
  - **Fluorescence spectroscopy**: with adjustable wavelength settings, this method allows targeted detection of pigments by selective excitation.
  - **Infrared spectroscopy**: allows identification of pigments based on particular structural features.
- **Chromatographic methods**: unlike spectroscopic methods, which are performed within crude extractions, chromatography allows separation of individual components from a mixture, and therefore gives higher resolutions.
  - **Open column**: open columns can be filled with calcium hydroxide or hydroxylapatite for the separation of different types of plant pigments.
  - **Thin-layer chromatography (TLC)**: feasible for separating compounds with distinguished structures, TLC can also be used as a pre-treatment for cleaning of total sample extractions.
  - **High-performance liquid chromatography (HPLC)**: requiring minimal sample preparation, HPLC ensures precise detection with high sensitivity, high resolution and less time cost.
- **Mass spectrometry (MS)**: modern MS technology is becoming increasingly popular for the characterization of plant pigments, as it allows precise, efficient identification and quantification within a single sample set.

Isolation and Synthesis of Quercetin

Quercetin is a unique bioflavonoid that has been extensively studied by researchers over the past 30 years. Bioflavonoids were first discovered by Nobel Prize laureate Albert Szent Gyorgyi in the year 1930. Flavonoids belong to a group of natural substances with variable phenolic structure and are found in the fruits, vegetables, grains, bark roots, stem, flowers, tea and wine. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for their attractive colors of flowers, fruits and leaves. Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring (A) condensed with a six membered ring (C), which in the 2-position carries a phenyl ring (B) as a substituent. The Flavonoids can be divided into various classes on the basis of their molecular structures. Six-member ring condensed with the benzene ring is either a-pyrone (flavonols and flavonones) or its dihydroderivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3- position). Flavonols differ from flavonones by hydroxyl group the 3-position and C2-C3 double bonds. Flavonoids are often hydroxylated in position 3, 5, 7, 2', 3', 4', 5'. Methylethers and acetylesters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be Lrhamnose, D-glucose, glucor-hamnose, galactose or arabinose. Flavonoids are mainly divided into seven major groups. One of the best described flavonoids, Quercetin is a member of this group.



Quercetin is found in abundance in onions, broccoli, apples and berries. The second group is flavanones, which are mainly found in citrus fruits. An example of a Flavonoid in this group is naringinin. Flavonoids belonging to the catechins are mainly found in green and black tea and in red wine, whereas, anthocyanins are found in strawberries, other berries, grapes, wines and tea.

Quercetin, the most abundant of the flavonoids (the name comes from the Latin –quercetum, meaning oak forest, quercus oak) consists of 3 rings and 5 hydroxyl groups. Quercetin is a member of the class of flavonoids called flavonoles and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, hesperidins, Naringenin and tangeritin. It

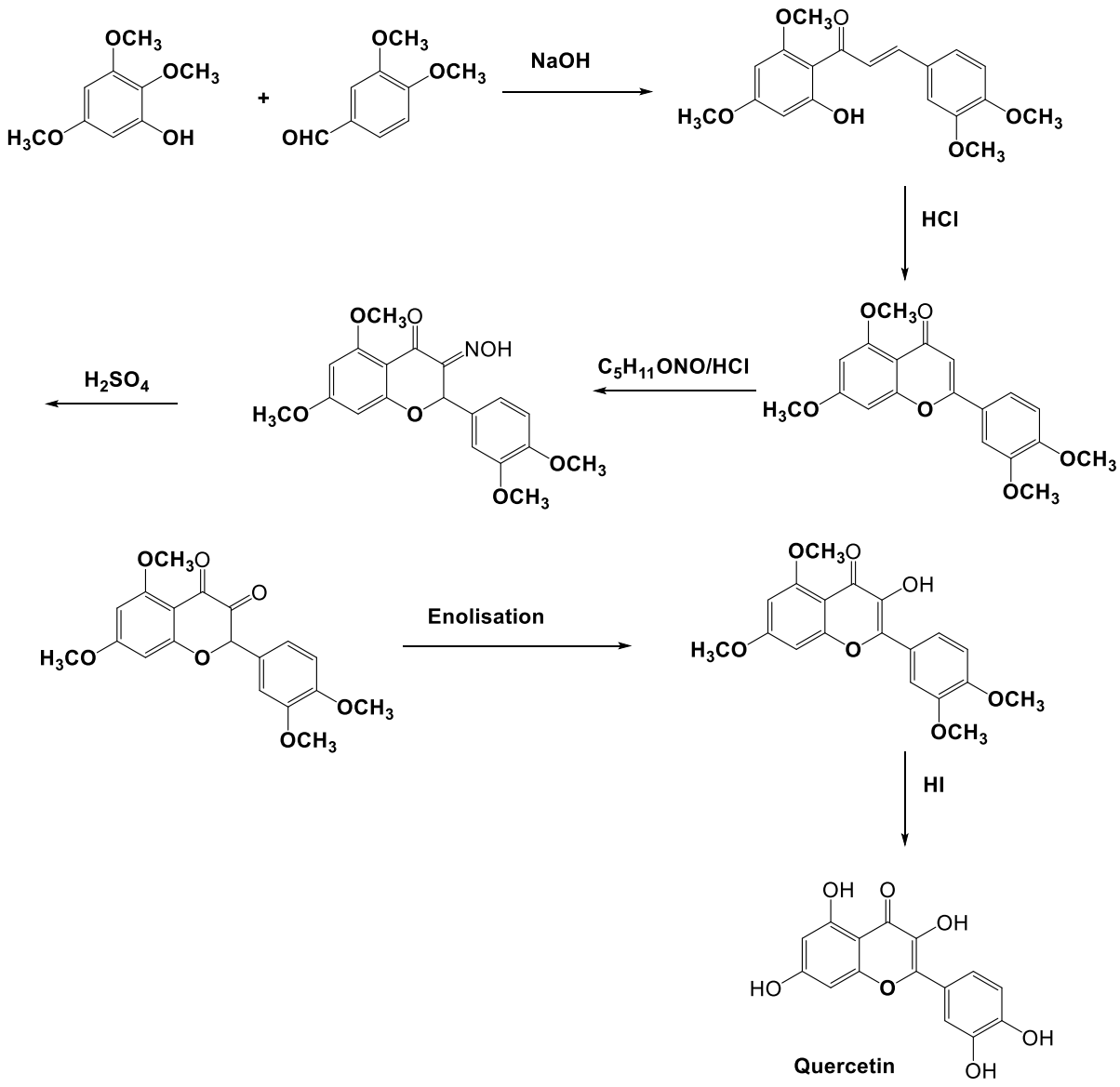
is widely distributed in the plant kingdom in rinds and barks. Quercetin itself is an aglycon or aglucone that does not possess a carbohydrate moiety in its structure.

Quercetin is typically found in plants as glycone or carbohydrate conjugates. Quercetin glycone conjugates include rutin and thujin. Rutin is also known as Quercetin-3-rutinoside.Thujin is also known as quercitrin, Quercetin-3-L-rhamnoside and 3-rhannosyl qurcetin. Onions contain conjugates of Quercetin and carbohydrate iso rhamnetin including Quercetin-3-4'-di-o-beta glucoside, isorhamnetin-4'-o-beta glucoside and Quercetin-4'-o-beta glucoside.

Isolation

The powdered flower petals (300 g) were subjected to soxhlet extraction for 10 hours with methanol (2 lit). The extract was concentrated to a viscous solid mass under vacuum. The dried methanolic extract (104 g) was suspended in 100 ml distilled water and shaken in ultrasonic bath for 15 min. The viscous liquid was extracted successively with hexane (100 ml x 5) and ethylacetate (100 ml x10) in separatory funnel. The ethyl acetate layer was collected and the solvent was evaporated under reduced pressure in rotavapor to get crude ethyl acetate extract. The ethyl acetate extract (9 g) was chromatographed on a Sephadex LH-20 column filled with 100 g Sephadex and eluted with methanol. The sub-fractions were collected consisting of 10-12 ml. Each fraction was monitored by TLC (Silica gel GF254) using solvent system, butanol-acetic acid-water, BAW (4:1:5), ethylacetate-methanol-water, EMW (100:15:10), chloroform-acetic acid-water, CAW (10:9:1) and toluene-ethylacetate-formic acid, TEF (10:8:1). The sub-fractions were pooled into four major fractions on the basis of TLC character. The quercetin accumulated fraction F-4 (2.5 g) was further chromatographed on Sephadex LH-20 column and eluted with 75% methanol. sub-fractions were collected and pooled into four major fractions on the basis of TLC character. The fraction F-4-D which was found to contain quercetin was concentrated and recrystallized from hot water to get 37 mg yellow powder

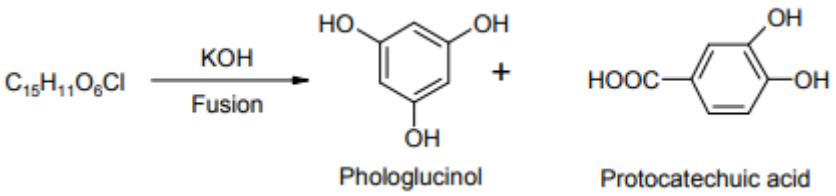
Synthesis



Cyanidin Chloride

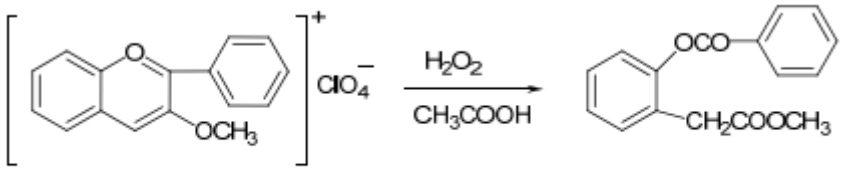
The sugar free anthocyanins are known as anthocyanidins. Anthocyanidins are isolated b hydrolysis of anthocyanins with HCl which give anthocyanidin chloride e.g. cyanidin chloride.

1. The molecular formula is $C_{15}H_{11}O_6Cl$.
2. Cyanidin chloride on treatment with $FeCl_3$ gives colour which indicate the presence of phenolic group.
3. Cyanidin chloride on methylation or acetylation produces pentamethyl cyanidin chloride or pentaacetyl cyanidin chloride, which confirms the presence of five phenolic groups in cyanidin chloride.
4. Cyanidin chloride on fusion with KOH gives phloroglucinol and protocatechuic acid.



Phloglucinol Protocatechuic acid

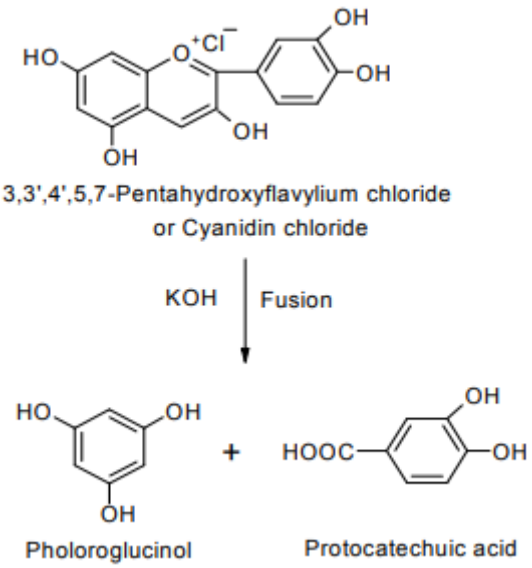
5. The formation of phloglucinol and protocatechuic acid confirms the presence of these two rings in cyanidin chloride. It also confirms the positions of four phenolic OH groups i.e. at 3', 4'(protocatechuic acid) and 5,7 (pholoroglucinol).



3-Methoxybenzopyrylium chlorate o-Benzoylhydroxyphenyl acetate

6. The position of fifth OH group in bezopyrylium nucleus has been proved by Diethey et.al. who oxidised 3-methoxy benzopyrylim chlorate with H2O2 in presence of acetic acid which gives O-benzoylhydoxyphenyl acetate.

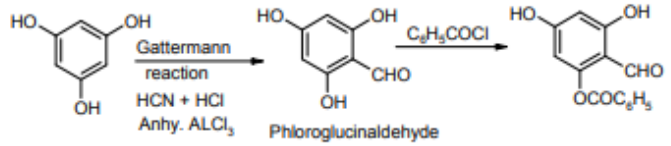
7. This oxidation rupture the ring between 2nd and 3rd carbon atom and thus OCH3 group is attached in resulting compound which confirms that 5th hydroxy group is present at position 3 in cynidin chlride. Therefore cyanidin molecule is made up of two phenyl rigs united through benzopyrylium nucleus. Thus cynidin chloride has following structure which explain the degradation reaction. Its structure is confirmed by its synthesis.



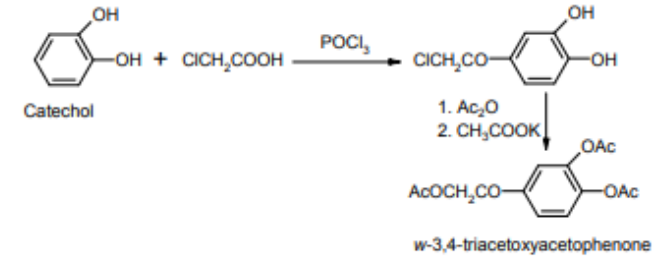
Synthesis

The above structure of cyanidin chlorde was confirmed by its synthesis. Robinson's Synthesis The various steps involved in synthesis are as follows:

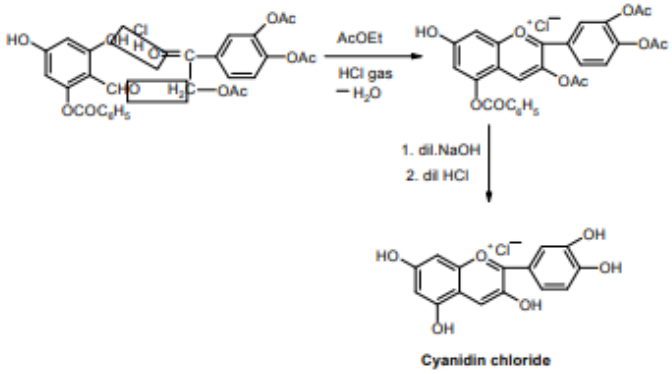
(I) Synthesis of 2-Benzoylphloroglucinaldehde

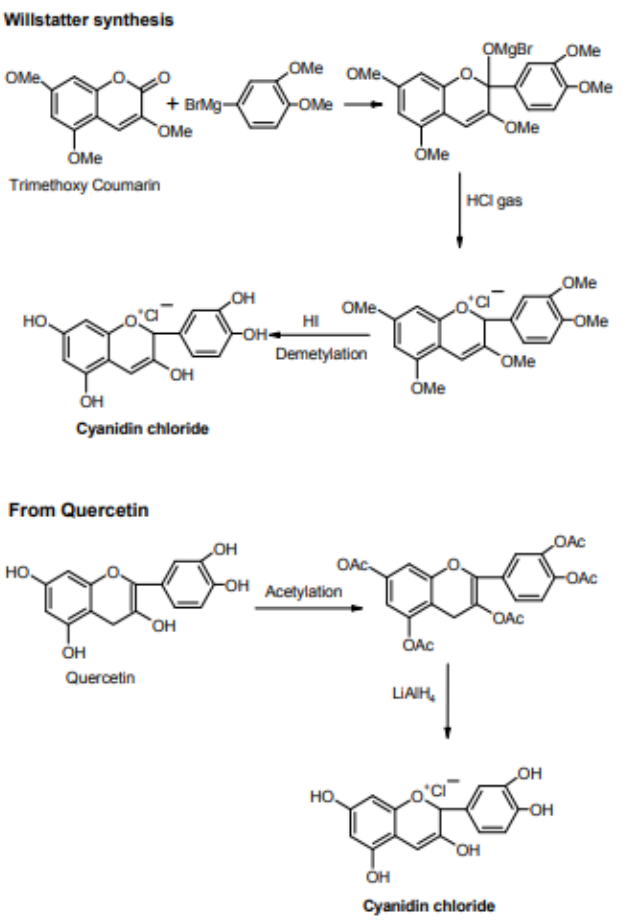


(II) Synthesis of ω - 3,4-Triacetoxyacetophenone



(III) Synthesis of Cyanidin chloride from (I) and (II)





Marine drugs -Selected Drug Molecules

Cardiovascular active Substances

Cardiovascular Active Drugs

During the past three decades a huge number of extracts, fractions and pure isolates from thousands of marine organisms were subjected to thorough cardiovascular screening in various research laboratories around the world. Interestingly, most of these compounds did exhibit cardiovascular activities perhaps as frequently as observed antibiotic and antineoplastic activities. Unfortunately, as on date hardly any compound could surface out and obtain the FDA approval as a potential drug.

The cardiovascular active drugs may be broadly classified under the following two categories, namely:

(a) Cardiotonics, and

(b) Hypotensive compounds.

These two categories shall now be treated separately as under:

(a) Cardiotonics

The cardiotonic compounds (or cardiotonics) showing positive response in either in vivo and/or in vitro inotropic activities on whole or part of the heart are included in this section. In general, the cardiotonics may be further sub-divided into two groups, namely:

(i) Marine peptides, and
(ii) Marine glycosides

(i) Marine Peptides

The age-old belief and classical concept that the steroidal nucleus present in the aglycone residues of either digitalis or strophanthus could only exhibit cardiotonicity was virtually turned down when 'marine peptides', obtained from coclenterates, such as: Anthopleura Xanthogrammica producing anthopleurins A, B and C* (also known as AP-A, B, C); and Anthopleura elegantissima giving AP-C.

Out of these three anthopleurins, only AP-A has been reported to be showing the most promising cardiotonic activity both on the isolated and in situ heart of different species. AP-A has a positive edge over the known cardiac glycosides because of its unique abilty to afford a sustained significant initropic effect under the ischemic conditions.

Furthermore, the anemonia toxin II (also termed as: Cardiotoxin-ll, ATX-II) isolated from Anemonia sulcata (Sea Anemone) comprising of at least 47 amino acids demonstrated a very close resemblance to AP-A. Besides, ATX-II was shown to exhibit a significant dose-dependent cardiotonic activities in different mammalian heart experiments.

The cardinal points of difference between AP-A and ATX-II peptides are as follows:

S.NO	Anthopleurin-A (AP-A)	Anemonia Toxin-II (ATX-II)
1	Alanine is present at the residue 38 .	Lysine is present at the residue 38.
2	No similar studies as for ATX-II where carried out.	Modification of the acidic-COOH function at the 38 residue leads to absolute inactivation of cardiotonic potency.
	If administered systemically gives an immune response; and	No such effect has been

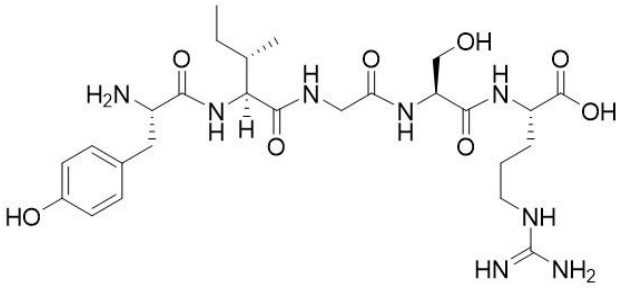
3	when given orally loses its activity due to gastric juice (acidic) in stomach.	observed.

Likewise, the two compounds viz., AP-A and ATX-II, display a number of similarities, namely: (a) conformation of the two peptides are almost identical; (b) amino-acid sequence similar; (c) disulphide bridges are alike; and (d) they have the same basicity. Based on these striking points of similarities one may rightly justify the very identical profiles of cardiotonic activity of the said two peptides.

A host of other compounds showing cardiotonic activities belonging to the class of 'marine peptides' are, namely: laminin, octopamine, saxitoxin and autonomium chloride. These compounds shall now be treated individually in the sections that follow:

A. Laminin:

Chemical Structure



Biological Source: Laminin is obtained from a marine algae Laminaria angustata.

Characteristic Features:

1. It is the abundant structural component of the basal lamina.

2. It is critical to the stability of the extracellular matrix and to the adhesion of cells to the basement membrane.

3.It belongs to the family of heterotrimeric glycoproteins composed of a heavy chain, designated as a (also known as A) and 2 light chains, designated as β (B1) and γ (B2), which are linked by disulphide bonds to form an asymetrical cross-shaped structure.

4. Eight genetically distinct laminin subunits have been identified, namely: $\alpha_1, \alpha_2, \alpha_3$, $\beta_1, \beta_2, \beta_3, \gamma_1$ and γ_2 .

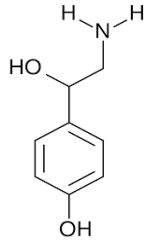
Uses :

l. It shows hypotensive effect.

2. It also exhibits diverse biological activities.

Octopamine

Chemical Structure



Biological Sources: Octopamine is found in the salivary glands of Octopus vulgaris, Octopus maropus and of Eledone moschata.

Characteristic Features

1. It is obtained as crystals from hot water that gets changed at about 160^0 to a compound which melts above 250^0 (decomposes).

2. Its specific optical rotation $[\alpha]_D^{25}$ - 56.0^0 (0.1 N.HCI); and — 37.4^0 (water).

DL (±)-Octopamine Hydrochloride ($C_8H_{11}NO_2.HCL$) (Epirenor, Norden, Norfen): It is obtained as crystals which gets decomposed at 170^0C. It is freely soluble in water.

Uses:

l. The natural D (-) form is almost 3 times more potent than the L(+) from in producing cardiovascular adrenergic responses in anaesthetized dogs and cats.

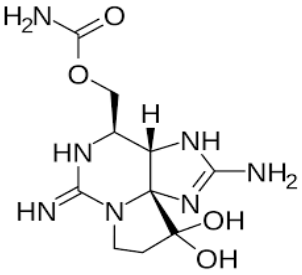
2. It gives distinct adrenergic responses.

3. In invertebrate nervous systems octopamine may function as a neurotransmitter.

C. Saxitoxin

Synonyms Mussel poison; Clam poison; Paralytic shellfish poison; Gonyoulax toxin; STX.

Chemical Structure



Biological Sources Saxitoxin is the powerful neurotoxin produced by the dinoflagellates Gonyaulax catenella or G. tamarensis, the consumption of which causes the California sea mussel Mytilus Californianus, the Alaskan butterclam Saxidomus gigantens and the scallop to become poisonous.

Characteristic Features

Savitoxin Dihydpochloride [$(C_{10}H_{17}N_7O_4)^{2+}.2HCL$]:

l. It is obtained as a white hygroscopic solid.

2. It has pka in water: 8:24, 11.60.

3. Its specific optical rotation $[\alpha]_D^{25}$ + 130^0.

4. It is extremely soluble in water, methanol; sparingly soluble in ethanol, glacial acetic acid; practically insoluble in lipid solvents.

5. It is found to be fairly stable in acid solutions; and decomposes rapidly in an alkaline media.

6. On boiling for 3 to 4 hours at pH 3 usually causes loss of activity.

Uses:

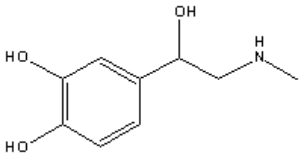
1. It exhibits a hypotensive effect.
2. It is invariably employed as a tool in the neurochemical research.

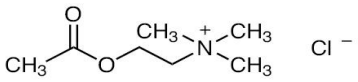
D. Autonomium Chloride:

Biological Source It is found in Verongia fistularis.

Characteristic Features

Autonomium chloride possesses an isosteric structure of adrenaline and acetylcholine as given below:





Adrenaline Acetylcholine

Uses:

l. It exerts both u- and ß-adrenergic effects.

2. Autonomium chloride also exhibits cholinergic action.

3. It distinctly shows CNS stimulant activity in mice which action is evidently exhibited by an apparent substantial increase in the spontaneous motor activity (SMA).

4. By virtue of the unique dual effects of adrenergic and cholinergic it may prove to be an asset in the control, management and regulation of the behaviour of heart i.e., cardiotonic effect.

S.No	Biological Source	Compound	Uses
1	Actinia equine	A polypeptide with 147 amino acids.	Exhibits bradycardia, rapid hypotension, and respiratory arrest in the rat.
2	Condylactis gigantea	A polypeptide with 195 amino acid residue.	Exerts a haemolytic action in rabbits.
3	Parasicyonis action stoloides	A poptide with very less number of amino acids.	Shows a neurotoxic action.

In addition to the above and cited examples of the marine peptides, there are quite a few polypeptides which have been isolated and characterized from a wide spectrum of sea anemones. A few such typical examples are given as under:

(ii) Marine Glycosides

The marine glycosides, in general, are of two types, viz., nonsulphated and sulphated ones. However, holothurins and astrosaponins are two typical examples of marine glycosides that generally display cardiotoxic activity having an unusual narrow margin between the effective dose (i.e., ED_{50}) and the lethal dose (i.e., LD_{50}).

Holothurins: These are the aglyconic residues obtained from the family Holothuroidae of phylum Echinodermata and essentially possess a steroidal moiety that very much resemble to the aglycones of the digitalis glycosides. In the recent past a large number of the 'hydroxylated steroidal glycosides' have been isolated and characterized from the holothuroids, also known as the sea cucumbers

.**Astrosaponins:** These are the marine glycosides obtained from the star fishes belonging to the family Asteroidae.

In fact, both these marine glycosides (i.e., sulphated and non-sulphated) inhibit Na^+, K^+, Mg^{2+} and ATPases, but the holothurins are reported to be far more active on the Na^+, K^+ and ATPase.

Interestingly, the astrosaponins are found to exert an altogether different type ofpharmacological actions, such as: antiinflammatory, analgesic, haemolytic, hypotensive; besides having the cytolytic activity on account of its interference in neuromuscular blocking effects and the protein metabolism.

However, the holothurins are found to exert both cardiotonic and ichthyotoxic actions. Besides, they also exhibit haemolytic activity.

Eledoisin; $[C_{54}H_{85}N_{13}O_{15}S]$

Chemical Structure 5-oxoPro-Pro-Ser-Lys-Asp-Ala-Phe-lle-Gly-Leu-MethNH_2

Biological Source: Eldoisin is obtained from the posterior salivary glands of eledone spp. (small octopus spp.) Eledone moschata.

Characteristic Features

l. Eledoisin is obtained as a sesquihydrate powder that gets decomposed at 230^0C.

2. Its specific optical rotation $[\alpha]_D^{22}$ - 44^0 (C = 1 in 95% acetic acid).

3. It is found to lose its activity gradually when incubated in blood.

Uses:

1. Its physiologic action resembles that of the other tachykinins, substance P and physalaemin.

2. It is found to stimulate extravascular smooth muscle.

3. Eledoisin acts as a potent vasodilator and hypotensive agent.

4. It causes salivation, and enhances capillary permeability in certain specific species.

5. It also stimulates lacrimal secretion.

(b) Hypotensive Compounds

There are quite a few potent hypotensive compounds that have been derived from a variety of marine organisms. These newer range of medicinally active chemical entities may be categorized into two groups,

Namely:

(i) Marine nucleosides, and

(ii) Hypotensive peptides and other compounds.

The various 'marine biomedicines' belonging to each of these two categories shall now discussed as under:

(i) Marine Nucleosides Nucleosides

Marine Nucleosides Nucleosides are formed by the combination of a purine pyrimidine base in glycosidic linkage with a sugar moiety, such as: adenosine, thymidine etc.; whereas, the 'phosphate ester' of a nucleoside is known as nucleotide e.g., 5'-guanylic acid; 3'-cytidylie acid.

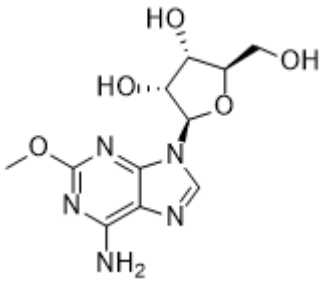
In the past two-and-a-half decade a plethora of marine nucleosides have been isolated and characterized, and also evaluated for their therapeutic efficacy.

Cryptotethia crytpa, the well-known Caribbean sponge made a spectacular success in the history of marine biomedicine that indeed produced a very rare and unique arabinosylnucleoside which resulted on slightest structural manifestation the wonderful drug ofchoice used for antileukemic & ıreatment cytarabine or Ara-C. A good number other nucleosides have been derived from C. crypta that showed varying interests. However, spongosine, being one such a drug that gained meaningful legitimate recognition because of its highly potential hypotensive activities. Doridosine, is another most promising and potent hypotensive nucleoside reported. These two compounds shall now be discussed as under:

A. Spongosine

It is a nucleoside and the methoxy derivative of adenosine.

Chemical Structure

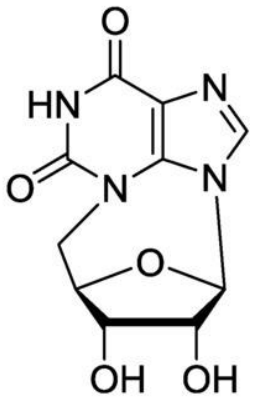


Biological Source: It is obtained from the Caribbean sponge Cryptotethia crypta and with a minor structural modification of the parent isolated nucleoside known as arabinosylnucleoside.

Uses:

1. It exhibits various coronary effects resembling to those of adenosine, for instance: coronary vasodilation and negative inotropy.
2. It is found to exert more marked and pronounced long-acting effects.
3. It acts as a hypotensive at such as dose level at which adenosine is observed to be absolutely inactive.
4. It reduces the rate as well as the force of contraction of heart.

B. Doridosine



Biological Source: It is obtained from the nudibranch Anisodoris nobilis:

Characteristic Features

1. It has been revealed on the basis of the kinetics of the enzymatic degradation of these nucleosides that doridosine is not only the most active but also the long-lasting nucleoside.

2. Doridosine does not undergo oxidative deamination in vitro on being subjected to incubation in the presence of adenosine deaminase, while adenosine disappears very fast.

Perhaps the intensity and the duration of the cardiovascular effects of doridosine is directly related to its half-life in vivo.

Note: The intermediate acting, spongosine happens to disappear in a gradual manner.

Uses:

1. It is the most potent hypotensive marine nucleoside known so far.

2. It also exerts hypothermic effect i.e., it lowers the normal temperature of the body.

Hypothermic Effect Interestingly, both spongosine and doridosine, when administered intracerebroventricularlv to guinea pigs, they lower the body temperature by several degrees for a duration ranging between 4-7 hours. However, this hypothermic effect seems to have no bearing to their prevailing systemic actions.

5'-Deoxy-5-iodobericidin: It is also a nucleoside which has been observed to lower the body temperature of mice**. It has been isolated from Hypnea valentiae (Red Algae). Perhaps this is the first and foremost compound which possesses the following two specific criteria, namely:

(a) First iodinated nucleoside discovered, and

(b) First 5'-deoxyribosyl nucleoside discovered in nature.

Uses:

l. It may prove to be a vital biochemical research tool of immense interest.

2. It is found to be a potent inhibitor of adenosine kinase.

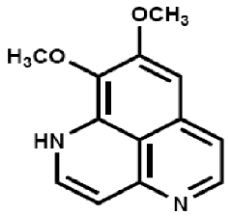
(ii) Hypotensive Peptides and Other Compounds

A number of peptides have been isolated from the marine organisms that exclusively showed distinct and significant hypotensive activities in experimental laboratory animals.

There are certain typical examples, such as: aaptamine, hymenin and uro-tensins I and II which shall now be discussed briefly in the sections that follows:

A. Aaptamine

Chemical Structure



Biological Source: Aaptamine is obtained from Aaptos aaptos.

Characteristic Features It has an inherent interesting heterocyclic nucleus besides characteristic and molecular size which may render this molecule a good candidate drug for future intensive as well as extensive pharmacological studies provided it should exhibit a bare minimum level of toxicity.

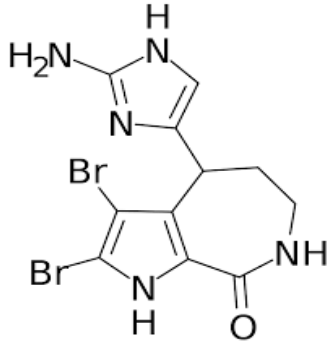
Uses:

1. It has an α-adrenergic blocking effect.

2. In case, it does not undergo a rapid metabolism in mammalian species in situ, it may prove to cause a hypotensive effect.

B. Hymenin

Chemical Structure



Biological Source: Hymenin is obtained from Hymeniacidon aldis.

Its characteristic features and uses are very much similar to those of aaptamine.

C. Urotensins I and II (U I, U II)

Biological Sources Urotensins I and II are obtained from the specific caudal neurosecretory system of Giltichthys miralilis (Telecost); and also from Catostomus commersoni - a fish.

Characteristic Features Both UI and UII are naturally occurring poly-peptides which distinctly possess appreciable hypotensive activity as evidently shown in rat, dog, sheep and squirrel monkey.

Uses:

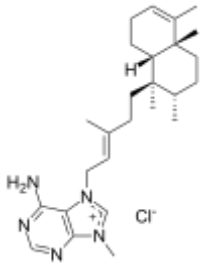
1. These polypeptides exhibit hypotensive effect that seems to be on account of their vasodilatory action.
2. Nevertheless, the discoverers of these two vasoactive peptides proposed that they may possibly prove to be clinically potential drugs, but till date no evidence for their clinical efficacy has yet been reported.

Cytotoxic compounds

Agelasines

Agelasines are 7,9-dialkylpurinium salts isolated from marine sponges (*Agelas* sp.). They are considered secondary metabolites. Their contribution to the sponge is assumed to be some sort of protection against microorganisms. At the present time a total of eleven 9-methyladeninium salts, agelasine A–I, epiagelasine C and agelin B, are known. All

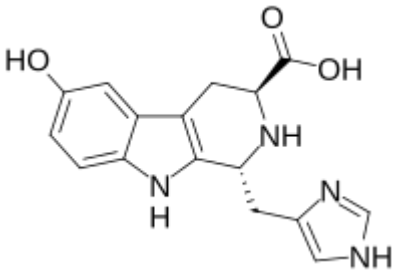
compounds carry a diterpenoid side chain in the adenine 7-position. The agelasines are closely related in structure with the agelasimines.



Hyrtioreticulins A and B

Hyrtioreticulins A and B are indole alkaloids extracted from the marine sponge Hyrtios reticulatus that are used to inhibit the ubiquitin-activating enzyme (E1):

- Inhibiting E1: Hyrtioreticulins A and B have been shown to inhibit E1 with IC50 values of 0.75 and 11 μg/mL, respectively.
- Anti-inflammatory activities: Hyrtioreticulins have been shown to have anti-inflammatory activities.



Hyrtioreticulin A

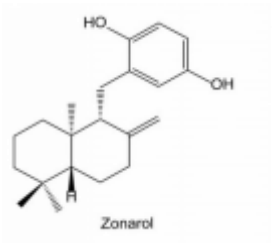
Antimicrobial Compounds

Antimicrobial drug substance have been isolated, characterized and studied extensively over the past three decades particularly from the vast domain of marine organisms. A few examples are brown algae and red algae (viz., three different species), gorgoniun corals; (viz., two variant species). The chemical substance from these species shall now be discussed briefly as under:

1.Zonarol

Biological source:- Zonarol which was discovered in the Dictyopteris zonaroides (Brown algae) as a marine natural product.

Chemical structure:



Molecular formula: $C_{21}H_{30}O_2$

Average mass: 314.469

Uses:

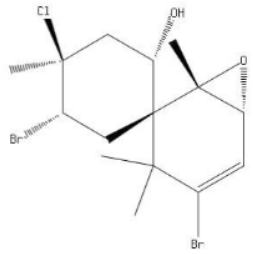
It is used as a antimicrobial agent.

Provide neuroprotection by activating the Nrf2/ARE pathway.

2.Prepacifenol

Biological sources: It is a halogenated epoxy sesquiterpene and antimicrobial agent that can be isolated from two different species of Red Algae Laurecia pacifica and Laurencia filformis.

Chemical structure:



Prepacifenol

Molecular formula: $C_{15}H_{21}Br_2ClO_2$

Molecular weight: 428.58 g/mol

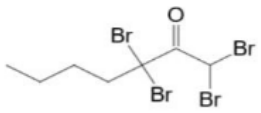
Uses:

It is used as an antimicrobial agent.

Tetrabromo-2-Heptanone

Biological source: It is oobtained from yet another species of Red Algae Bonnemaisonia hemifera.

Chemical structure:



Molecular formula: $C_7H_{12}Br_4O$

Molecule weight: 411.89 g/mol

Appearance: Colorless to pale yellow liquid or solid.

Boiling point: Approximately250-300°C (depending on the purity and condition)

Density: Around 2.0-2.2 g/cm3

Solubility: soluble in organic solvents, such as dichlorimethane, chloroform and acetone.

Uses:

Intermediate in organic synthesis, particularly in the production of pharmaceuticals, agrochemicals, and specialty chemicals.

Precursor for synthesizing other brominated compounds, such as flame retardants and biocides.

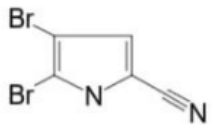
Potential use in material science, such as in the development of new polymers and resins.

5. 2-Cyano-4,5-dibromopyrrole

It is perhaps one of a chemical entity isolated from a marine organisms which contains a cyano (-CN) functional group.

Biological source: It is obtained from Agelas oroides, a specific type of sponge found in marine sources.

Chemical structure:



Molecular formula: $C_5H_2Br_2N_2$

Molecular weight: 249.89 g/mol

Appearance: Solid or crystalline powder

Melting point: Approximately 150-200°C (depending on purity)

Solubility: Soluble in organic solvents like DMSO, DMF, and chloroform

Uses:

Intermediate in organic synthesis, particularly in pharmaceuticals and agrochemicals

Potential building block for synthesizing:

Heterocyclic compounds

Dyes and pigments

Biologically active molecules (e.g., inhibitors, receptors)

Research applications in material science, medicinal chemistry, and chemical biology

6. Aeroplysinin-1(+) and Aeroplysinin-1(-)

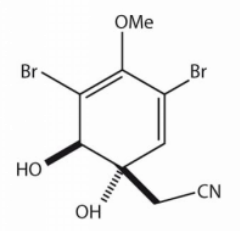
Biological source: Aeroplysinin-1 is a natural compound found in marine sponges, specifically in the genus Aplysina. It exists in two enantiomeric forms:

Aeroplysinin-1 (+) (also known as Aeroplysinin-1 R)

Aeroplysinin-1 (-) (also known as Aeroplysinin-1 S)

These enantiomers have the same molecular formula ($C_9H_9Br_2NO_3$) but differ in their three-dimensional arrangement of atoms.

Chemical structure:



Molecular weight: 338.98 g/mol

Appearance: White or off-white solid

Solubility: Soluble in organic solvents like methanol, ethanol, and DMSO

Biological activities:

Aeroplysinin-1 (+) and (-) have been reported to exhibit:

Antimicrobial activity against bacteria and fungi

Antiviral activity against certain viruses

Cytotoxic activity against cancer cell lines

Inhibition of protein kinases and other enzymes

Research applications:

Studying the biological activities and mechanisms of action of Aeroplysinin-1 enantiomers

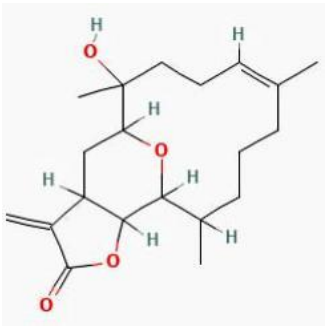
Developing new pharmaceuticals and therapeutics based on Aeroplysinin-1

Exploring the potential of Aeroplysinin-1 as a lead compound for drug discovery

7. Eunicin

Biological source: Eunicin is obtained from Eunicia mamosa the well known Gorgonian Corals.

Chemical structure:



Molecular formula: $C_{20}H_{30}O_4$

Average mass: 334.456

Uses:

- Antimicrobial agent
- Antibiotics.

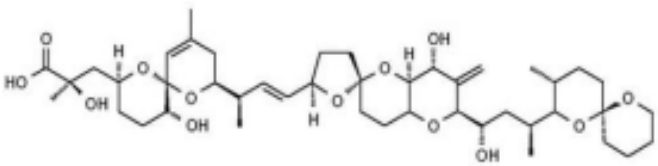
Antibiotic compounds

The marine chemist across the globe have been able to evolve a few antibiotics from the various marine organisms, namely: **Okadaic acid, Acanthifolicin and Norhalichondrin A**. These naturally occurring marine antibiotics shall be discussed in the section that follows:

1. Okadaic Acid

Synonym: Halochondrine A

Chemical structure:



Molecular Formula:

$$C_{44}H_{68}O_{13}$$

Molecular Weight:

805.0g/mol

Biological Sources:

It is obtained from Halichondria (okadai or melanodocia) (Marine black sponges).

Characteristic Features:

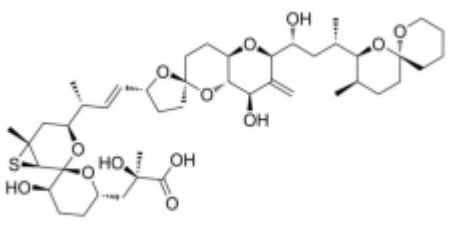
- ❖ It is the first ionophoric polyether identified in marine organisms.
- ❖ It is obtained from dichloromethane/hexane having melting point 171-175 ^{o}C.
- ❖ It has specific optical rotation $[\propto]_D^{25}$ +21^{o} C (C=0.33 in chloroform).
- ❖ It is also reported as crystals from benzene chloroform mixture having melting point 164-166 ^{o}C; and $[\propto]_D^{25}$ +25.4 ^{o}C (C=0.24 in chloroform).

Uses:

- ➢ It is an important biochemical tool as tumour promoter are probe of cellular regulation.
- ➢ It was found to be far more cytotoxin to KB-cells and to mice than compared to another novel ionophoric marine substance acanthifolicin.
- ➢ It is able to transport divalent cations e.g., Ca^{2+} across the lipoidal membrances conveniently.
- ➢ Okadaic acid uniquely causes a prolonged contraction of the human umbilical artery and rabbit aorta without the presence of extracellular Ca^{2+}; and it does not affected the Na^{2+} and K^{+} ATPase.

ACANTHIFOLICIN

Chemical structure:



Molecular Formula:

$$C_{44}H_{68}O_{13}S$$

Molecular Weight:

837.1g/mol

Biological Source:

Acanthifolicin is obtained from Pandaros acanthifolium (Sponge).

Characteristic Features:

- ❖ It possesses an antibacterial activity.
- ❖ It also exerts cytotoxin actions.
- ❖ It is found to be lethal to mice at low dose level of 0.14mg kg^{-1} i.v.

Uses:

- ➢ It is an important biochemical tool as tumour promoter are probe of cellular regulation.
- ➢ It was found to be less cytotoxin to KB-cells and to mice than compared to another novel ionophoric marine substance Okadaic aid.
- ➢ It is able to transport divalent cations e.g., Ca^{2+} across the lipoidal membrances conveniently.

Anti-inflammatory Agents

Anti- inflammatory marine agents are substances that reduce inflammation in the body, such as redness, swelling and pain. They can be found in marine invertebrates, algae and other marine organisms.

6-n-Tridecyl salicylic acid :

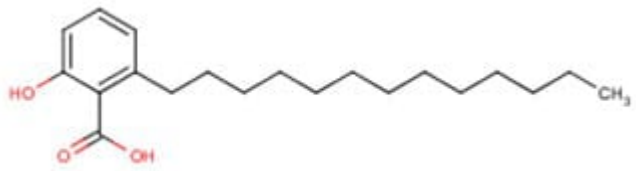
Caulocystis cephalornithos

It is one of the anti-inflammatory marine agents. It is derived from Caulocystis cephalornithos. It is a species of marine algae , specifically a type of brown algae .

Molecular formula :

$C_{20}H_{32}O_3$

Molecular structure :



Molar mass :

320.47g/mol

Melting point :

$41\text{-}42^0c$

Appearance :

White to off white crystalline powder.

Solubility :

Soluble in organic solvent like ethanol,methanol and dichloromethane . Insoluble in water.

Reactivity :

Reacts with strong oxidizing agents ,bases and reducing agents .

Uses :

- Reduces swelling,pain.
- May help alleviate conditions like arthritis,gout or tendinitis.
- Effective against skin conditions like acne ,psoriasis or eczema.
- Also used to treat chronic disorders

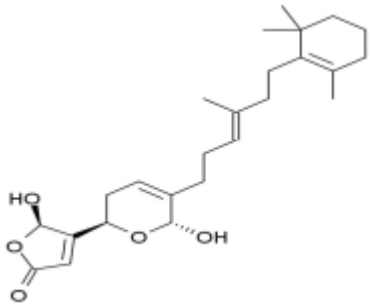
Side effects:

- Skin irritation : Reddness, itching, burning or dryness
- Allergic reactions : Rarely ,some individuals may experience allergic reactions like hives,itching or difficulty breathing .
- Nephrotoxicity : May affect kidney functions especially with long term use or high doses.

2. Manolide:

It is a bio active compound , derived from Luffariella variabilis . It is a type of marine sponge.

Molecular structure :



Molecular formula :

C25H36O5

Molecular weight :

416.55g/mol

Stability :

Monolide is sensitive to light, heat and oxygen ,which can lead to degradation and loss of biological activity .

Uses :

- May help reduce inflammation in mucous membranes such as in the respiratory or gastrointestinal tracts.
- Effective against skin deseases.
- Used to heal wounds

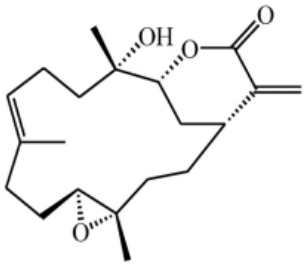
Side effects :

- Head aches, muscle weakness
- Vomiting

3.Flexibilide :

It is also a type of marine organism .It is used to treat inflammation . It is a type of marine coral. Flexibilide is derived from Sinularia flexibilis .It is a type of soft coral.

Molecular structure :



Molecular formula :

$C_{20}H_{30}O_4$

Uses :

- Used also anti microbial agents ,anti-inflammatory agents
- Used in pharmaceuticals, neuroprotection , cosmetics.
- Flexibilide has been shown to exhibit antimicrobial properties ,effective against bacteria and fungi.

Side effects :

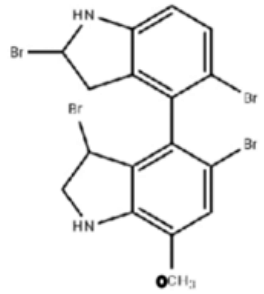
It works against cancer cells sometimes its also affect the healthy cells .

4.Rivularia firma :

Rivularia firma is a species of cyanobacteria that is commonly found in aquatic environments, particularly in fresh water habitats. First described by Carl Adolph Agardh in 1824. It is a class of Cyanophyceae and the family is Rivulariaceae.

A series of bio indole derivatives isolated from marine cyanobaterium Rivularia firma has shown potential anti-inflammatory activity in the models of Carrageenan induced rat paw oedema.

Molecular structure :



Chemical compositions :

Carbohydrates ,Protein , Lipids (Glycolipids) , Pigments(Chlorophyll) , Nucleic acids(DNA,RNA), Minerals(Ca, Mg, K, Na)

Uses :

- Reduces swelling,pain.
- May help alleviate conditions like arthritis,gout or tendinitis.
- Effective against skin conditions like acne ,psoriasis or eczema.
- Also used to treat chronic disorders.

Side effects :

Vomitting, diarrhea, allergic reaction,skin irritation.

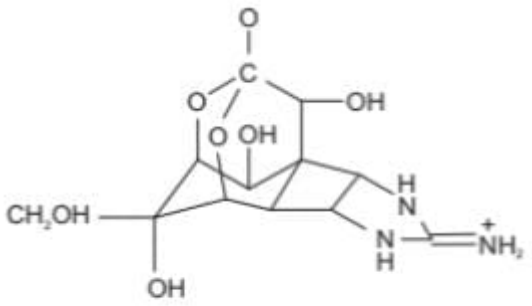
Marine toxins

Many marine organism produce potentially toxic compound which may work for their safety and protect them from predators these toxins may pose potential hazards to human health. Many of these toxins had also shown remarkable biological activities in comparatively lower does some of these marine toxins are discussed below.

Tetrodotoxin

Tetrodotoxin is a potent neurotoxin produced by the pufferfish of family Tetraodontidae. It is present in other animal including Gobius Crinigar, Taricha torosa, Atelopus chiriquensic marine crabs and also produced by marine bacteria. This dangerous toxin shows the cardiovascular and neurophysiological activity in experimental animal. Tetrodotoxin

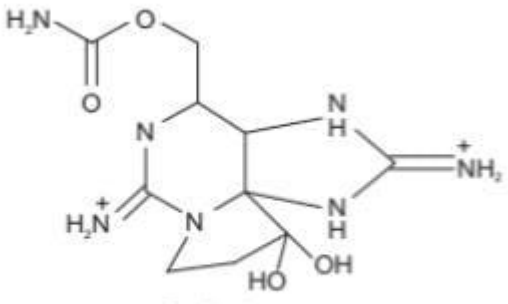
containing puffer fish is considered as a delicacy in Japan but great care is exercised to avoid the toxin during its preparation for culinary purpose.



Tetrodotoxin

Saxitoxin

Saxitoxin is a purine skeleton containing toxic compound produce by the batter clam Saxidomus giganteus and California mussel Mytilus californianus, It is also found in two toxic species of mollusc, Zolimus aeneus and platipodia granulosa saxitoxin is identical with toxin isolated from the dinoflagellate gonyaulax calenenalla upon which the butter clam feeds. In lower doeses this toxin produces a marked.



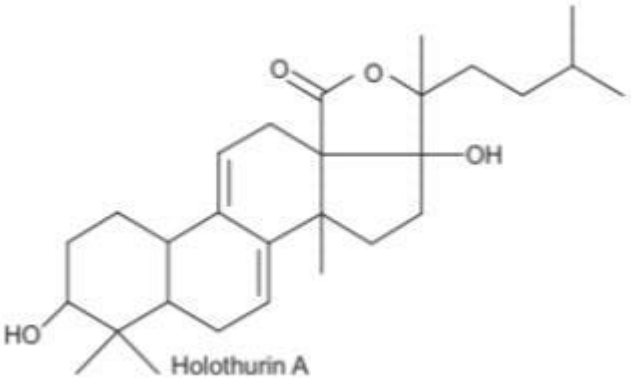
Sexitoxin

Ciguatoxic

Ciguatoxin is the poisonous compound found in the dinoflagellate gambiderdiscus toxicus. In many cases it is responsible for Ciguatera fish poisoning associated with the utilization of tropical fish resource. Ciguatoxin show the cardiovascular and neurophysiological properties. Another toxin manitotoxin present in G. toxicus is found to be powerful calcium channel activator in a very low does of pico-to nano-molar range.

Holothurin A

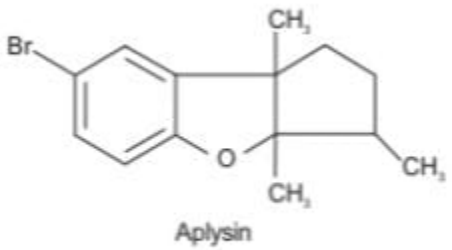
Holothurin A is a toxic saponin isolated from the sea cucumber of holothurian group helix pomatia. It is recognized as mixture of titerpenic aglycones which are linked to four sugar molecules and a molecules and a molecule of Sulphuric acid as a sodium salt. It has shown hemolytic and neurotoxic properties.



Holothurin A

Aplysins

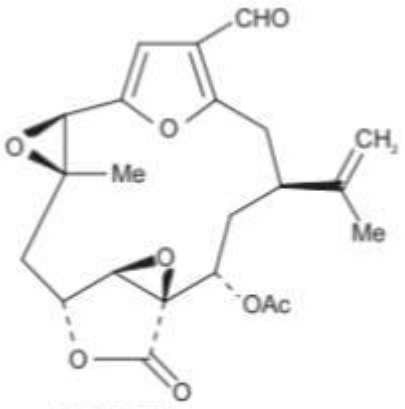
Aplysins is a group of toxic compounds isolated from mediterranean sea hares Aplysia depilans. Aplysins contains an unpleasant colourless fluid secreted by the skin these are the white viscous liquid by opaline gland and purple secretion from another gland present in sea hares. Aplysins causes paralysis when injected cold-blooded animals.



Aplysin

Lophotoxin

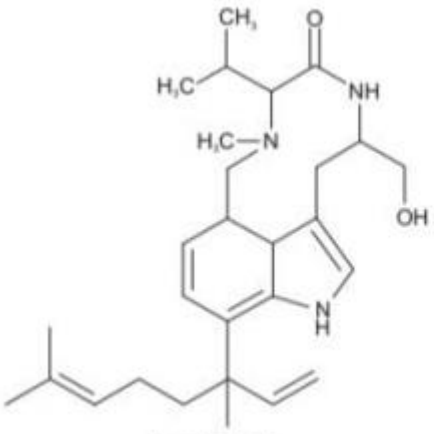
Lophotoxin is a diterpene lactone present in the gorgonian corals of the genus Lophogorgia. It produces an irreversible postsynaptic blockage at neuromuscular Junction.



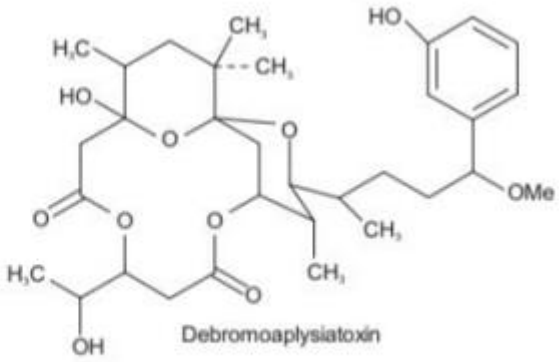
Lophotoxin

Lyngbyatoxin

Lyngbyatoxin is an indole group of alkaloid produced by the marine cyanobacterium Lyngbya majuscule. It is responsible for the contact dermatitis know as sweemers itch' in the humans. Some other organism of the same species L. Majuscula have been found produce totally unrelated skin irritant debromoaplysiatoxin winch also demonstrates antineoplastic activity.



Lyngbyatoxin



Debromoaplysiatoxin