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FACULTY OF **SCIENCE**

DEPARTMENT OF PURE AND APPLIED SCIENCES

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INTRODUCTION

Natural Products Chemistry (CHM 422) aims at getting you acquainted with as much information as possible on organic compounds produced by living organisms and their importance in today's world. Compounds occurring naturally

may be broadly divided into three categories: primary metabolites, secondary metabolites and macromolecules. In this course, we will look at the individual chemical composition of plants. We will also look at the organic components of higher plants.

WHAT YOU WILL LEARN IN THIS COURSE

This course presupposes that you have taken a course on natural products before now. However, in this follow up course our approach will be to reveal the logic of natural products by being selective in the topics we cover, as well as thorough and patient in developing them. To this effect, you will be presented information in natural products in a structured way to make learning easier.

The course comprises two modules of five units. The course starts with the definition of terminologies that will be encountered in the process of studying this course and then goes on to give a brief historical background to the course. The simple classification of natural products is given and detail discussion on each of the classes is done.

COURSE AIMS

This course broadly aims at providing you with knowledge about how and why plants produce a vast array of metabolites, give you new insights into how plants use these compounds and the importance these compounds have in human medicine and in human social action and behaviour.

COURSE OBJECTIVES

On completion of the course, you should be able to:

• give a brief history and background of natural products

• distinguish between primary and secondary metabolites

• discuss the role of natural products as new sources of drugs

• give a broad classification of natural products into the main classes

• describe the stages involved in isolation and structure determination of natural products.

WORKING THROUGH THIS COURSE

In order to be able to successfully complete this course, you are required to carefully study each unit along with recommended textbooks and other materials that may be provided by the National Open University of Nigeria. You may also need to exploit other e-reading sources such as the Internet for further useful information on the course. Each unit contains self-assessment exercise and at certain points in the course you would be required to submit assignment for grading and recording purposes. You are also to participate in the final examination at the end of the course. It is recommended that you devote abundant time to reading and comprehension. It is highly necessary that you avail yourself the opportunity of attending the tutorial sessions where you will be able to compare your understanding of the course contents with your colleagues.

THE COURSE MATERIALS

You are provided with the following sets of course materials:

- A course guide which spells out the details of the natural product chemistry including the aims and objectives.
- The study units with detailed learning information.

Each study unit has a set of performance objectives along with other relevant learner guide. In unit 1 of module 1, the various types of compounds found in plants were introduced with a view to providing basis for understanding natural products. Unit 2 of module 1 presents a detailed treatment of a class of compounds derived biosynthetically from isopentenyl diphosphate terpenes. In unit 3 of module 1, we will consider nitrogen containing compounds widely distributed in different plant groups known as alkaloids.

Unit 1 of module 2 provides information about how plant natural products are isolated using traditional, analytical and preparative methods and the structure of the isolated compounds determined by means of both classical and modern methods of structural elucidation and characterisation by spectroscopy.

In unit 2 of module 2, a brief mention of biosynthesis of some natural products is made, while a highlight of other natural products of pharmaceutical importance is given.

STUDY UNITS

The modules and study units in this course are as follows:

Module 1

Unit 1 - Types of Compounds Found in Plants

Unit 2 - Terpenes

Unit 3 - Alkaloids

Module 2

Unit 1 - Isolation and Structure Determination of Natural Products

Unit 2 - Biosynthesis

ASSESSMENT

The course assessment consists of three aspects; namely, the self-assessment exercise, the Self-Assessment Exercise and the end of course examination. It is essential that you attempt all exercises and assignments and submit appropriately to the course facilitator for grading. Let your answers be concise and as accurate as possible. You are expected to consult other materials in addition to your course materials in order to be able to present accurate answers to the questions. Kindly note that the Self-Assessment Exercise covers only 30% of the total marks for the course.

SELF-ASSESSSMENT EXERCISES (SAE)

The SAE is a continuous assessment component of your course. It accounts for 30% of the total score. You will be given a number of SAEs to answer. They must be answered before you are allowed to sit for the end of the course examination. The SAEs will be given to you by your facilitator and returned after you have done the assignment. Note that these assignments are already contained in the assignment file to be given to you. You may do yourself good by reading and researching well before you attempt to answer the questions.

FINAL EXAMINATION AND GRADING

The end of the course examination is intended to cover information from all parts of the course. Avail yourself the opportunity of the time between completion of

the course content and the beginning of the examination to revise as much as possible the whole course materials, the exercises and assignments.

FACILITATORS/TUTORS AND TUTORIALS

There are hours of tutorials provided in support of this course. You will be informed appropriately of the name, telephone number and e-mail address of your facilitator. In addition, the time, dates and location of the tutorial lessons will be communicated beforehand. You are required to mail or submit your Self-Assessment Exercise to your facilitator, at least two working days, before the schedule date. Note that the facilitator with further comments that can improve on your performances will duly mark all the submitted assignments. The facilitator will from time to time take record of accomplishment of your comprehension, progress and difficulty in the course. Be kind enough to attend tutorial lessons at the fixed appointment. It is probably the only avenue to meet face to face and discuss with your facilitator. There, you will be able to ask questions or seek clarification on seemingly grey areas in the course material. You may as well have prepared questions and comments for your facilitator before the due date. An active participation during the tutorial lessons will be an added advantage to boost your confidence level. In case any of the situations listed below arises, do not hesitate to intimate your facilitator using his or her telephone number or via e-mail address:

- You do not understand any part of the study or the assigned readings
- You are not skill enough to attempt the self-assessment exercise
- The questions in the SAEs are not clearly understood.

Accept my best wishes in the course and I do hope that you benefit considerably from its application.

Module 1

Unit 1 - Types of Compounds found in Plants

Unit 2 - Terpenes

Unit 3 – Alkaloids

UNIT 1 - TYPES OF COMPOUNDS FOUND IN PLANTS

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1.0 Introduction

2.0 Objectives

3.0 Main Content

- 3.1 History and Background of Natural Products
- 3.2 Classification of Natural Products

4.0 Self-Assessment Exercise

5.0 Conclusion

6.0 Summary

7.0 References/Further Reading

1.0 INTRODUCTION

According to online medical dictionary, natural products are defined as compounds occurring naturally, which are products of secondary metabolism; they are unique compounds for specific organism or classes of organisms. In general, every biological molecule is a natural product. However, the term is applied normally to secondary metabolites, which are small molecules with molecular weight up to 1500 amu produced by an organism. Such molecules are not strictly essential for the survival of the organism. They differ from the more widespread macromolecules for example polysaccharides, proteins and nucleic

acids, which form the basic machinery for the more fundamental processes of life.

Furthermore, natural products are organic compounds that living organisms produce. Key areas of organic chemistry focus on the structural elucidation, chemistry, synthesis and biosynthesis of these products. Compounds occurring naturally may be categorised into three broad groups:

1. **Primary metabolites:** These compounds occur in all cells and play a principal role in cellular metabolism and reproduction. For example, sugars, nucleic acids and the common amino acids.
2. **Polymeric materials:** These are high-molecular-weight polymeric materials, which form the cellular structures. They include cellulose, the lignins and the proteins.
3. **Secondary metabolites:** These are compounds produced by a living system, which are not directly involved in normal growth, development or reproduction of the organism. Specific secondary metabolites are characteristic of a limited range of species.

The primary metabolites contrast the secondary ones in that they exert their biological effects intracellularly or within the organism that is responsible for their production; on the other hand, secondary metabolites exert their biological effect on other organisms thus making them to attract interest. Numerous biologically active ingredients of plants (medicinal, commercial and poisonous) that have been studied are secondary metabolites.

More than 40% of medicines originate from these natural products. New drugs such as taxol, an anticancer agent, have emerged from a number of screening programmes for bioactive compounds. In addition, natural products play an important ecological role as regulators of interactions between microorganisms, plants, insects and animals. They often act as antifeedants, attractants, defensive substances and pheromones.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- Explain the concept of natural products

- Give a brief history of natural products
- Classify natural products.

3.0 MAIN CONTENT

3.1 History and Background of Natural Products

More than half of all drugs in clinical use are obtained from natural products, their derivatives and analogues. Man has been using plant-based medicines in the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations since centuries. The plant-based indigenous knowledge was passed down from generation to generation in various parts of the world throughout its history and has significantly contributed to the development of different traditional systems of medicine. The use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century and subsequently led to the isolation of early drugs such as cocaine, codeine, digitoxin and quinine.

Many natural products were known to humankind for thousands of years. Ethanol produced by fermentation has its origin in pre-history, natural dyes have been known for long, sucrose the most common and well known natural product has its history of use since the time of Alexander the Great. With the discovery of salicin from willow tree extracts and the development of aspirin in 1899, the art of exploiting natural products became a molecular science. The discovery of penicillin in 1928 and its subsequent development as an anti-infective agent represents another milestone in the history of natural products, and marked the beginning of a new era in drug discovery, in which bacteria and fungi were added to the plant kingdom as sources for biologically active compounds.

Before the middle of the 20th century, chemists in this field were intent merely on isolating and determining the structure of natural products by classical methods. However, after 1945 and over the next several decades, scientists developed a host of analytical tools that greatly improved the study of these natural materials. Advancement like new separation techniques, spectral methods of identification and new synthetic tools led to major advances in the analysis of natural products. This resulted in isolation and synthesis of thousands of these products.

Natural products also inspired chemists and physicians and their rich structural diversity and complexity prompted synthetic chemists to produce them in the laboratory, often with therapeutic applications in mind, and many drugs used today are natural products or natural-product derivatives. Recent years have seen considerable advances in our understanding of natural product biosynthesis. Coupled with improvements in approaches for natural-product isolation, characterisation and synthesis, these could be opening the door to a new era in the investigation of natural products in the academia and industry.

In-text Questions

To which of the following is, the term natural product reserved? (a). Primary metabolites (b). Secondary metabolites (c). Both.

Answer: *(b) Secondary metabolites*

3.2 Classification of Natural Products

There is no rigid scheme for classifying natural products, their immense diversity in structure, function, and biosynthesis is too great to allow them to fit neatly into a few simple categories. In practice, however, workers in the field often speak of five main classes of natural products:

- Terpenoids and steroids
- Alkaloids
- Polyketides and fatty acids
- Phenylpropanoids
- Specialised carbohydrates.

Terpenoids and steroids

These are a vast group of substances, more than 35,000 are known, they are derived biosynthetically from isopentenyl diphosphate. Terpenoids have an immense variety of apparently unrelated structures, while steroids have a common tetracyclic carbon skeleton and are modified terpenoids that are biosynthesised from the triterpene lanosterol.

Alkaloids

Like terpenoids, alkaloids constitute a large and diverse class of compounds, with more than 12,000 examples known at present. They contain a basic amine group in their structure and are derived biosynthetically from amino acids.

Fatty acid and polyketides

It is a class of which more than 10,000 are known. They are biosynthesised from simple acyl precursors such as acetyl CoA, propionyl CoA, and methylmalonyl. Non-ribosomal polypeptides are peptide-like compounds that are biosynthesised from amino acids by a multifunctional enzyme complex without direct RNA transcription. The penicillins are good examples.

4.0 SELF-ASSESSMENT EXERCISE

1. What are natural products?
2. Explain the concept of natural products.
3. Give a brief history of natural products.
4. List the various classes of natural products

Answer:

1. *Natural products are secondary metabolites, which are small molecules with molecular weight up to 1500 amu produced by an organism.*
4. *Main classes of natural products include (i) Terpenoids and steroids (ii) Alkaloids (iii) Polyketides and fatty acids (iv) Phenylpropanoids (v) Specialised carbohydrates.*

5.0 CONCLUSION

The term “natural product” is perhaps something of a misnomer. Strictly speaking, any biological molecule is a natural product, but the term is usually reserved for secondary metabolites, small molecules (mol wt ≥ 1500 amu approx) produced by an organism but that are not strictly necessary for the survival of the organism. They are different from the more prevalent macromolecules such as proteins, nucleic acids, and polysaccharides that make up the basic machinery for the more fundamental processes of life.

6.0 SUMMARY

Primary metabolites are natural products that are found in all cells, but secondary metabolites are natural products that are restricted in their occurrence. Secondary metabolites have been studied because of their biological activity, or

for chemosystematic or ecological reasons. Secondary metabolites may be classified as polyketides, terpenoids and steroids, phenylpropanoid (C6-C3) compounds, alkaloids and carbohydrates, based on their biosynthetic building blocks. Acetyl co-enzyme A, isopentenyl pyrophosphate and shikimic acid are the building blocks for the polyketides, terpenoids and phenylpropanoid compounds, respectively.

Many of the products of metabolism are readily isolable organic compounds and have historical importance in organic chemistry. These compounds are grouped together under the broad heading of **natural product**. There are many different classes of naturally occurring compounds such as fats, carbohydrates, proteins and nucleic acids, which have obvious roles in the functioning of organisms. These natural products, together with a relatively small number of related substances, occur in almost all organisms; they are called **primary metabolites**.

A second class of natural product is called **secondary metabolites**. They are not necessarily of secondary importance to the organism, but their distribution in nature tends to be more species-dependent. They are the product of **secondary metabolic processes** of the organism. Examples of such secondary metabolites are the terpenes, the steroids, and the alkaloids. This is the main class for which the term **natural product** is reserved.

7.0 REFERENCES/FURTHER READING

1. Leland, J. C. *et al.* (2006). *Natural Products from Plants.* (2nd ed.). Boca Raton: CRC Press Taylor & Francis Group.

2. Hanson J. R. (2003) *Natural products: The Secondary metabolites*, The Royal Society of Chemistry, Cambridge.

UNIT 2 - TERPENES

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1.0 Introduction

2.0 Objectives

3.0 Main Content

- 3.1 Isolation of Terpenes
- 3.2 Properties of Terpenes
- 3.3 Classification of Terpenes

4.0 Self-Assessment Exercise

5.0 Conclusion

6.0 Summary

7.0 Reference/Further Reading

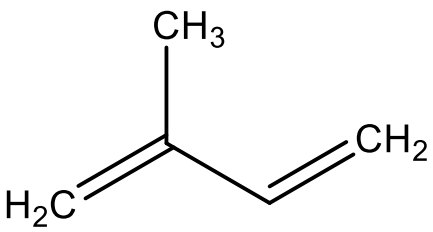
1.0 INTRODUCTION

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers though also by some insects such as termites or swallowtail butterflies, which emit terpenes from their osmeterium. They are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine". When terpenes are modified chemically (by oxidation or rearrangement of the carbon skeleton), the resulting compounds are generally referred to as terpenoids. Terpenoids are also known as isoprenoids.

Some authors use the term "terpenes" more broadly, to include the terpenoids. These terpenes are frequently found in plant essential oils which contain the "*Quinta essentia*", the plant fragrance. They are universally present in small amounts in living organisms, where they play numerous vital roles in plant physiology as well as important functions in all cellular membranes. Terpenes and terpenoids are the primary constituents of the essential oils of many types of

plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene. Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature. The aroma and flavor of hops, highly desirable in some beers, comes from terpenes.

Terpenes may be defined as a group of molecules whose structure is based on various but definite number of isoprene units (2-methylbuta-1,3-diene, named hemiterpene, with 5 carbon atoms).



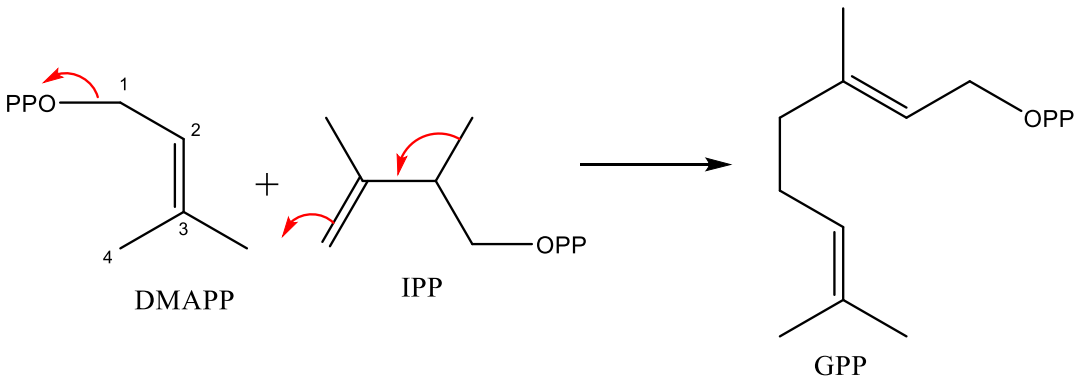
Isoprene

The **terpenes** have been prized for their essential oils and their use as fragrances for over two thousand years. An archaeological investigation in Egypt in 1997 unearthed boswellic acids from the resin of frankincense (*Boswellia* spp.) dating from 400 to 700 AD. Records from the Middle Ages of terpene-based essential oils were preserved, and chemical analysis of the oils began early in the nineteenth century. Commerce in essential oils and aromatherapy continues today. For example, rose (*Rosa* spp.) fragrance has enchanted many. Bulgarian rose oil requires over 4000 kg of petals to produce 1 kg of steam-distilled oil. Over 260 constituents have been identified, many of which are olfactory relevant. It should be apparent that even the simple terpenes found in fragrances have a considerable amount of structural diversity.

Fortunately, despite their diversity, the terpenes have a simple unifying feature by which they are defined and by which they may be easily classified. This

generality, referred to as the **isoprene rule**, was postulated by Otto Wallach in 1887 and it states that ***Terpene skeleton are formed by linking together isoprene units through carbon atoms 1 and 4 (head-to-tail), carbon atoms 1 and 1 (head-to-head), carbon atoms 4 and 4 (tail-to-tail) or combination thereof***.

This rule describes all terpenes as having fundamental repeating five-carbon isoprene units. The head-to-tail arrangement is most common. This isoprene rule has proved to be of great value in deriving the structure of terpenes. Thus, terpenes are defined as a unique group of hydrocarbon-based natural products that possess a structure that may be hypothetically derived from **isoprene**, giving rise to structures that may be divided into isopentane (2-methylbutane) units. The actual biosynthetic route to terpenes is not quite so simple. Two different biosynthetic pathways produce the main terpene building block, **isopentenyl diphosphate** (IPP). The first is referred to as either the MEP (methylerythritolphosphate) or DOX (1- deoxy-D-xylulose) pathway. Here, IPP is formed in the chloroplast, mainly for the more volatile mono and diterpenes. The second biosynthetic route is known as the MVA (**mevalonic acid**) pathway. This takes place in the cytosol, producing sesquiterpenes. Terpenoids are extraordinarily diverse but they all originate through the condensation of the universal phosphorylated derivative of hemiterpene, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) giving geranyl pyrophosphate (GPP).



2.0 OBJECTIVES

At the end of this unit, you should be able to:

- Define terpenes and terpenoids
- State the isoprene rule
- List and give examples of various types of terpenes
- Mention some uses of terpenes.

3.0 MAIN CONTENT

3.1 Isolation of Terpenes

Terpenes are isolated from essential oils present in plants. The essential oil is extracted from the plant tissues by four methods: (1) steam distillation; (2) digestion with solvents; (3) expression; and (4) adsorption in purified fats.

Method 1 (steam distillation) is the one most widely used. The plant tissue is macerated and then steam distilled. If a particular terpene is decomposed under these conditions, it may be removed by extracting with light petrol at 50°C and the solvent distilled under reduced pressure. Alternatively, the method of adsorption in fats may be employed. For example, the flower petals are spread over molten fat until the latter is saturated with essential oil. The fat is then digested with ethanol to remove the essential oil from it.

Essential oils obtained from plants as above usually contain a number of terpenes which are separated by *fractional distillation* or *chromatography*. Gas chromatography has been particularly useful.

3.2 Properties of Terpenes

Physical

Most of the terpenes are colourless fragrant liquids having a boiling point between 150°C and 200°C. They are lighter than water and are readily volatile in steam. They dissolve in organic solvents, but usually not in water. Most of them are optically active although there is no prevailing direction of rotation; some are *dextro*, others are *laevo.*

Chemical

Most of the terpenes being unsaturated hydrocarbons, are highly reactive. They undergo addition reactions with hydrogen bromide, bromine, hydrogen, nitrosyl chloride and ozone. They also form characteristic addition compounds with NO_2

and NOBr, which are used in their identification. Most terpenes are oxidised easily and tend to resinify upon exposure to air.

3.3 Classification of Terpenes

A rational classification of the terpenes has been established based upon the number of isoprene (or isopentane) units incorporated in the basic molecular skeleton; a prefix in the name indicates the number of terpene units needed to assemble the molecule. The number of five-carbon units they contain thus classifies terpenes:

- **Hemiterpenes (C_5)** consist of *a single isoprene* unit. Isoprene itself is considered the only hemiterpene, but oxygen-containing derivatives such as prenol and isovaleric acid are hemiterpenoids.
- **Monoterpenes (C_{10})** consist of *two isoprene* units and have the molecular formula $C_{10}H_{16}$. Examples of monoterpenes are: geraniol, limonene and terpineol.
- **Sesquiterpenes (C_{15})** consist of *three isoprene* units and have the molecular formula $C_{15}H_{24}$. Examples of sesquiterpenes are: farnesenes, farnesol. (The *sesqui* prefix means one and a half).
- **Diterpenes (C_{20})** are composed for *four isoprene* units and have the molecular formula $C_{20}H_{32}$. They are derived from geranylgeranyl pyrophosphate. Examples of diterpenes are cafestol, kahweol, cembrene and taxadiene (precursor of taxol). Diterpenes also form the basis for biologically important compounds such as retinol, retinal, and phytol. They are known to be antimicrobial and antiinflammatory.
- **Sesterterpenes (C_{25})**, terpenes having 25 carbons and *five isoprene* units, are rare relative to the other sizes. (The *sester* prefix means half to three, i.e. two and a half). An example of a sesterterpene is geranylfarnesol.
- **Triterpenes (C_{30})** Consist of *six isoprene* units and have the molecular formula $C_{30}H_{48}$. The linear triterpene squalene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Squalene is then processed biosynthetically to

generate either lanosterol or cycloartenol, the structural precursor to all the steroids.

- **Tetraterpenes (C_{40})** Contain *eight isoprene* units and have the molecular formula $C_{40}H_{64}$. Biologically important tetraterpenes include the acyclic lycopene, the monocyclic gamma-carotene, and the bicyclic alpha-carotene and beta-carotene.
- **Polyterpenes** Consist of long chains of *many isoprene* units. Natural rubber consists of polyisoprene in which the double bonds are cis. Some plants produce a polyisoprene with trans double bonds, known as gutta-percha.

Table 1. Classification of terpenes

S/N	Class of Terpene	Isoprene unit	Number of carbon
1	Monoterpenes	2	10
2	Sesquiterpenes	3	15
3	Diterpenes	4	20
4	Sesterpenes	5	25
5	Triterpenes	6	30
6	Carotenoids	8	40
7	Rubber	>100	>500

Monoterpenes: C_{10}

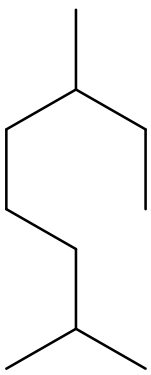
A bewildering assortment of isoprene-based decane arrangements exist in nature. This gives the term “terpenoid” a particularly elastic meaning and is reminiscent of some of the current combinatorial efforts employed in the pharmaceutical industry. The **monoterpenoids** are the major component of many essential oils and, as such, have economic importance as flavors and perfumes. They are the terpenes that have been known for several centuries as components of the fragrant oils obtained from leaves, flowers and fruits. Monoterpenes, with sesquiterpenes, are the main constituents of essential oils. While a few, such as camphor, occur in a near pure form, most occur as complex mixtures, often of isomers difficult to separate. These essential oils have numerous actions, such as allelochemical functions between plants as well as between plants and predators, a role in wound healing and many monoterpenes possess antitumor activity in animals. Common acyclic examples include myrcene, geraniol, and linalool. Cyclic structures include many well-known compounds, including menthol, camphor, pinene, and limonene. Most of the monoterpenes come from common sources with which most of us are familiar.

The **thujone** diastereomers are rapidly metabolised convulsants. They act as noncompetitive blockers of the γ-aminobutyric acid (GABA) gated chloride channel. Myrcene is found in the essential oil of bay leaves (*Laurus nobilis*) as well as hops (*Humulus lupulus*). It is used as an intermediate in the manufacture of perfumes. Geraniol, which is isomeric with linalool, constitutes the major part of the oil of geraniums (*Pelargonium graveolens)* and is also found in essential

oils of citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus* or *C. flexuosus*), and others. Lavandulol is one of the principal ingredients of oil of lavender (*Lavandula augustifolia*), commonly used in male perfumes. Perillene can be found in the perilla (*Perilla frutescens*), native to South and East Asia. Menthol is a well-known monoterpene that is found in the essential oil of peppermint (*Mentha piperita*) and other members of the mint family (Lamiaceae). Carvone is a common monoterpene. It is one of the main olfactory components of caraway seed (*Carum carvi*), and it shows antifungal activity. 3-Carene is a cyclopropane containing monoterpene, derivatives of which have shown anesthetic activity. α-Pinene, the major ingredient in turpentine, may play a significant role in the activity of hydrocarbon-degrading bacteria in nature. Linalool is one of the principle constituents of coriander (*Coriandrum sativum*), a common spice. It is also one of the most common floral scent compounds found in flowering plants, and it is a common flavor compound in various teas. Safranal is chiefly responsible for the characteristic odor of saffron (*Crocus sativus*). Eucalyptol (1,8-cineole) is the main component of the essential oil of eucalyptus leaf (*Eucalyptus globulus*). Eucalyptol along with camphor, form the major constituents of rosemary oil. Recent research showed that eucalyptol is effective in reducing inflammation and pain and in promoting leukemia cell death.

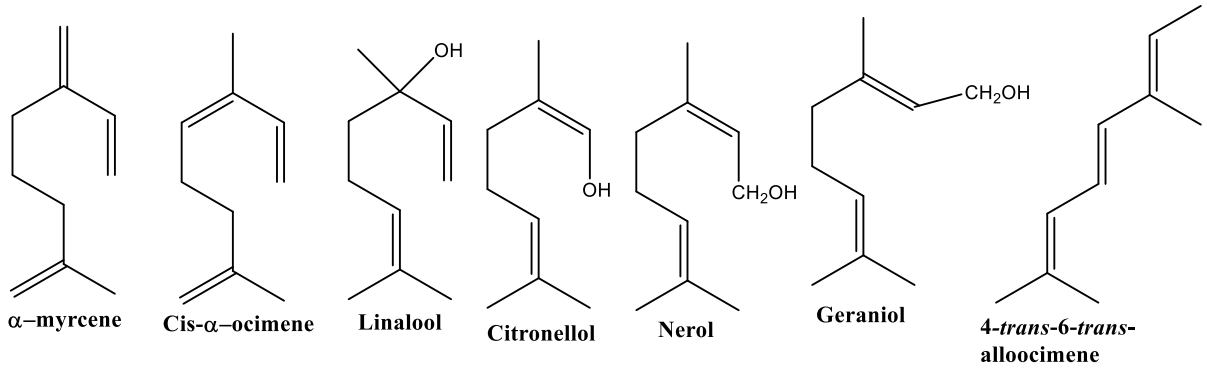
(a) Acyclic monoterpenes

They can be considered as derivatives of 2,6-dimethyloctane.



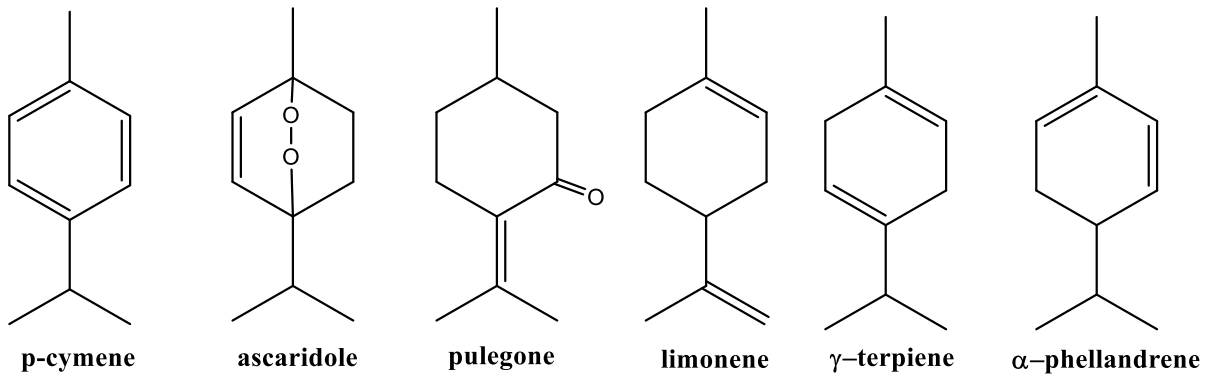
2,6-dimethyloctane

Among natural molecules, the followings are well known and have several structural isomers.

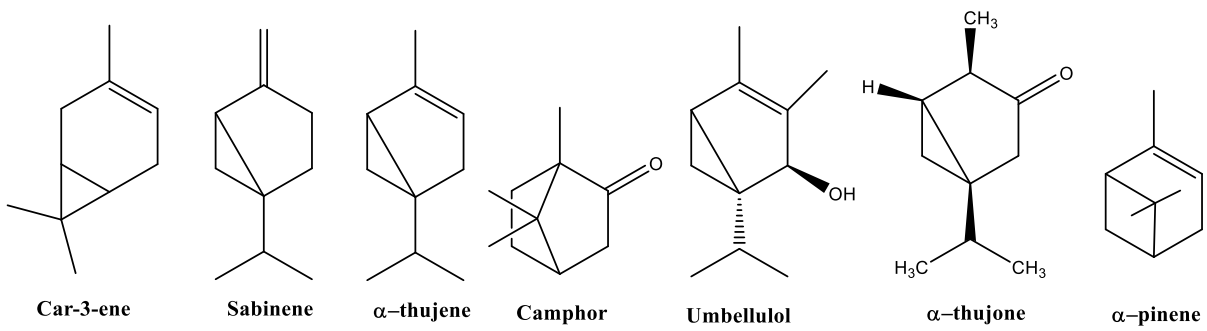


(b) Monocyclic monoterpenes

They are derived from cyclohexane with an isopropyl substituent. The most typical are:



(c) Bicyclic monoterpenes



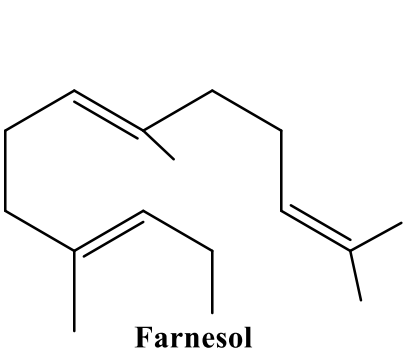
Sesquiterpenes: C_{15}

Sesquiterpenoids are defined as the group of 15 carbon compounds derived from three isoprene units, the C_{15} sesquiterpenes exist in aliphatic, bicyclic, and tricyclic frameworks. Like the monoterpenes, most of the sesquiterpenes are components of the essential oil of the plant from which they are derived. An important member of this series is **farnesol**, with pyrophosphate that serves as a key intermediate in terpenoid biosynthesis. They are found mainly in higher plants but also in invertebrates. Sesquiterpenes, with monoterpenes, are an

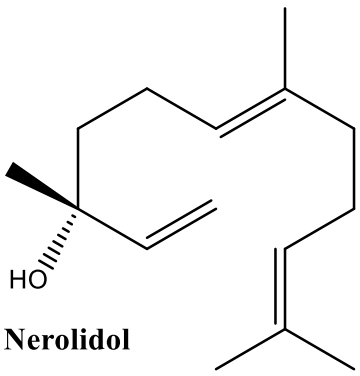
important constituent of essential oils in plants. They are the most diverse group of isoprenoids. In plants, they function as pheromones and juvenile hormones.

(a) Acyclic compounds

The acyclic representative are also called **farnesans**, term derived from the basic structure, farnesol. Farnesol and nerolidol are very common and are isolated from essential oils of various sources.



Farnesol

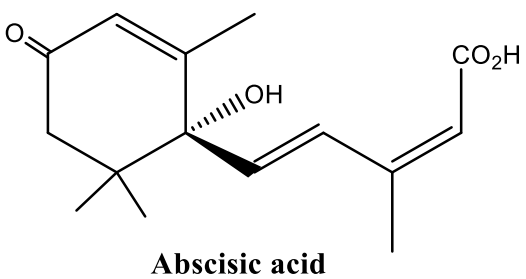


Nerolidol

Farnesol is widely distributed in many essential oils such as citronella, neroli, cyclamen, lemon grass, tuberose, rose, musk, and balsam. It is used in perfumery to emphasise the odors of perfumes. Moreover, it is a natural pesticide for mites and is also a pheromone for several insects and mammals, including elephants (territorial marking, individual recognition, mate attraction).

(b) Cyclic compounds

Abscisic acid plays a key role in plants in the regulation of stomatal closure by regulating ion channel activities and water exchanges across the plasma membrane of guard cells.



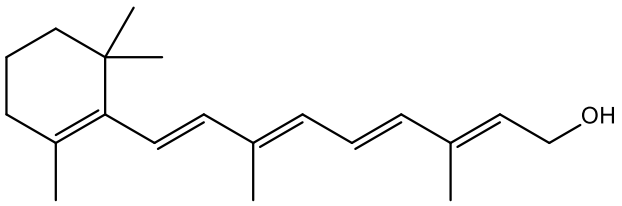
Abscisic acid

Abscisic acid has also a variety of roles in plant development, bud and seed dormancy, germination, cell division and movement. It induces also storage protein synthesis in seeds and may be involved in defense against insect attack.

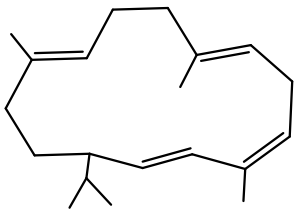
Diterpenes: C_{20}

They have 20 carbon atoms and are derived from geranylgeraniol pyrophosphate. They are of fungal or plant origin and are found in resins, gummy exudates, and in the resinous high-boiling fractions remaining after distillation of essential oils. Diterpenoid groups that are physiologically active include: vitamin A (retinol), phytohormones that regulate plant growth and germination, e.g. gibberellin, fungal hormones that stimulate the switch from asexual to sexual reproduction, e.g. trisporic acid; disease resistance agents (phytoalexins), e.g. casbene and podocarpic acid, the anticancer drug, taxol, from the bark of the yew tree, the cancer promoter, phorbol, and natural cannabinoids. The diterpenes have exceptionally open chain, as found in geranylgeraniol or phytol, which forms a part of chlorophyll and the side chain of vitamin E and K, and crocetin, which is a diacid diterpenoid, and the lipid part of the crocins, glycosylated derivatives present in saffron.

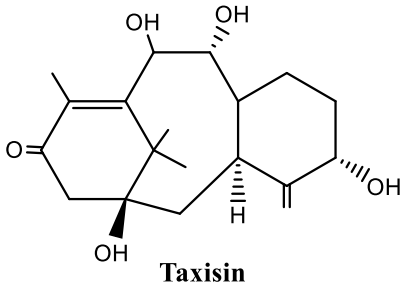
Examples of diterpene substances are given below:



All-*trans* retinol



Cembrene



Taxisin

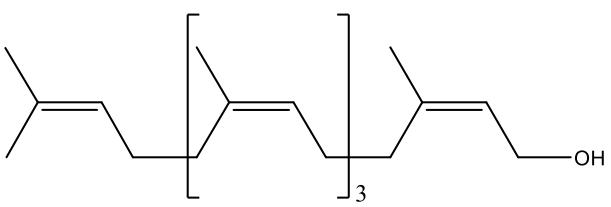
The diterpenes are a widely varied group of compounds based on four isoprene groups. Because of their higher boiling points, they are not considered to be essential oils. Instead, they are classically considered to be resins, the material that remains after steam distillation of a plant extract. Many interesting examples may be mentioned here. The cyclic ether zoapatanol is derived from the Mexican

zoapatle plant (*Montanoa tomentosa*). It has been used as an abortifacient. A number of clerodanes were isolated from *Ajuga*, *Salvia*, and *Teucrium* species.

They have been found to possess insect antifeedant activity. A variety of cytotoxic lactones were isolated from *Podocarpus* species. These podolactones and nigilactones have plant regulatory properties as well as antileukemic activity. The gibberellins comprise an important group of widely distributed plant hormones. These fall into two series, including a C_{20} family represented by gibberellin and a C_{19} series for whichgibberellic acid (GA3) is typical. Marrubiin is a diterpene lactone from white horehound (*Marrubium vulgare*). It has been used as a vasorelaxant. Taxol® or paclitaxol (derived from needles and bark of *Taxus* spp., yews) is a wholly unique antimitotic agent used to treat breast cancer. Chemically, it is made up of a diterpenoid core with an alkaloid side group. It binds to microtubules and stabilises them, as opposed to all other antimitotics of the tubulin-binding type, such as vincristine, the podophyllotoxins, and colchicines.

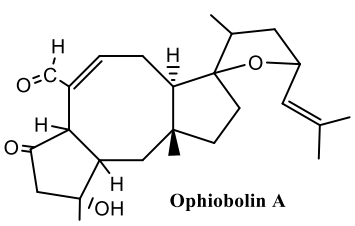
Sesterpenes: C_{25}

They are derived from geranylfarnesol pyrophosphate and have 25 carbon atoms. They were isolated from insect protective waxes and from fungal sources.

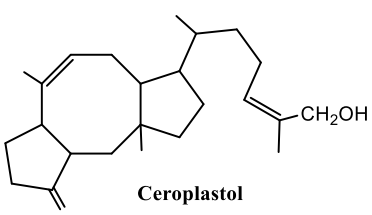


Geranylfarnesol

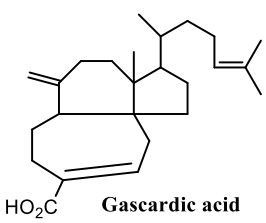
Three examples of sesterpenes are shown below.



Ophiobolin A



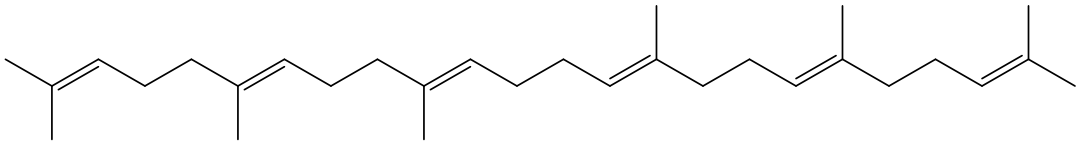
Ceroplastol



Gascardic acid

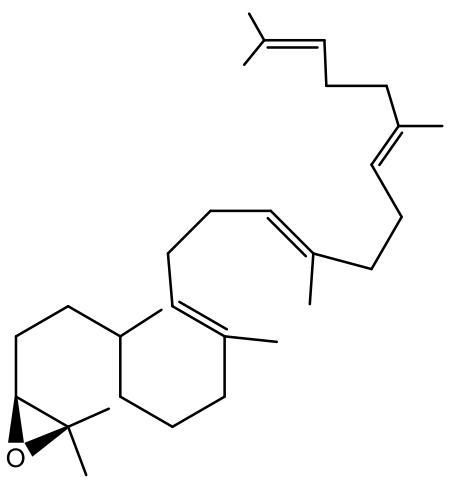
Triterpenes: C_{30}

They form a large group of natural substances, which includes steroids and consequently sterols. Squalene is the immediate biological precursor of all triterpenoids.



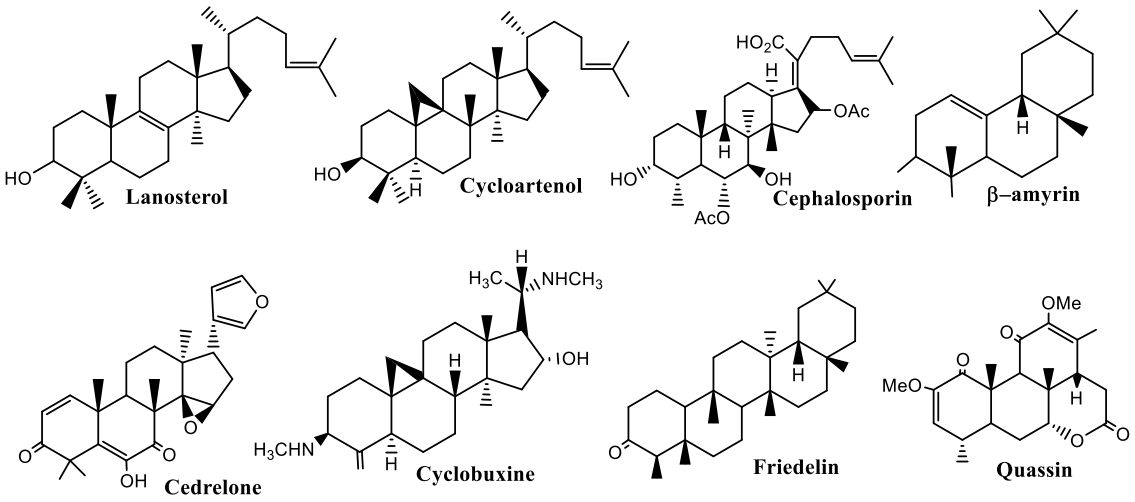
Squalene

Squalene epoxide (2,3-oxidosqualene) is produced by the enzyme squalene epoxidase which use NADPH and oxygen to oxidise squalene. This metabolic step is the first in sterol biosynthesis leading to the formation of lanosterol or cycloartenol.



Squalene epoxide

Squalane is a completely saturated derivative of squalene. Present in sebum, it is largely used as a component in many cosmetic products. It is obtained by hydrogenation of squalene extracted from olive oil. Among the large number of triterpenoid structures, some of them are shown below.

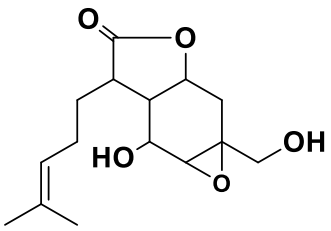


In-text Question

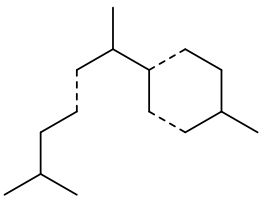
1. A terpene composed of four isoprene units and has the molecular formula $C_{20}H_{32}$ belongs to what class of terpenes?

   Answer: *The molecule is a diterpene.*

2. *Andrographis paniculata* produces a terpene derivative, paniculide B, as a product of tissue culture. Identify the number of isoprene units in the compound and state to what class of terpenes it belongs.



Paniculide B



Paniculide B skeleton

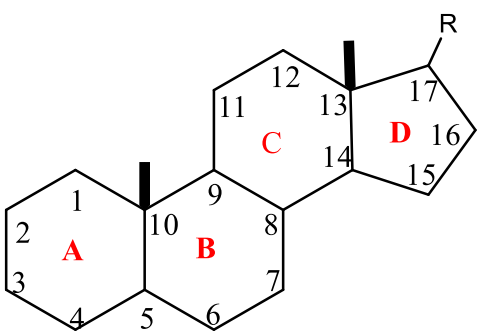
Hint: Use the attached skeleton of the compound to identify the isoprene units.

The C_{30} terpenes are based on six isoprene units and are biosynthetically derived from squalene. They are often high-melting colourless solids and are widely distributed among plant resins, cork, and cutin. There are several important groups of triterpenes, including common triterpenes, steroids, saponins, sterolins, and cardiac glycosides. Among these is azadirachtin, a powerful insect

antifeedant derived from seeds of the neem tree (*Azadirachta indica*). Only a few of the common triterpenes are widely distributed among plants. These include the amyrins and ursolic and oleanic acid, which are common on the waxy coatings on leaves and as a protective coating on some fruits. Other triterpenes include the limonins and the cucurbitacins, which were found to be potent insect steroid hormone antagonists.

Steroids

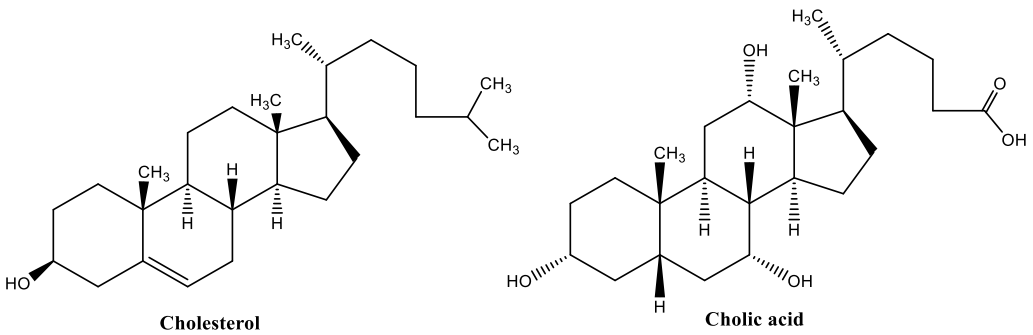
The important class of lipids called **steroids** is actually metabolic derivatives of terpenes which derived also from squalene by cyclisation, unsaturation and substitution, but they are customarily treated as a separate group. Steroids may be recognised by their tetracyclic skeleton, consisting of three fused six-membered and one five-membered ring, as shown in the diagram to the below. The four rings are designated A, B, C & D as shown in the carbon skeleton, and the peculiar numbering of the ring carbon atoms is the result of an earlier assignment of the structure. The substituents designated by R are often alkyl groups, but may also have functionality. The R group at the A:B ring fusion is most commonly methyl or hydrogen, that at the C:D fusion is usually methyl. The substituent at C-17 varies considerably, and is usually larger than methyl if it is not a functional group. The most common locations of functional groups are C-3, C-4, C-7, C-11, C-12 & C-17. Ring A is sometimes aromatic. Since a number of tetracyclic triterpenes also have this tetracyclic structure, it cannot be considered a unique identifier.



The steroid carbon skeleton

Steroids are widely distributed in animals, where they are associated with a number of physiological processes. Examples of some important steroids are shown in the following diagram. Norethindrone is a synthetic steroid, all the other

examples occur naturally. A common strategy in pharmaceutical chemistry is to take a natural compound, having certain desired biological properties together with undesired side effects, and to modify its structure to enhance the desired characteristics and diminish the undesired. The generic steroid structure drawn above has seven chiral stereocenters (carbons 5, 8, 9, 10, 13, 14 & 17), which means that it may have as many as 128 stereoisomers. With the exception of C-5, natural steroids generally have a single common configuration. This is shown in the last of the toggled displays, along with the preferred conformations of the rings.



Typical animal steroids

Practically all plant steroids are hydroxylated at C-3 and are, in fact, **sterols**. In the animal kingdom, the steroids have profound importance as hormones, coenzymes, and provitamins. However, the role of the **phytosterols** is less well understood. There is evidence that some of the phytosterols are effective against cardiovascular disease.

Chemical studies of the steroids were very important to our present understanding of the configurations and conformations of six-membered rings. Substituent groups at different sites on the tetracyclic skeleton will have axial or equatorial orientations that are fixed because of the rigid structure of the trans-fused rings. This fixed orientation influences chemical reactivity, largely due to the greater steric hindrance of axial groups versus their equatorial isomers. Thus an equatorial hydroxyl group is esterified more rapidly than its axial isomer.

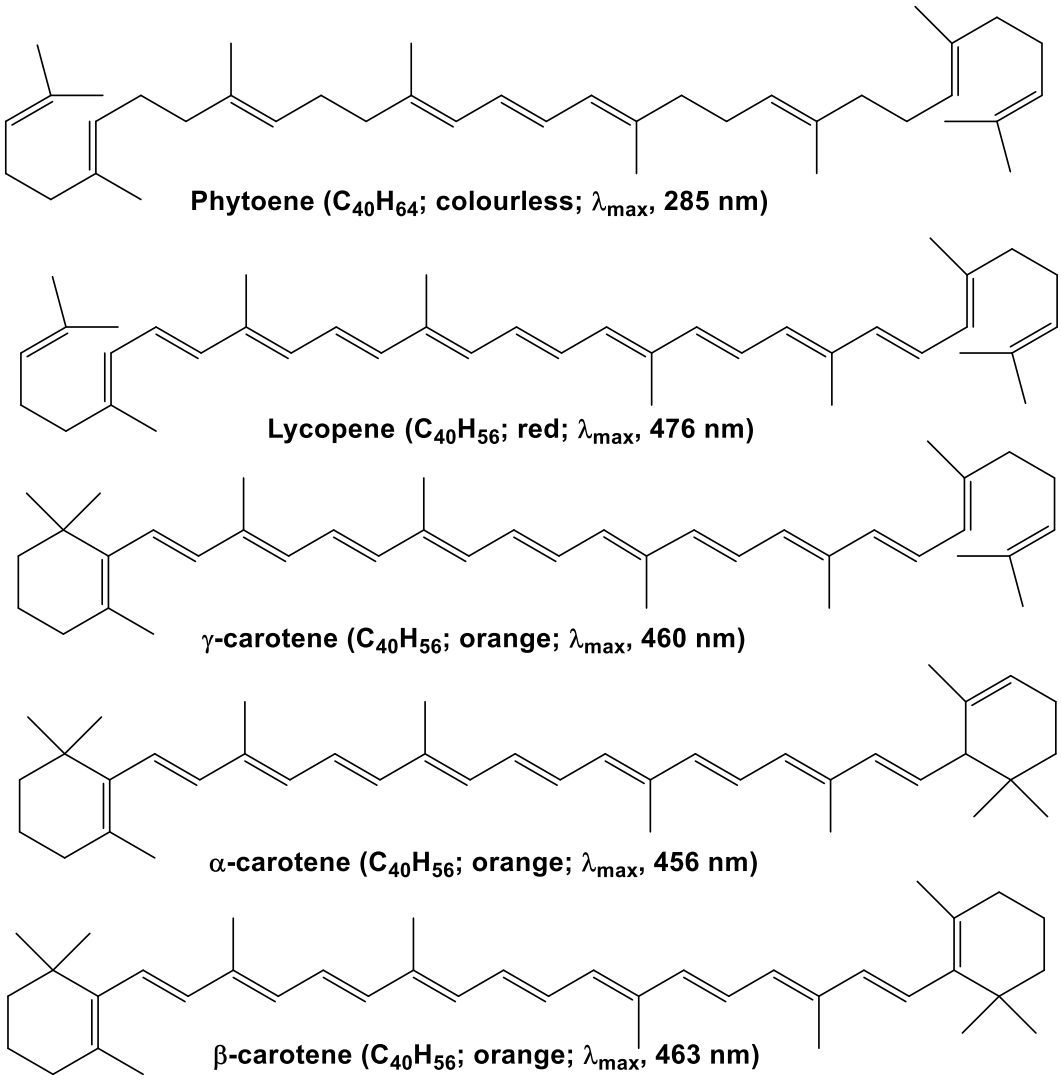
Tetraterpenes: C_{40}

The most common tetraterpenoids are the **carotenoids** a widely distributed group of C_{40} compounds. Whereas the structures of the di-and triterpenes can have a wide variety of fascinating structures, the carotenoids are generally derived from **lycopene**. Cyclisation at one end gives γ**-carotene** and at both ends provides β**-carotene**. This pigment was first isolated in 1831. The nature of these compounds was discovered during the 19th century. In 1831, Wachenroder H. proposed the term "carotene" for the hydrocarbon pigment he had crystallised from carrot roots. Berzelius J. called the more polar yellow pigments extracted from autumn leaves "xanthophylls" and Tswett M., who separated many pigments by column chromatography, called the whole group "carotenoids". It is virtually universal in the leaves of higher plants. As is evident from this polyene structure, numerous double-bond isomers are possible for these basic structures, all of which can provide brightly coloured pigments. In plants, carotenoids serve as necessary pigments in photosynthesis, where they are believed to protect plants from over oxidation catalysed by other light-absorbing pigments, such asthe chlorophylls. They are also responsible for colours varying from yellow to red in both flowers and fruits. This colouration attracts **pollinators** (flowers) and serves as a source of food for animal **herbivores** (fruits), thus aiding in **seed dispersal**. Among this important group, the numerous compounds consist of C_{40} chains (tetraterpenes) with conjugated double bonds, they show strong light absorption and often are brightly coloured (red, orange). They occur as pigments in bacteria, algae and higher plants.

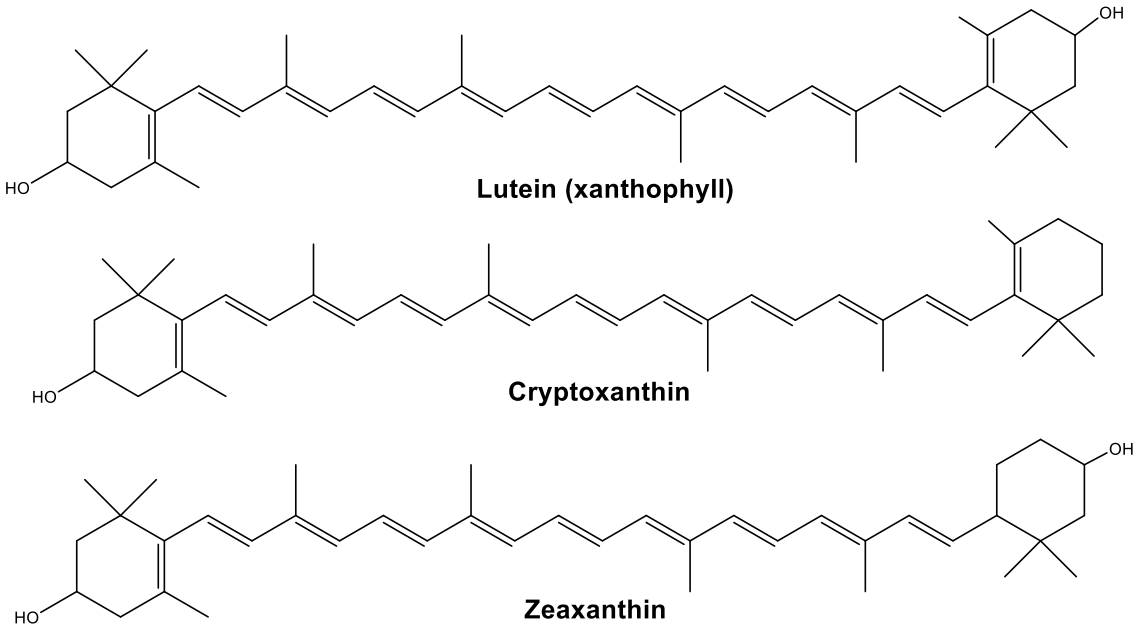
The hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls. Carotenoids are important components of the light harvesting in plants, expanding the absorption spectra of photosynthesis. The major carotenoids in this context are lutein, violaxanthin and neoxanthin. Additionally, there is considerable evidence which indicates a photoprotective role of xanthophylls preventing damage by dissipating excess light. In mammals, carotenoids exhibit immunomodulatory actions, likely related to their anticarcinogenic effects. β-Carotene was thus shown to enhance cell-mediated immune responses. The decrease in prostate cancer risk has been linked to the consumption of tomatoes, vegetable rich in lycopene, as prostatic

tissues. While there is yet limited direct evidence linking lycopene and prostate cancer, several observations, including the ability of the prostate to concentrate lycopene, suggest a special protection of lycopene against that pathology. Carotenoids consist of eight isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the centre of the molecule so that the two central methyl groups are in a 1,6-position relationship and the remaining non-terminal methyl groups are in a 1,5- position relationship. They are, by far the predominant class of tetraterpenes. They may be also classified in the terpenoids. Carotenoids can be considered derivatives of **lycopene**, found in tomatoes, fruits and flowers. Its long straight chain is highly unsaturated and composed of two identical units joined by a double bond between carbon 15 and 15'. Each of these 20 carbon units may be considered to be derived from 4 isoprene units. Lycopene is a bioactive red coloured pigment naturally occurring in plants. Interest in lycopene is increasing due to increasing evidence proving its antioxidant activities and its preventive properties toward numerous diseases. *In vitro, in vivo* and *ex vivo* studies have demonstrated that lycopene-rich foods are inversely associated to diseases such as cancers, cardiovascular diseases, diabetes, and others.

Carotenoids may be acyclic (seco-carotenoids) or cyclic (mono- or bi-, alicyclic or aryl). Oxyfunctionalisation of various carotenoids leads to a large number of **xanthophylls** in which the function may be a hydroxyl, methoxyl, carbonyl, oxo, formyl or epoxy group. Only some of the most common carotenes and xanthophylls are given below:



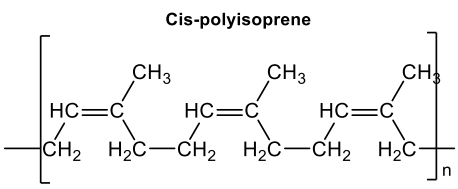
In human serum, several carotenes and xanthophylls have been detected. If α-, β-carotene and lycopene are frequently quoted in specialised papers, some others are now determined with precise HPLC methods (lutein, zeaxanthin, cantaxanthin and β-cryptoxanthin). These compounds originate from ingested fruit, green leaves, berries and yellow corn.



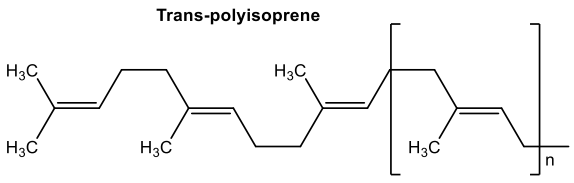
Some examples of xanthophylls

Polyterpenes: (C_n)

The two most important polyterpenes are **natural rubber** and **gutta-percha**. In natural rubber, the double bonds of the repeating isoprene units are *cis*; the molecules are able to bend back and forth a little so that the rubber can stretch. Gutta-percha has the *trans* structure. It is hard and non-rubbery. Gutta-percha is extensively used in electrical insulation and dentistry.



Chemical structure of Natural rubber



Chemical structure of Gutta-percha

In 1829, Faraday Michael showed that rubber produced by *Hevea brasiliensis* was made up solely of carbon and hydrogen and had the empirical formula C_5H_8. In 1860, the English chemist, Greville Williams C., obtained a liquid with the same formula by distilling rubber, he called it, isoprene. In 1879, Bouchardat G. obtained isoprene from natural rubber and found that heating isoprene with HCl produced a rubber-like polymer after distillation. He said that this new product had "the elasticity and other properties of rubber itself." This was the first

production of artificial rubber. After the early demonstration that each isoprene unit has one double bond and that rubber has a high molecular weight, the idea that the rubber molecule consisted of long chains formed by the regular linking of isoprene units was only slowly established after the works of Harries C.D. (between 1902 and 1905) and mainly of Staudinger H. (in 1920) who coined the term "macromolecule". It was determined that the hydrocarbon chains were composed of an initial group ω' formed by two or three *trans*-isomer units, a long chain formed by a great number of *cis*-isomer units, and a not yet completely determined terminal group α.

The ω-terminal linking to proteins was suggested to form physical cross-links, whereas the α-terminal linking to phospholipids to form chemical cross-links with long chain fatty acid ester groups. It was shown that the linked fatty acids were composed of saturated and unsaturated C_{10} to C_{22} fatty acids, the composition of which was similar to that of mixed fatty acids. It seems that the mechanical properties of natural rubber could be dependent on the composition of these fatty acids. If natural rubber is formed by *cis*-isomer units, the material known as gutta-percha produced by *Palaquium gutta* (Sapotaceae), and balata, formed by *Mimusops globosa*, are polyisoprenes having all *trans* structure. The polymer chains of rubber are very long and have an average molecular weight more than a million. As these long chains are not naturally cross-linked, rubber is soluble in non-polar solvents and thus may be considered as lipids.

Goodyear C. found in 1830 the way to harden the natural rubber in heating the raw product with elementary sulfur, process which creates chain cross-links and is now known as vulcanisation.

It must be noticed that while natural rubber is mainly produced today from *Hevea* tree, it may also be obtained from guayule (*Parthenium argentatum*), a xerophytic shrub that has been exploited as commercial source of rubber since the pre-Columbian times when Indians of Mexico used it to form balls for their games.

In-text Question

What is the main structural difference between natural rubber and gutta-percha?

Answer

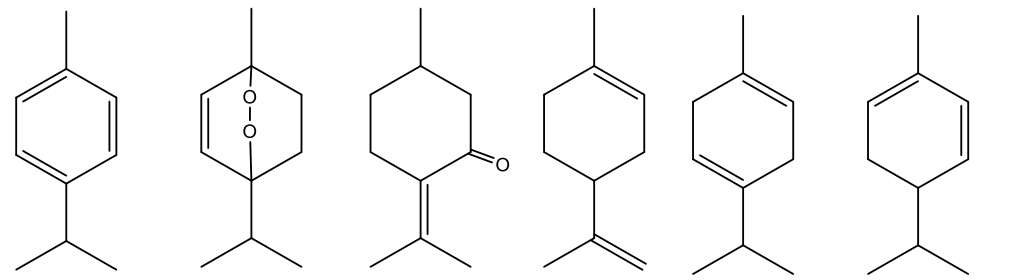
In natural rubber, the double bonds of the repeating isoprene units are cis while gutta-percha has the trans structure.

4.0 SELF-ASSESSMENT EXERCISE

1. Give two examples each of monocyclic and bicyclic monoterpenes.
2. List all the classes of terpenes with the number of isoprene units and carbon atoms each is made of.
3. State the isoprene rule.
4. Explain briefly the processes involved in isolation of terpenes from plants.

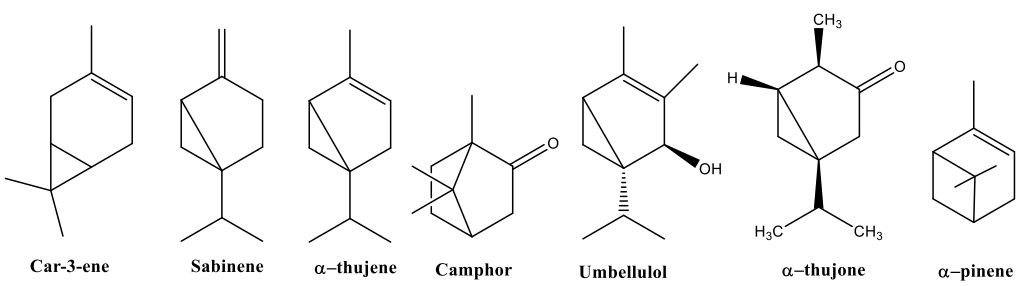
Answers:

1. *Examples of monocyclic monoterpenes include:*



Choose any two.

Examples of bicyclic monoterpenes include:



Choose any two.

2. Classes of terpenes with the number of isoprene units and carbon atoms.

S/N	Class of Terpene	Isoprene unit	Number of carbon
1	Monoterpenes	2	10
2	Sesquiterpenes	3	15
3	Diterpenes	4	20

4	Sesterpenes	5	25
5	Triterpenes	6	30
6	Carotenoids	8	40
7	Rubber	>100	>500

3. *The isoprene rule states that "Terpene skeleton are formed by linking together isoprene units through carbon atoms 1 and 4 (head-to-tail), carbon atoms 1 and 1 (head-to-head), carbon atoms 4 and 4 (tail-to-tail) or combination thereof."*
4. *See section 3.1*

5.0 CONCLUSION

In this unit you learnt the definition of terpenes and possible classifications of this class of compounds. You also learned that the structure of terpenes is based on various but definite number of isoprene units.

6.0 SUMMARY

What you have learned in this unit concerns the chemistry that distinguishes terpenes from other classes of secondary metabolites. It has served to introduce you to the basic unit of this class of compounds and to give a basis for the various classifications.

7.0 REFERENCE/FURTHER READING

1. Leland, J.C. *et al.* (2006). *Natural Products from Plants.* (2nd ed.). Boca Raton: CRC Press Taylor & Francis Group

UNIT 3 ALKALOIDS

CONTENTS

1.0 Introduction

2.0 Objectives

3.0 Main Content

- 3.1 Occurrence
- 3.2 Nomenclature
- 3.3 Isolation of Alkaloids
- 3.4 Properties
- 3.5 Chemical Tests for Alkaloids
- 3.6 Classification

4.0 Conclusion

5.0 Summary

6.0 Self-Assessment Exercise

7.0 References/Further Reading

1.0 INTRODUCTION

Alkaloids constitute a class of basic, nitrogen-containing plant products that have complex structures and possess significant pharmacological properties. The term alkaloid, meaning ‘alkali-like,’ was first proposed by the pharmacist W. Meissner in the early nineteenth century before anything was known about the chemical structures of the compounds.

The term alkaloid has been used to designate the compounds of plant origin having one or more basic nitrogen atoms in heterocyclic ring systems, which induce pronounced physiological activity in animals and man.

The above definition of the alkaloids is by no means perfect and does not cover all compounds classed as alkaloids for reasons such as; piperine, the alkaloid of pepper,

is not basic and has practically no physiological activity and ephedrine is a straight-chain alkaloid that is produced by animal glands and has marked physiological activity.

In fact, no precise definition of the term ‘alkaloid’ is possible but in general it designates compounds having the following common features:

(a) They are found in plants, although a few are of animal origin.

(b) They are basic in character and show marked physiological activity.

(c) They have heterocyclic rings containing nitrogen as a part of their structures.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- Give a simple definition of the term alkaloid
- Explain the occurrence of this class of compounds
- Mention some of their general properties
- Give the basis for the classification of alkaloids
- Itemise the steps involved in their isolation.

3.0 MAIN CONTENT

3.1 Occurrence

Alkaloids occur chiefly in plants of the dicotyledons families and are localised in seeds, leaves, bark or root of the plant. Each site may contain closely related alkaloids. They occur largely as salts of common plant acids such as acetic acid, oxalic acid, lactic acid, malic acid, tartaric acid, citric acid or of certain special organic acids.

3.2 Nomenclature

There is no systematic nomenclature for alkaloids but some of the methods adopted in their naming are mentioned below:

- **According to their source:** Some are named according to the family in which they are found, e.g. papavarine from papaveraceae family, punarnavin, ephedrin.
- **According to their physiological response:** Others obtained their names from their physiological response; e.g. morphine means *God of dreams*; emetine means *to vomit*.

- **According to their discovery:** The pelletierine group has been named after its discoverer, P.J. Pelletier.

3.3 Isolation of Alkaloids

The extraction of alkaloids is based upon their basic character and solubility pattern. The normal procedures followed are to treat moistened drug with alkali so as to set free the base as it exists in salt form and then to separate the free base with organic solvent. Though the methods of extraction vary, the following general procedure is applied for small scale extraction of alkaloids. For extraction of alkaloids, the plant material is macerated. If the material is rich in fat first it is defatted with ligroin or petroleum ether, especially in case of seed and leaf forms of drugs. Before applying this treatment the alkaloid should be tested for its solubility in petroleum ether. Otherwise, the drug should be pretreated with acid so as to convert alkaloids into the salts. This happens in case of extraction of ergotamine from ergot.

In the second stage the drugs may be extracted with polar solvents like water, ethanol, methanol, aqueous alcohol mixture or with acidified aqueous solution and the cellulose material separated by filtration. By this treatment, alkaloid salts are transferred to polar solvent. It also helps in removing pigments, sugar and other organic constituents in the following stage. The filtrate is evaporated to thick syrup to give the crude plant extract. This is then dissolved in dilute acid and is subjected to partitioning between aqueous acid solution and an organic solvent such as ether. After continuous extraction with organic solvent for some time the aqueous phase is made alkaline with either sodium carbonate or ammonia and extracted with ether. Evaporation of ether solution gives a solid mixture of crude alkaloids. It is then subjected to fractional crystallisation for separation into individual pure alkaloids. In modern practice, the isolation is effected by column chromatography, gas chromatography and by counter current distribution. The general scheme for extraction of alkaloids is illustrated in Fig. 1.

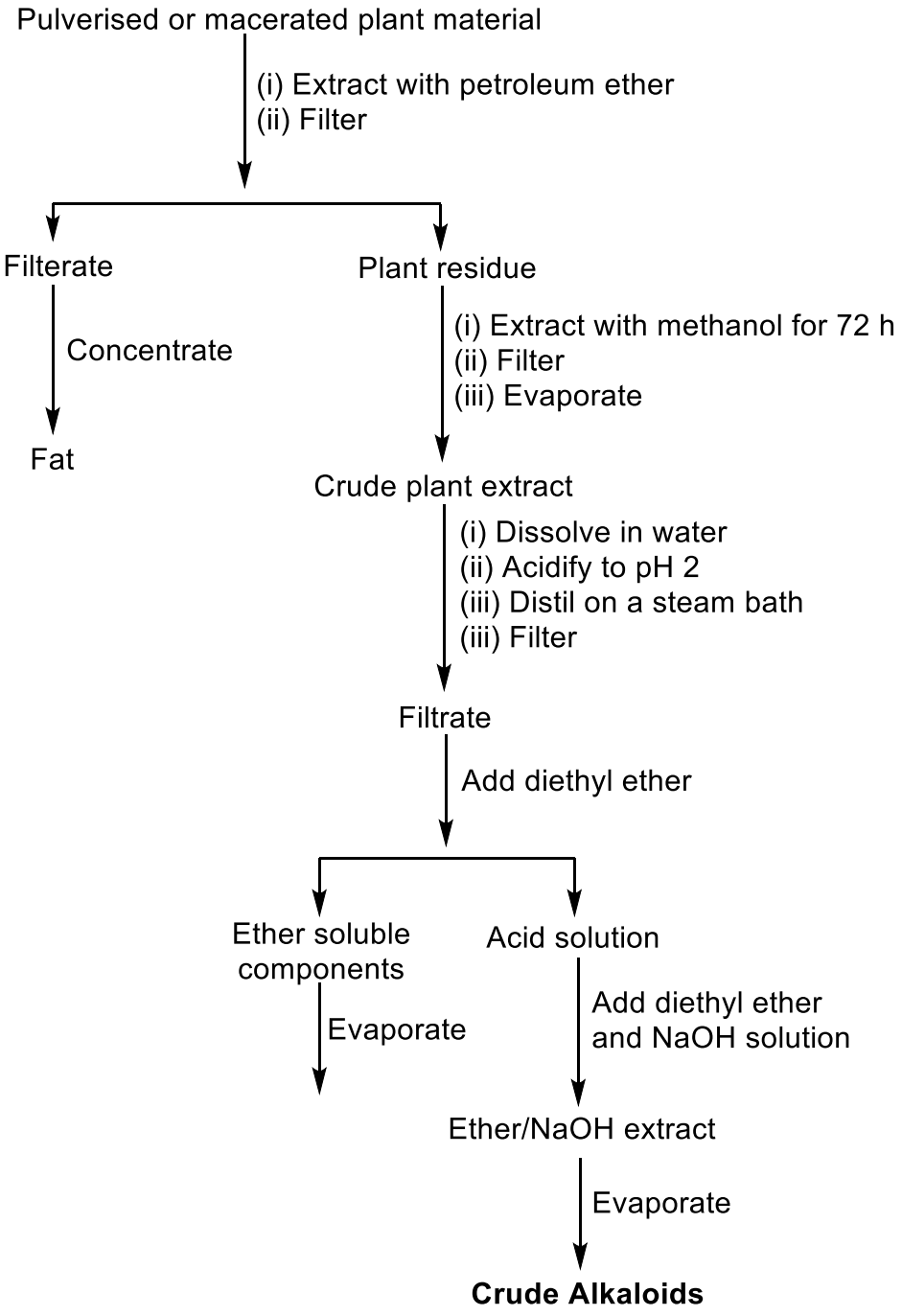


Figure 1: Isolation of alkaloids

Properties

Physical properties

With few exemptions, all the alkaloids are colourless, crystalline solids with sharp melting points or decomposition range. Some alkaloids are amorphous gum, while others such as coniine, spartine and nicotine are liquid and volatile in nature. Some

alkaloids are coloured in nature, e.g. betanidin is red, berberine is yellow and its salts are copper-red in colour.

In general, the free bases of alkaloids are soluble in organic, non-polar solvents. The salts of most alkaloids are soluble in water. In contrast, free bases are insoluble in water and their salts are also sparingly soluble in organic solvents. The alkaloids containing quaternary bases are only water soluble. Some of the pseudoalkalods and protoalkaloids show higher solubility in water. For examples, colchicine is soluble in alkaline water, acid and water and caffeine (free base) is freely soluble in water. Quinine hydrochloride is highly soluble in water; i.e. one part of quinine hydrochloride is soluble in less than one part of water, while only one part of quinine sulphate is soluble in 1000 parts of water. The solubility of alkaloids and their salts is useful in pharmaceutical industry for the extraction and formulation of final pharmaceutical products.

Chemical properties

Most of the alkaloids are basic in reaction, due to availability of lone pair of electrons on the nitrogen in the heterocyclic ring. The basic character of alkaloids is enhanced if the attached functional groups are electron releasing. The alkaloid turns to be neutral or acidic when the attached functional groups are electron withdrawing like amide group which reduces availability of lone pair of electrons. Free alkaloids exhibit basic characters and are very much prone to decomposition and this causes a problem during their storage. Their salt formation with inorganic acid prevents many a time their decomposition. The alkaloids may contain one or more number of nitrogen and it may exist in the form of primary ($R\text{-}NH_2$), e.g. mescaline, secondary amine ($R_2\text{-}NH$), e.g. ephedrine; tertiary amine ($R_3\text{-}N$), e.g. atropine; and quaternary amine ($R_4N^+X^-$), e.g. tubocurarine chloride. In the last type, their properties vary from other alkaloids, owing to quaternary nature of nitrogen. Alkaloids exist in nature either in free form, as amine or as salt with acid or alkaloids N-oxides.

3.4 Chemical Tests for Alkaloids

- Test by Dragendorff reagent (potassium-bismuth-iodide solution): Alkaloids give **reddish-brown precipitate** with this reagent.
- Test by Mayer reagent (potassium-mercuric-iodide solution): Alkaloids gives **cream colour precipitate** with this reagent.

- Test by Wagner reagent (iodine-potassium-iodide solution): Alkaloids give **Brown colour precipitate** with this reagent.
- Test by Hager reagent (saturated solution of picric acid): alkaloids give **yellow colour precipitate** with this reagent.
- Test by Tannic acid: Alkaloids gives **buff colour precipitate** with this acid.
- Test by Picrolonic acid: Alkaloids give **yellow colour precipitate** with this acid.

3.5 Classification

Alkaloids are so numerous and involve such a variety of molecular structure that their rational classification is difficult. However, the best approach to the problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule. Very often, a number of alkaloids having similar structure are found in the same plant and also show resemblance in the properties. Alkaloids are also named as a group after the name of the plant from which they are isolated.

For historical reasons as also because of their structural complexities, the nomenclature of alkaloids has not been systematised. The names of individual members are, therefore, generally derived from the name of the plant in which they occur, or from their characteristic physiological activity.

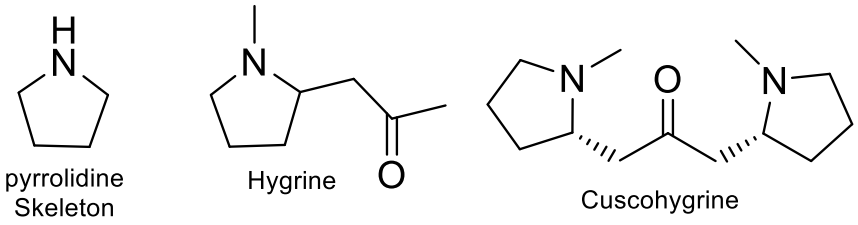
The various classes of alkaloids according to the heterocyclic ring system they contain are listed below:

- *pyrrolidine*
- *piperidine (tropane)*
- *pyridine-pyrrolidine*
- *pyridine-piperidine*
- *quinoline*
- *isoquinoline*
- *indole alkaloids.*

In the following treatment we will discuss the above classes taking examples of the familiar members. In most of these cases we will also give concisely the structure, the source and the characteristic physiological activity.

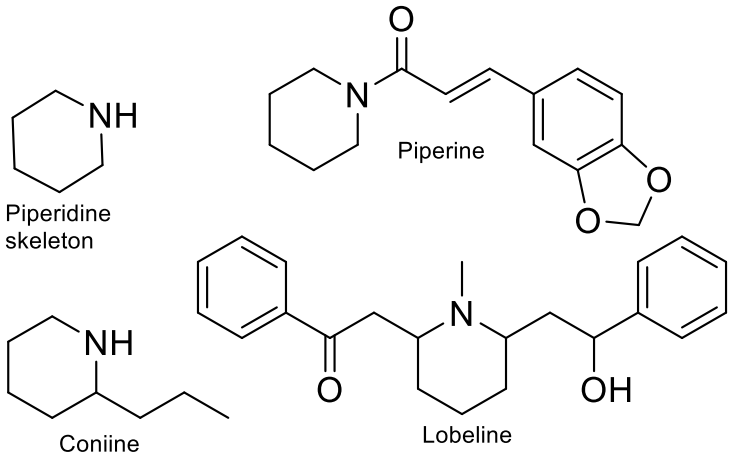
Pyrrolidine alkaloids

They contain the pyrrolidine (tetrahydropyrrole) ring system, e.g. *Hygrine* and *Cuscohygrine.* Hygrine is isolated from the leaves of Peruvian coca shrub. *Cuscohygrine* is found in '*cusco leaves'.*



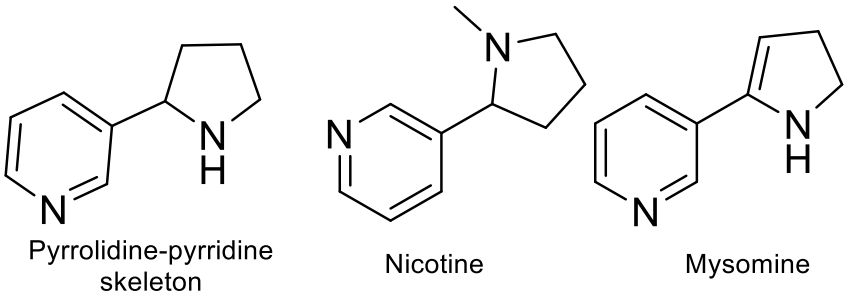
Piperidine alkaloids

They have piperidine (hexahydropyridine) as the heterocyclic structural unit, e.g., Coniine, Isopelletierine, Lobeline and Piperine. Coniine occurs in the oil of hemlock and is poison to humans. Isopelletierine is isolated from bark of pomegranate tree. Lobeline, obtained from the seeds of Indian tobacco is used in medicine as a respiratory stimulant and as a tobacco substitute.



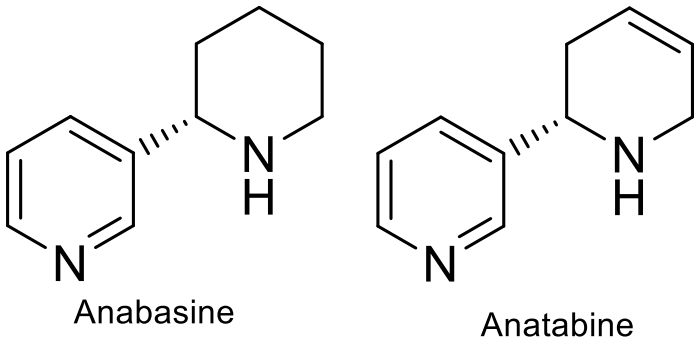
Pyrrolidine-pyridine alkaloids

The heterocyclic ring system present in these alkaloids is pyrrolidine-pyridine. Examples are nicotine or mysomine. Nicotine is isolated from tobacco leaf. It is highly toxic which in very small doses causes respiratory stimulation and in large doses causes respiratory failure and death. Mysomine also occurs in tobacco, and the aroma of tobacco smoke is due to it.



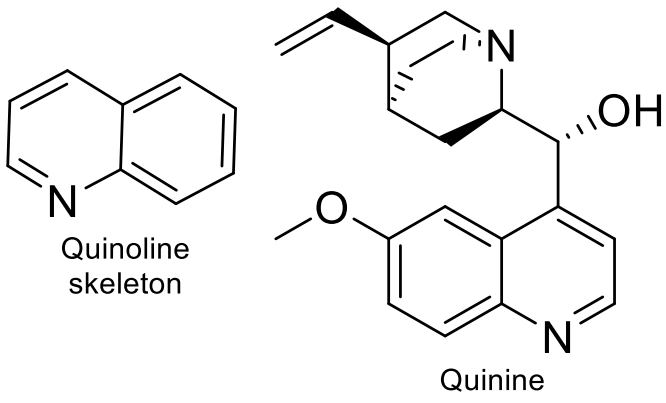
Pyridine-piperidine alkaloids

This family of alkaloids contains a pyridine ring system joined to a piperidine ring system. The simplest member is Anabasine, the chief alkaloid isolated from the poisonous Asiatic plant, *Anabasis aphyllan.* Another alkaloid of this class is Anatabine.



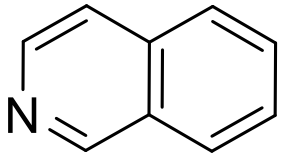
Quinoline alkaloids

These have the basic heterocyclic ring system quinoline; e.g., quinine and primaquinine. Qiunine occurs in the bark of cinchona tree. It has been used for centuries for treatment of malaria. Synthetic drugs such as primaquinine have largely replaced quinine as an anti-malarial. A recently isolated alkaloid of this group is camphtotecin found in Chinese tree *Camphotheca acuminata* Nyssaceae. This is known to possess anti-leukemic and anti-tumor activities in animals.

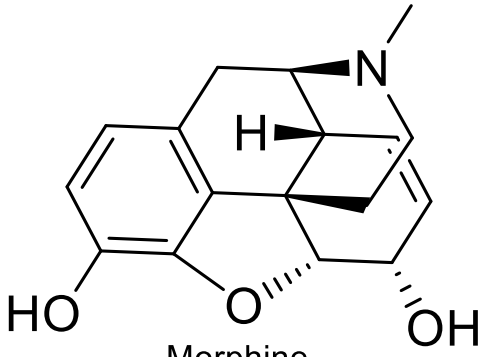


Isoquinoline alkaloids

They contain the heterocyclic ring system isoquinoline; e.g., papverine, narceine, emetine, cephaline and morphine. Papaverine is isolated from the unripe seed capsule of the opium poppy. It finds uses as medicine in the treatment of spasm of the stomach or intestines. Morphine is isolated from opium. It is used in medicine as hypnotic (sleep producing) andanalgesic (pain relieving). Unfortunately, prolonged intake of morphine leads to addiction and hence, its use is sharply restricted.



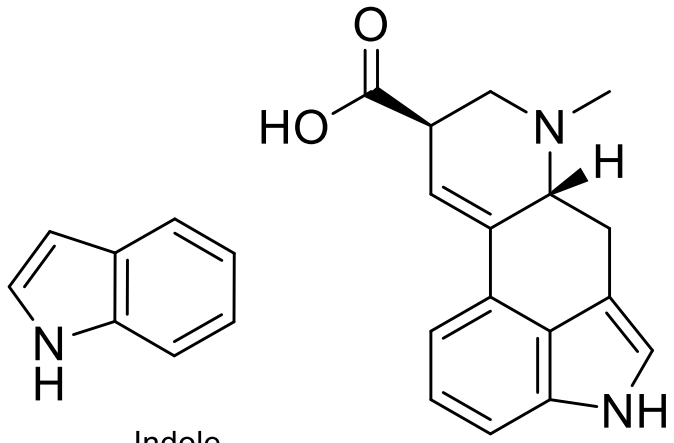
Isoquinoline skeleton



Morphine

Indole alkaloids

Alkaloids based upon the indole skeleton are widely distributed in nature. The physiological action of these substances may rest in the fact that derivatives of indole play important roles in the chemistry of the brain. Lysergic acid, isolated from *Claviceps purpurea,* is the best known alkaloid of this class. In small regulated amounts, it is used in the treatment of migraine headaches as also for the induction of uterine contraction in child birth. Another indole alkaloid is strychnine which is isolated from the seed of *Strychnos nuxvomica*. It is extremely poisonous and is used for exterminating vermin. Others include yohimbine, aspidospermine, vinblastine and vincristine.



Indole skeleton

Lysergic acid

In-text Question

An example of isoquinoline alkaloids is (a) hygrine (b) vinblastine (c) papaverine (d) mysomine.

Answer: *(c) papaverine*

4.0 SELF-ASSESSMENT EXERCISE

1. Give a precise definition of the term ‘alkaloid’. Comment on the degree of perfection of this definition.
2. Classify alkaloids based on the nature of the heterocyclic ring system present in their molecular structure. Give the name and structure of at least one example in each class.

Answer:

1. *Alkaloids are a class of compounds of plant origin having one or more basic nitrogen atoms in heterocyclic ring systems, which induce pronounced physiological activity in animals and man.*

   The definition above is not perfect and does not cover all compounds classed as alkaloids for the following reasons: (a) piperine is not basic and has practically no physiological activity and ephedrine is a straight-chain alkaloid that is produced by animal glands and has marked physiological activity. In fact, no precise definition of the term ‘alkaloid’ is possible.
2. *The various classes of alkaloids according to the heterocyclic ring system they contain are listed below: (i) pyrrolidine (ii) piperidine (tropane) (iii) pyridine-*

pyrrolidine (iv) pyridine-piperidine (v) quinoline (vi) isoquinoline (vii) indole alkaloids. (See section 3.5 for examples of each class)

5.0 CONCLUSION

Alkaloids are a chemically heterogenous group of natural substance and compose more than 6000 basic nitrogen containing organic compounds which occur in about 15% of all vascular terresterial plants and in more than 150 different plant families. The alkaloids exhibit diversity of structure and also show an extraordinary spectrum of pharmacological activities. Because of these characters, they are important for chemical, physiological, taxonomical and biogenetic studies.

6.0 SUMMARY

Alkaloids may be classified in terms of the nitrogen-containing ring system which may reflect the amino acids (lysine, ornithine, tyrosine and tryptophan) which are involved in their biosynthesis.

7.0 REFERENCES/FURTHER READING

1. Bruneton, J. (1999). *Pharmacognosy, Phytochemistry, Medicinal Plants.* (2nd ed.). United Kingdom: Lavoisier Publishers.

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Module 2

Unit 1 Isolation and Structure Determination of Natural Products

Unit 2 Biosynthesis

UNIT 1 ISOLATION AND STRUCTURE DETERMINATION OF NATURAL PRODUCTS

CONTENTS

1.0 Introduction

2.0 Objectives

3.0 Main Content

- 3.1 General Methods of Extraction
- 3 2 Fractionation
- 3.3 Partition Chromatography
- 3.4 Gas Chromatography
- 3.5 Characterisation of Natural Products

4.0 Conclusion

5.0 Summary

6.0 Self-Assessment Exercise

7.0 References/Further Reading

1.0 INTRODUCTION

Secondary metabolites, with some exceptions, occur in amounts that are less than 0.01 % of the dry weight of the plant. Extraction of 1 kg of dry plant material is likely to yield less than 100 mg of a natural product. These compounds may be unstable and present as part of a complex mixture. The isolation, separation and purification of these natural products require considerable skill. The source of a secondary metabolite requires proper identification and a voucher specimen needs to be retained. Within the same species there are sometimes chemotypes, each with a particular

composition. Some compounds are found in the roots, some are components of the bark, and others may be found in the leaves, the flowers or the fruit. Some compounds play a seasonal role in the plant, for example as insect antifeedants. Thus, the part of the plant and the place and date on which the plant was collected should all be recorded.

Chemistry of natural products involves the study of these chemicals produced in plants, and other organisms describing their:

- extraction
- purification
- identification
- structure determination
- synthesis.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the motivation for examining the structures of natural products
- discuss the chemistry underlying the methods of isolating natural products
- mention the stages in the elucidation of the structure of a natural product
- mention the difference between isolation and characterisation.

3.0 MAIN CONTENT

3.1 General Methods of Extraction

Aqueous and organic-solvent extraction methods

Extraction methods to be chosen should be based on knowledge of several physicochemical properties of the compound of interest. These include partition coefficients in water or organic solvents, relative polarity of the molecule, stability of the molecule in light or dark, as well as the temperature employed during the extraction process. So, if the compound of interest is highly soluble in water, we employ hot or cold water to obtain an **aqueous extract**. If, on the other hand, the compound is highly soluble in a particular organic solvent, we employ that solvent to obtain an **organic-solvent extract**.

Aqueous extraction of compounds

Traditional methods of aqueous extraction

The preparation of **herbal remedies** based on traditional methods of water extraction utilises two different approaches. If extracting **herbaceous tissues** of leaves, roots, and flowers, or soft-textured fruits with a relatively high water content (in the range of 60 to 95% water) with hot water or cold water, relatively mild physical conditions are used to obtain what is called an **infusion**. However, for woody, highly lignified tissues with relatively low water content (in the range of 5 to 50% water), such as roots, barks, twigs, and some dry fruits, we need to employ more vigorous physical extraction procedures, using longer extraction times and boiling water, to obtain what is called a **decoction**.

There is an almost endless list of methods for preparing infusions and decoctions, but all are based on the same principles.

Laboratory methods of organic-solvent extraction of compounds

Maceration

Powdered crude material is placed in a stoppered container with the solvent and allowed to stand for a period of 24 to 48 hours with frequent agitation until soluble matter is dissolved. The mixture is then stained; the marc (the damp solid material) pressed and may be macerated again. The combined liquid is clarified by filtration or decantation after standing.

Percolation

Performed in a cone shaped vessel, (called percolator) the narrow side of which is fitted with a suitable filter and a stopcock, plant material allowed to macerate with the specified menstrum for a specified period in the percolator after which the stopcock is opened so that the solvent drips slowly from the bottom. The collected solvent is evaporated in a rotary evaporator and recovered solvent is put back to percolator.

Hot continuous extraction (Soxhlet extraction)

If the compounds of interest are not soluble in water because of their **non-polar** nature, select an organic solvent (e.g., acetone, methanol, ethanol, chloroform, diethyl ether, methylene chloride, or a combination of more than one organic solvent) to carry out the extraction. The temperature of this extraction depends on the boiling point of the solvent chosen and must be carefully watched due to the special equipment that

is used. One can use a **Soxhlet extractor**, which is basically a specialised glass refluxing unit that is used for such organic-solvent extractions. The method involves continuous extraction by boiling organic solvents. Natural products may be obtained from the crushed biological material by extraction with a solvent such as petroleum ether, chloroform (trichloromethane), ethyl acetate (ethyl ethanoate) or methanol. Several solvents of increasing polarity may be used. Thus lipid material (waxes, fatty acids, sterols, carotenoids and simple terpenoids) can be extracted with non-polar solvents such as petroleum ether, but more polar substances such as the alkaloids and glycosides are extracted with methanol, aqueous methanol or even hot water.

In the following example (using a 1:1 mixture of methylene chloride/methanol), the temperature must be maintained at 40°C for 8hrs in order to obtain complete extraction of each sample. If the temperature falls below this, extraction will be slow. If the temperature goes above this, the risk of degrading the compounds of interest becomes great.

With the presence of higher temperatures, there is always the risk of degrading some of the active compounds; thus, low-boiling-point solvents, such as dichloromethane or diethyl ether, are usually the best choice. The basic procedure is outlined below. Preliminary cleanup procedures are usually necessary before samples are analysed by HPLC or by any of the other chromatographic techniques.

The example we use here employs methylene chloride. It is highly toxic, and therefore, needs to be used in a fume hood to avoid breathing the toxic fumes. It should also be handled carefully, using latex gloves so that none of this solvent comes in contact with the skin. The extraction methods are as follows:

1. Weigh out a standard quantity of plant tissue sample that was previously stored in deep freeze at –80°C.

2. Grind frozen plant tissues to a fine powder using small amounts of liquid nitrogen in a ceramic **mortar** and grinding with a ceramic **pestle**.

3. Weigh out a known quantity of powdered tissue sample in a **cellulose thimble** in a Soxhlet extractor containing enough solvent or solvent mixture such as a 1:1 mixture of methylene chloride/methanol.

4. Reflux the sample for 8hrs at 40°C using a condenser (with running cold water) attached to the top of the Soxhlet. This condenser drops the temperature quickly, allowing the solvent to condense on the sides of the glass and drop back into the cellulose thimble.

5. Allow the solvent to cool to room temperature, and filter with a 40 µm filter to remove any particulate matter.

6. Blow the collected liquid to dryness with a stream of N_2 gas, and store the sample in the refrigerator at 4◦C until it is used to separate and identify the kinds and amounts of compounds present by means of HPLC/MS or other appropriate procedures.

Newer methods include:

Supercritical fluid extraction (SFE)

A substance is in its **supercritical fluid state** when both the temperature and pressure equal or exceed the critical point (31°C and 73 atm for carbon dioxide). In its supercritical state, CO_2 is neither a gas nor a liquid and is best described as intermediate to the two extremes. This dual characteristic of supercritical fluids provides the ideal conditions for extracting compounds with a high degree of recovery in a short period of time. Supercritical fluid extractions (SFEs) are currently being carried out with carbon dioxide on a large scale for the decaffeination of green coffee beans (*Coffea arabica*) and the extraction of hops (*Humulus lupulus*) for beer production. Carbon dioxide is the most adopted supercritical fluid due to its low cost, environmentally benign nature, lack of flammability, and reactivity. There are several advantages of SFE compared to traditional solvent extractions. Supercritical fluids have lower viscosities and higher diffusivities than conventional solvent systems; therefore, extraction rates are enhanced and less degradation of solutes occur.

SFE with carbon dioxide is carried out at a relatively low temperature, ca. –40°C, avoiding the decomposition of thermally labile components. In addition, extractions can be selective to some extent, by controlling the temperature or pressure of the medium. Because of the high pressures required to perform CO_2 extractions, the equipment is expensive and must be handled with care. However, commercial SFE instruments are available.

SFEs with CO_2 were used as alternatives to **hydrodistillation** for isolating essential oils.

Because of the non-polar nature of carbon dioxide, supercritical fluid extraction is an attractive technique for the isolation of relatively non-polar natural products, such as artemisinin from *Artemisia annua*, azadirachtin from neem (*Azadirachta indica*), phloroglucinols from St. John's wort (*Hypericum perforatum*), or triterpenoids and steroids from cork (*Quercus suber*). In order to efficiently extract more polar components, the addition of a small amount of a liquid modifier can significantly enhance the extraction of these materials. Typical modifiers include methanol, ethanol, dichloromethane, and acetonitrile. For example, vindoline was efficiently extracted from rose periwinkle (*Catharanthus roseus*) using SFE with 3% methanol as a co-solvent; flavanones and xanthones were extracted more efficiently from Osage orange (*Maclura pomifera*) using 20% methanol rather than CO_2 alone; and the polyphenolics catechin and epicatechin were isolated from grape seeds using 40% methanol.

In-text Question

A specialised glass refluxing unit that is used for hot organic-solvent extractions is called--------.

Answer: *Soxhlet apparatus*

3.2 Fractionation

The extracts are then subjected to fractionation by partition among solvents of different polarities or can be directly put to chromatographic separation. A typical fractionation is set out in figure 2. All separation processes involve the division of a mixture into a number of discrete fractions. These fractions may be obvious, physically discrete divisions, such as the two phases of a liquid-liquid extraction, or they may be the contiguous eluate from a chromatography column that is artificially divided by the extractor into fractions. The type of fractionation depends on the individual sample and the aims of the separation. Typically, a column is run and the eluate divided into a manageable number of even-sized fractions, followed by analysis of the fractions to determine which contain the desired compounds. Obviously, collecting the eluate as a large number of very small fractions means that each fraction is more likely to contain a pure compound. However, it requires more work in analysing every fraction; this also runs the risk of spreading the target compound over so many fractions that, if originally

present in only low concentrations, it may evade detection in any one of the fractions. If the separation process is relatively crude, it is probably more sensible to collect only a few large, relatively crude fractions and quickly home in on those containing the target.

Alternatively, one may monitor “online” and fractionate the eluate accordingly.

This is generally used at the later stages of separation for separations of less complex mixtures, typically on high-performance liquid chromatography (HPLC) separations monitored by UV, where one can identify and isolate material corresponding to individual peaks.

Fractionation by partitioning between solvents

A solution of the extract in an organic solvent (such as ethyl acetate) is shaken with an inorganic base (such as aqueous sodium hydrogen carbonate) to remove the carboxylic acids as their water-soluble sodium salts. The weaker acidic phenols may only be extracted with a sodium hydroxide solution. Extraction of the original solution with an acid such as dilute hydrochloric acid will remove the bases such as the alkaloids as their salts. The neutral compounds remain behind in the organic phase. The acids and the phenols may be recovered from the aqueous solution of their sodium salts by treatment with dilute hydrochloric acid and re-extraction with an organic solvent, and the bases may be recovered by treatment of their salts with ammonia and re-extraction.

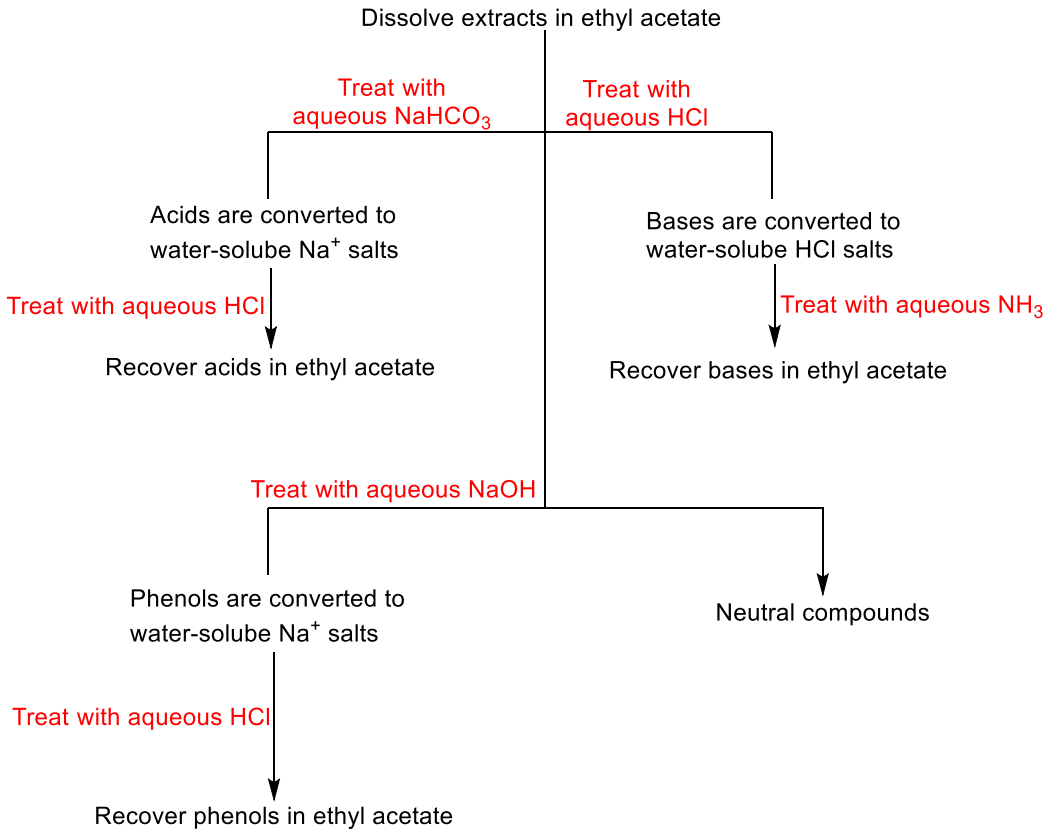


Figure 2: A typical fractionation scheme

Fractionation by chromatography

Chromatography is one of the most useful means of separating mixtures of compounds, as a technique to both purify the components and identify them. In chromatography, the mixture is separated by differential distribution of the components between a stationary phase and a mobile phase. Primary methods of chromatography in isolation and analysis of natural products include the following: *thin layer chromatography (TLC), liquid column chromatography (LC), gas chromatography (GC), high-performance liquid chromatography (HPLC), fast protein liquid chromatography (FPLC).* There are probably five major separation *mechanisms of chromatography.* They include adsorption chromatography, gas chromatography, liquid-liquid partition chromatography, ion-exchange chromatography, and size-exclusion chromatography.

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds. A **gas chromatograph** consists of a gaseous mobile phase, an injection port, a separation column containing the stationary phase, and a detector. The organic compounds are separated due to differences in their partitioning

behavior between the mobile gas phase and the stationary liquid phase in the column. The volatilities of the compounds, which strongly correlate to their boilingpoints, are mostly responsible for the partitioning between the liquid phase and the gas phase.

Liquid–liquid partition chromatography is based on the different solubilities of compounds in two liquid phases. Here, the stationary phase and the mobile phases are liquids. The oldest known method of liquid–liquid partition chromatography is paper chromatography. More recent implementations are **droplet countercurrent chromatography** (**DCCC**), **centrifugal droplet countercurrent chromatography** (**CDCC**), **high-speed countercurrent chromatography** (**HSCC**), and **elution extrusion countercurrent chromatography** (**EECC**). While paper chromatography is widely used in analytical separations, the other methods are often used for preparative purposes.

In **ion-exchange chromatography,** separation is based on ionic interactions of the individual components of a mixture with a stationary phase that is an ionically charged surface of opposite charge to the sample ions. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute it from the column. Typical applications in natural products chemistry are in the separation of fruit acids.

In **size-exclusion chromatography**, the separation of mixtures is based strictly upon size. The column is filled with material that has precisely controlled pore sizes, and the sample is simply screened or filtered according to its solvated molecular size. Because larger molecules cannot get into the small pores of the packing material, they are rapidly washed through the column. Smaller molecules, however, penetrate inside the porous packing particles, which leads to a longer path through the column, and as a result, makes them elute later. Typical applications for size-exclusion chromatography are separations of biomacromolecules, such as proteins.

The first type of chromatography, **adsorption chromatography**, can be further subdivided into **normal-phase chromatography** and **reversed-phase chromatography**. In normal-phase chromatography, the stationary phase is of a polar nature (hydrophilic), typically silica, and the solvent is of a non-polar nature (hydrophobic). In order to achieve separation, the polarity of the solvent is adjusted to

the polarity of the mixture. It ranges from non-polar hexanes to very polar methanol or even water. Polar samples are retained on the polar surface of the column packing longer than less-polar materials. On the other hand, reversed-phase chromatography consists of modified silica surfaces, such that the nature of the stationary phase becomes unpolar. Themobile phase is a polar solvent, such as water–methanol mixtures or water–acetonitrile mixtures. The separation process in adsorption chromatography is typically accomplished by probing the various functional groups in the molecules to be separated. An exemplary case could be a very polar and hydrophilic carboxylic acid function attached to a long alkane chain that is hydrophobic. Attempts to separate molecules of various chain lengths and complexity should then focus on the hydrophobic part.

For the separation process, these different types of behaviour compete. To resolve variations in different molecules, a delicate balance between those different effects, hydrophobic versus hydrophilic interaction, is needed. Because we can vary only the mobile phase during a separation, after choosing a stationary phase, we have to accomplish minute variations by choosing proper solvent systems. As a result, we often end up with fairly complex mobile phases that address different parts of our molecules. In many cases, however, we have no prior knowledge of these different groups, either because we do not know exact structures or because we do not know exact composition of the mixture, and we are left with simple trial-and-error attempts based on our experience with a specific group of plants or plant constituents.

Adsorption chromatography

In adsorption chromatography, finely divided inert adsorbent materials (e.g., silica gel or alumina) serve as the stationary phase, and organic solvents serve as the mobile phase. Separation of the mixture, then, is achieved by differences in polarity of the individual components. Depending on the pore size of the material, different techniques can be used to achieve separations.

(a) Thin-layer chromatography (TLC)

In thin-layer chromatography (TLC), the adsorbent is coated on one side of a plate of glass or a strip of plastic or aluminum. Common adsorbents are **silica gel** and **alumina**. A few microliters of a solution of the sample to be analysed are spotted onto the plate as a single small dot near one end of the plate using a microcapillary tube.

The plate is developed by placing it in a jar or developing chamber that contains a small amount of solvent. The solvent rises up to the plate by capillary action, carrying the components of the sample with it. The different compounds are separated based upon their interaction with the adsorbent coating.

Commercially available TLC plates, 60 Å silica gel, 250 μm layer thickness, on either polyester or aluminum backing, either with or without a fluorescent indicator (e.g., Whatman® flexible-backed TLC plates), are suitable for the rapid analysis of crude plant extracts and for following the progress of preparative separations. These commercial plates can be cut with scissors or a paper cutter to the desired size. Samples are commonly applied with fine capillaries prepared from melting-point capillary tubes. Development of analytical TLC plates can be carried out either in wide-mouth round jars or rectangular TLC developing chambers. The detection of spots is generally achieved using an ultraviolet (UV) lamp (if the TLC plates have the fluorescent indicator) or iodine vapor. The detection of components of TLC plates may also be accomplished by spraying the plates with a suitable reagent (e.g., chromic acid solution or 2,4-dinitrophenylhydrazine reagent). Preparative TLC plates, also commercially available (e.g., Sorbent Technologies, 20 cm × 20 cm, glass-backed, 60 Å silica gel, 1000 μm layer thickness, with fluorescent indicator), were used for preparative separations. The sample is applied as a line rather than a spot onto the bottom of the plate with a capillary, and the plates are developed in a solvent chamber. Visualisation of the components is carried out using a UV lamp. The desired components are scraped off of the glass with a razor blade. The compound is separated from the adsorbent by dissolving with an appropriate solvent and filtering from the silica gel.

Analytical as well as preparative TLC is performed by first spotting the mixture under study onto a dry TLC plate. This task is best performed by adding small amounts of sample at a time, such that only a small area of the TLC plate is covered with the mixture. In the case of comparative studies of several mixtures, or mixtures with reference compounds, care has to be taken that all mixture spots are lined up parallel to the edge of the plate. The mobile phase should be placed into the developing chamber prior to the separation. The chamber should have a lid that closes tightly enough to keep a constant gas pressure in the chamber. Essential for good and reproducible separations is that the solvent system be allowed to equilibrate with its

vapor phase. Chamber walls are often covered with filter-type paper to ensure the evaporation of the solvent and a fast equilibrium. The depth of the solvent should be less than the distance of the sample spots from the bottom of the plates.

Finally, the plate is placed in the chamber and allowed to develop. Capillary forces will move the mobile phase into the TLC plate. On their way through the TLC plate, they move the different molecules at different distances through the plate. Development should be stopped before the solvent front reaches the other end of the plate. The exact end-point of the solvent front should be marked.

(b) Preparative column chromatography

Column chromatography is most often used for the preparative scale separation of components from a crude plant extract. In practice, avertical column, usually glass, is packed with a suspension of adsorbent in a nonpolar solvent (e.g., hexane). The crude extract to be separated is added to the top of the column, and a suitable solvent or solvent mixture, which should be as nonpolar as poss, is allowed to flow down through the column, eluting the various components from the bottom of the column. Common adsorbents for column chromatography include aluminum oxide, Florisil® (a magnesium silicate), and silica gel. More recently, Sephadex™ LH-20 (a hydroxypropylated, cross-linked dextran) and bondedphase silica gel derivatives such as C8 or C18 (for reversed-phase separations) have become popular. In general, the ratio of mass of adsorbent/mass of extract should be around 40:1, and the ratio of column height/column diameter of the packed column of adsorbent should be around 15:1. For gross preparative separations of multigram quantities of crude plant extracts, we found that columns of silica gel (240 to 400 mesh), 85 cm length × 5 cm diameter, either gravity elution or with slight nitrogen pressure (flash chromatography), eluting with a solvent gradient (e.g., hexane/ethyl acetate or chloroform/ methanol) generally produce good results.

(c) Affinity chromatography

This is a powerful means of purifying proteins. The principle of this technique is based on the fact that some proteins have a very high affinity for specific chemical groups (**ligands**) covalently attached to a chromatographic bed material (the **matrix**). After loading and running the protein mixture sample through the column, only those proteins with a high affinity to the ligand can bind to the column matrix. Other proteins,

in contrast, run through the column, because they are unable to bind to the ligand. The bound proteins can then be eluted from the column by a solution containing a high concentration of the soluble form of the ligand or, in some cases, by changes in the buffer conditions that cause the bound proteins to change conformation. A number of affinity columns are commercially available, depending on the particular protein purification desired. One example is **ConA-Sepharose affinity chromatography** that is specific for glycoprotein purification. Another example uses columns with immobilised metal ion covalently linked to the column matrix. In this type of method, the proteins with affinity to the metal ion (including the proteins that have a HIS-Tag or a series of six to ten histidine residues) will bind to the column, while other proteins will be washed away.

(d) High-performance liquid chromatography (HPLC)

HPLC typically uses small particle sizes for the stationary phase, which results in a fairly large backpressure when the mobile phase is passed through this bed. As a consequence, the only way to achieve flow of themobile phase is to use pump systems. The pressure of HPLC typically reaches 150 to 200 bars. In certain cases, larger pressures are unavoidable. Modern instrumentation is able to handle up to 400 bars. Typically, an HPLC system consists of the following components: solvent reservoir, injection system, column, HPLC pump, detector, sample collector (optional), and a computer serving as a data station for the detector information as well as a way to control and automate the HPLC pump.

More sophisticated instruments consist of two or three pumps as well as a number of detectors to characterise the sample. The advantage of having more than one pump is that solvent gradients can be programmed, meaning that the solvent composition can be changed continuously throughout the chromatography. This allows for the separation of a much larger range of compounds because the mobile phase can be adjusted to the changing polarity of the mixture. As an additional benefit, chromatographic peaks get sharper - they elute in a smaller volume from the column - and the separation can be done in a much shorter time period. Almost every HPLC system is equipped with a UV detector in order to detect compounds of interest. Traditionally, these UV detectors were single-wavelength detectors; however, with cheaper hardware **photodiode-array** (**PDA** or **DAD**) **detectors** that permit the scanning of the full UV-visible range (210to 650 nm) are becoming more popular. For

the separation of natural products, this is of great advantage because depending on the compounds involved; there can be very large differences in their UV maxima.

In most cases, HPLC is implemented as an analytical technique. With either reversed-phase or normal phase materials, column sizes are typically 25 cm in length and 4.6 mm in diameter. The typical load for an analytical column is well below 1 mg for the overall mixture. However, up to 5 mg of sample are possible in favourable cases. Common flow rates for analytical separations are 1 ml·min–l. Semipreparative separations can be achieved using larger columns; here the diameter of the column is increased to 10 mm or more. For a 10 mm column, a maximum load of about 50 mg is possible. Preparative columns go up to 50 mm in diameter, which allows for the separation of a few grams of material. In the case of preparative separations, the larger column diameters require increased flow rates (up to 150 ml·min–1), and thus, HPLC pumps with larger pumping capabilities are required.

For normal-phase HPLC, there are a variety of different surface chemistries commercially available. Those most commonly used are silica columns; however, the use of alumina packing or modified silica columns, such as diol, cyano, or amino phases, is possible. Theadvantage of the modified silica phases is their ability to equilibrate quickly, contrary to silica or alumina phases.

Partition Chromatography

Partition chromatography, often called liquid–liquid partition chromatography, involves two liquid phases. The stationary phase is an adsorbed solvent held on the surface or within the grains or fibers of an inert solid supporting matrix. Examples of inert supports include sheets of paper (cellulose) as used in paper chromatography. The separation of mixtures is based on the partitioning of the individual components between two immiscible liquid phases - the solubility differences of each component in the mobile phase and the stationary phase. In paper chromatography, a thin film of water on the paper constitutes the stationary phase. Early liquid chromatography (LC) stationary phases were coated onto the inert support and packed into a column. The mobile phase was then passed through the column.

(a) Paper chromatography

Paper chromatography is usually carried out in a large glass tank or cabinet and involves either ascending or descending flow of the mobile-phase solvents.

Descending paper chromatography is faster due to gravity facilitating the flow of solvents. Large sheets of Whatman #1 or #2 filter paper (the latter is thicker) are cut into long strips (e.g., 22 × 56 cm long) for use in descending paper chromatography, or a wide strip of paper (e.g., 25 cm wide) of variable height is used for ascending paper chromatography. For descending liquid–paper chromatography, substances to be separated are applied as spots (e.g., 25 mm apart) along a horizontal pencil line placed down from the V-trough folded top of the paper.

The V-trough folded paper is placed in a glass trough, held down by a glass rod, and when the tank has been equilibrated (vapor-saturated) with “running solvents” (mobile phase), the same solvent is added to the trough via a hole in the lid covering the chromatography tank. The lid is sealed onto the chamber with stopcock grease in order to make the chamber airtight. After the mobile-phase trails to the base of and off the paper sheet, the paper is hung to dry in a fume hood, where it can then be sprayed with reagents (e.g., ninhydrin reagent for amino acids) that give colour to the separated compounds of interest in white or UV light. Some compounds of interest have their own distinctive colours (e.g., chlorophylls), and hence, can be purified using this technique. In other cases, the dyes used to stain the location of the compound or protein cause irreversible covalent changes to the compound. In these cases, purification is not possible. In ascending paper chromatography, thesame basic setup and principles apply, with the exception that the mobile phase is placed at the bottom of the tank. Separation is achieved when the mobile phase travels up the paper via capillary action.

Gas Chromatography

Gas chromatography was the first commercially available separation technique available. A gas chromatograph consists of an injector system, a column that is placed in a programmable oven, and a detection system. Currently, the columns most widely used are fused silica columns approximately 30 m in length. They provide excellent stability over a wide temperature range, so that compounds up to molecular weights of approximately 450 amu can be analysed by GC. The most common detector for GC is the **flame ionisation detector**, which provides excellent sensitivity for a wide range of compounds. A more sophisticated detector is a mass selective detector. In natural products, GC instruments are typically used for the evaluation of highly volatile essential oils. Because, in many cases, **retention time** is the only parameter to

distinguish individual compounds, and retention time highly depends on the column used as well as the specific temperature program incorporated, methods were developed to compare different analyses. In order to obtain a scaled retention time, a standard sample of a known mixture of hydrocarbons is used to evaluate the combination of temperature program and column characteristics under the exact conditions as the actual plant extract.

Using this standard mixture, **retention indices** (**RIs**) can be calculated, and a comparison of the actual sample with the work done by others is possible based on these RIs.

It is clear that the individual components are better separated with the slower temperature gradient. Furthermore, when inspecting the hydrocarbon mixture, it is apparent that the spacing between the individual components changes, indicating that it is very important to correlate separations under different conditions with standard mixtures.

In-text Question

What is the full meaning of each of the following chromatographic methods, (a) TLC (b) HPLC (c) GC?

Answer: *(a) TLC means Thin Layer Chromatography (b) HPLC means High Performance Liquid Chromatography (c) GC means Gas Chromatography.*

3.3 Characterisation of Natural Products

The bottleneck in natural products chemistry generally is separation/purification of compounds of interest from complex mixtures and structure elucidation of those compounds. The most important tools for structure elucidation of natural products are **nuclear magneticresonance** (**NMR**) and **mass spectroscopic** (**MS**) techniques. In addition, **infrared** (**IR**) and **ultraviolet-visible spectrophotometric (UV-Vis**) methods are of importance. We also include hyphenated techniques such as gas chromatography (GC)-MS, liquid chromatography (LC)- MS, and LC-NMR. These techniques provide separation methods coupled with structural (spectroscopic) information. A very powerful modern analytical method is x-ray crystallography.

The stages in structure elucidation

In examining the strategies for natural product structure determination, it is possible to discern four overlapping stages. First there is the preliminary **characterisation** in which the physical constants are determined, the molecular formula is established and the functional groups are identified. At this stage it is often possible to recognise the class of natural product to which the compound belongs. It may be apparent that the compound is known from another source or is just a simple derivative of another compound of known structure. A simple chemical interrelationship may then be enough to establish the structure of the unknown compound.

The second stage is one of **structural simplification** in order to identify the underlying **carbon skeleton**. Chemically, this work involves the selective removal of the various functional groups and the dissection of the carbon skeleton into identifiable fragments. Spectroscopically, it involves identifying adjacent groups of atoms by, for example, their NMR characteristics. Evidence may accumulate concerning the position of the functional groups on the carbon skeleton. The determination of the position and relative **stereochemistry** of the functional groups forms the third phase. There is an interesting contrast in the use of spectroscopic and chemical methods between the first and third stages of this strategy. In the first stage, the methods are used to obtain information about the separate functional groups, but in the third phase the methods are used to reveal interactions between groups. The fourth stage involves establishing the **absolute stereochemistry** of the molecule. Although this is sometimes assumed, it is important to confirm it in order to understand the biological activity of a natural product. A few natural products are found in both enantiomeric forms.

When a natural product or a simple derivative crystallises particularly well, X-ray crystallography can provide an unambiguous way of establishing the structure. Using a modern diffractometer, structures can be obtained in a matter of hours rather than days. However, unless a heavy atom such as bromine is present or a derivative is made with an optically active compound of known absolute stereochemistry, the structure determined this way will contain only relative stereochemical information. When a structure has been proposed, it is helpful to rationalise this in biogenetic terms. An unambiguous partial synthesis from a compound of known structure, or a total synthesis, will finally provide the ultimate proof of structure.

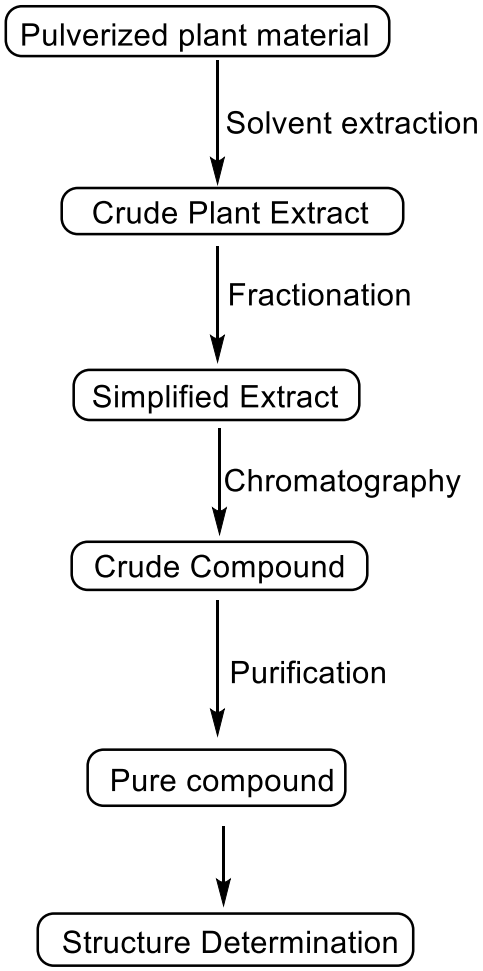


Fig. 2: The Stages Involved in Isolation and Characterisation of Natural Products

In-text Question

Which of the following is not a spectroscopic method of structure determination (a) NMR (b) HPLC (c) UV (d) IR?

Answer: *(b) HPLC*

4.0 SELF-ASSESSMENT EXERCISE

1. Show, by means of a flow chart, the stages involved in isolation and characterisation of natural products.
2. List 5 general methods of extraction of natural products.
3. Explain briefly, the procedure involved in fractionation by partitioning between solvents.

Answer:

1. *See Section 3.3*
2. *Maceration, percolation, Soxhlet extraction, supercritical fluid extraction and aqueous extraction*
3. *See Section 3.2*

5.0 CONCLUSION

Natural products are obtained from plant sources in quantities of 10-100 mg/kg dry weight by solvent extraction, partition into acidic, basic and neutral fractions, and chromatography. The elucidation of the structure of a secondary metabolite involves characterisation, determination of the carbon skeleton, establishing the position of the functional groups, and determining the relative and absolute stereochemistry. Bio-separation of natural products from plants can be relatively simple and inexpensive when applied to the preparation of natural and whole foods, herbal medicines, teas, and plant dyes. Most of these protocols involve hot or cold water extractions; preparation of salves, ointments, and decoctions; and the making of alcohol/water tinctures. No sophisticated laboratory equipment is needed. Such extractions are the cornerstone of folk medicine, preparation of home remedies, and natural plant dyeing of wool and other fibres. In contrast, when there is a need to identify particular constituents and their amounts in cell fractions, cells, tissues, organs (e.g., roots, stems, leaves, flowers, and fruits), and whole plants (e.g., seeds, seedlings, adult plants, or mycelial and cell cultures), one must employ many different chemical and physical separation methods. These may include grinding tissues in liquid nitrogen, centrifugations, Soxhlet extractions, freeze-drying, column and silica gel thin-layer chromatography, high-performance liquid chromatography, gas chromatography, and a variety of modes of electrophoresis. The analytical equipment of MS and NMR spectroscopy is extremely sensitive.

6.0 SUMMARY

Chemistry of natural products involves the study of these chemicals produced in plants, and other organisms describing their extraction, purification, identification, structure determination and synthesis.

Since the concentrate contains an enormous variety of compounds, early isolations involved selective crystallisation of the most dominant component in the mixture. Liquid natural products were distilled. Natural organic acids were isolated by aqueous

basic extraction and natural organic bases (alkaloids) were isolated by aqueous acidic extraction. Modern chromatographic methods have been greatly developed to isolate and purify a large number of different compounds in very small quantities: column, GC, TLC, HPLC, paper, electrophoresis, ion exchange, etc.

Natural products are usually given names that are derived from the species name of the plant or animal, or from the biological action, or property, of the compound. In the late 1800's, natural products were identified and analysed by mp, bp, [a] (optical rotation), hoping to find correlations between data and structure. This initiative was not successful in predicting structure, but useful data on natural products were obtained. Classical structural elucidation is done by:

- determination of functional groups
- determination of the carbon skeleton and the location of the functional groups
- degradation to smaller fragments (A-B-C ------> A + B + C)
- elemental analysis
- reactivity (leading to new reactions)
- stereochemistry
- synthesis of the smaller fragments (A, B, C) and the entire molecule (A-B-C)
- classification of the compound into a biogenetic family of compounds.

More modern structural elucidation and characterisation by spectroscopy include:

- 1930's UV (ultraviolet) light (cf. Woodward's Rules, 1941)
- 1940's IR (infrared) spectroscopy (note: penicillin structure problem in W.W.II)
- 1950's NMR (nuclear magnetic resonance) spectroscopy
- 1960's MS (mass spectrometry)
- ESR (electron spin resonance) spectroscopy.

In addition, by other methods like:

- ORD (optical rotatory dispersion)
- CD (circular dichroism)

- acidity and basicity measurements (pK)
- advanced synthetic and biosynthetic technology
- X-ray crystallography

Modern methods reduce the necessity of chemical degradation methods, so much less material is required.

7.0 REFERENCES/FURTHER READING

1. Leland, J. C. *et al.* (2006). *Natural Products from Plants.* (2nd ed.). Boca Raton: CRC Press Taylor & Francis Group.

2. “Natural Products.” (www.cem.msu.edu/~reusch/VirtualText/biomol.htm)

- A Collection of Information on Important Classes of Natural Products.

UNIT 2 BIOSYNTHESIS

CONTENTS

1.0 Introduction

2.0 Objectives

3.0 Main Content

3.1 Biosynthesis of Some Plant Metabolites

4.0 Conclusion

5.0 Summary

6.0 Self-Assessment Exercise

7.0 References/Further Reading

1.0 INTRODUCTION

In module 1, we presented a compilation of some of the many types of chemical compounds that plants produce. Now the question arises: How do plants synthesise these compounds, and why do plants synthesise such a vast array of compounds? These are the primary topics of this unit, and in the process of exploring the answers, we hope to shed some light on the factors that drive the evolution of the biosynthetic pathways that produce these compounds. For example, the simple fact that plants have roots results in very different selective pressures than those driving the evolution of animal metabolism. After all, very few plants have the ability to run away when another organism sees them as food. Consequently, plants have evolved ways to repel or, in some cases, attract other organisms. Their lack of movement also allows them to produce rigid compounds (such as cellulose or lignin) that, among other things, allow them to grow upward into new environmental niches.

To make such compounds as sugars, waxes, lignin, starch, pigments, or alkaloids, plants utilise specific enzymes, each of which catalyses a specific metabolic reaction. **Enzymes** are proteins that act as **organic catalysts**. They are coded by specific genes in the plant's DNA and are made via processes we call transcription (conversion of DNA to RNA via the enzyme RNA polymerase) and translation (conversion of RNA

to protein via the enzymatic action in complex structures called **ribosomes)**. When there is a series of enzymatically catalysed reactions in a well-defined sequence of steps, we have what is termed a **metabolic pathway**. Some enzymes may be involved in metabolic pathways requiring just a few enzymatic steps (as in synthesis of starch from the sugar nucleotide, **adenosine diphosphate [ADP]-glucose**) or many enzymatic steps (as in the synthesis of **gibberellins** hormones from **mevalonic acid**). Some enzymes may be involved in pathways that break down compounds (as in the hydrolysis of starch to sugars by α- and β-amylases). Still other enzymes may be involved in making storage forms of given compounds, such as glucosides, amides, or esters of the plant hormone **indole-3-acetic acid** (IAA).

These different enzymatic pathways involved in the synthesis, breakdown, and creation of storage forms of a compound regulate the level of the given compound. The regulation of each pathway and of each of its enzymes is, however, extremely complicated. Please note that not all proteins are enzymes. Many proteins within a given cell may be purely structural in function.

In the sections that follow, we aim to give the reader an understanding of the primary biosynthetic pathways that are known to occur in plants. We then give an overview of what is known about some of the best-known plant compounds and how they function within the plant. It should be noted, however, that there is a vast amount of information on these subjects, and new discoveries continue to be made.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- give a highlight on the biosynthetic pathways of natural products
- itemise some natural products of pharmaceutical relevance
- show a rough outline of the metabolic pathway of some of these compounds.

3.0 MAIN CONTENT

3.1 Biosynthesis of Some Plant Metabolites

Alkaloid biosynthesis

So far, we tried to touch upon each of the major categories of products produced by plants in general. We discussed the biosynthesis of the major cellular components found in the majority of plants, including primary storage compounds and key

compounds that start the carbon fixation process (chlorophylls). We used carotenoids to demonstrate the production of terpenoids and anthocyanins to give examples of phenolic compounds.

Now we will say a few words about nitrogen-containing compounds, which will be represented by the alkaloids. Most of these products are not considered to be essential to the growth and development of theplant, but some, such as pyrimidine nucleotides and tetrapyrroles, are essential. There are thousands of different plant products that have nitrogen in their structures. Perhaps the most diverse of these types of compounds (found in 20 to 30% of vascular plants) are the **alkaloids** that, like most other nitrogen-containing compounds, are synthesised from amino acids.

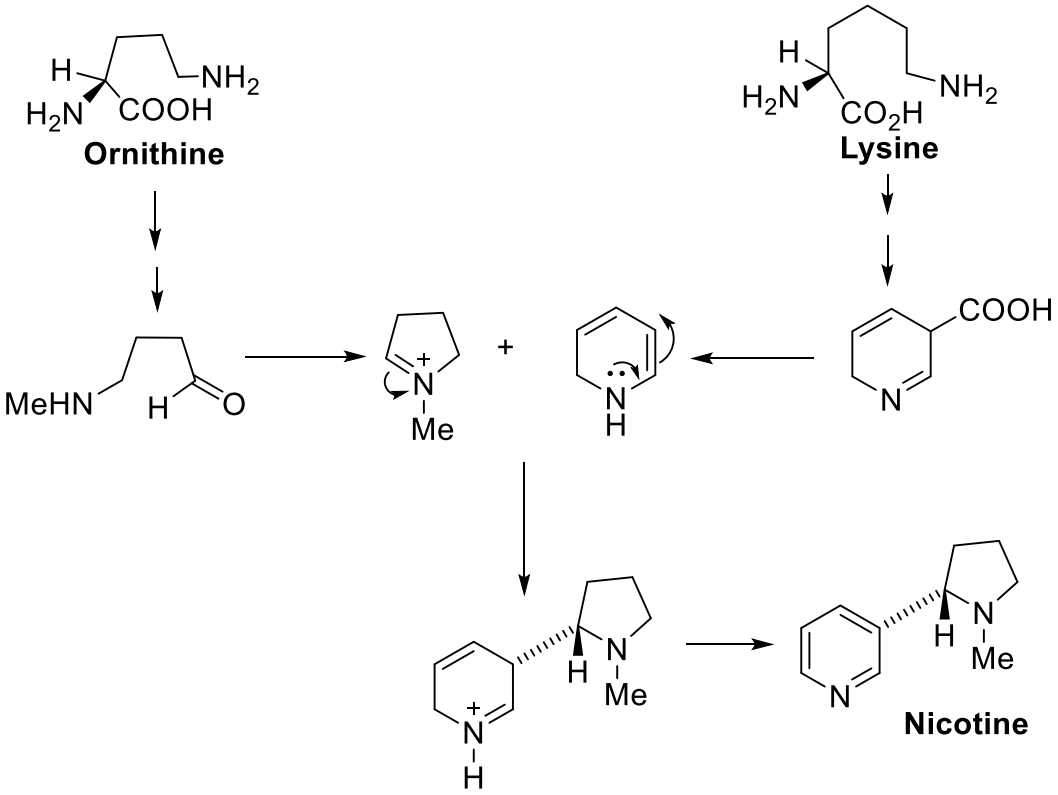
Alkaloids are especially interesting because they are toxic to both herbivores and humans; yet they have some very important medicinal properties for humans. The nitrogen atom, which in these substances is almost always part of a heterocyclic ring origin, is found innately in the structure of the amino acids from which they came or are the result of the circularisation of the given amino acid. This is the case with aspartic acid that combines with glyceraldehyde-3-phosphate in the production of nicotinic acid (a precursor of the alkaloid, nicotine) in plants such as tobacco (*Nicotiana tabacum*). Nicotine is well known as a toxic component of tobacco smoke. There are many categories of alkaloids, including pyrrolidine, tropane, piperidine, pyrrolizidine, quinolizidine, isoquinoline, and indole alkaloids. Much of the carbon skeleton of some of these alkaloids is derived from the mevalonic acid pathway, but it is beyond the scope of this unit to go into the details of the biosynthesis of all types of alkaloids. An old idea about the function of alkaloids in plants depicted them as waste products of plant metabolism. However, plants are energetically efficient organisms. They simply do not waste their energy in the production of compounds that they do not need - there always seems to be a reason for their production. The predominant activity of alkaloids in plants seems to be the deterrence of **herbivores**. Many livestock deaths are caused by ingestion of alkaloid-containing plants such as lupines (*Lupinus* spp.), larkspur (*Delphinium* spp.), and groundsel (*Senecio* spp.). They were also shown to be toxic to insects, bacteria, and fungi.

Alkaloids are a family of nitrogen-containing compounds with diverse structures. These structurally diverse bases are unified in their biosynthetic origin; they are

biosynthesized from a limited number of amino acids. Based on the precursor amino acid, the alkaloids can be categorized into (a) ornithine- or lysine-derived, (b) tyrosine or phenylalanine-derived and (c) tryptophan-derived alkaloids. A fourth category is the steroidal alkaloids obtained from steroid carbon skeleton. The introduction of the nitrogen heteroatom occurs after the formation of the final carbon skeleton.

Ornithine- or lysine-derived alkaloids

Pyrrolidine and piperidine-based alkaloids are derived from the basic amino acids ornithine and lysine. These basic amino acids form iminium ion in a Mannich-type condensation reaction. Nicotine, a pyrrolidine-pyridine alkaloid, is biosynthesized from nicotinic acid in a Mannich-type condensation reaction according to Scheme 1.

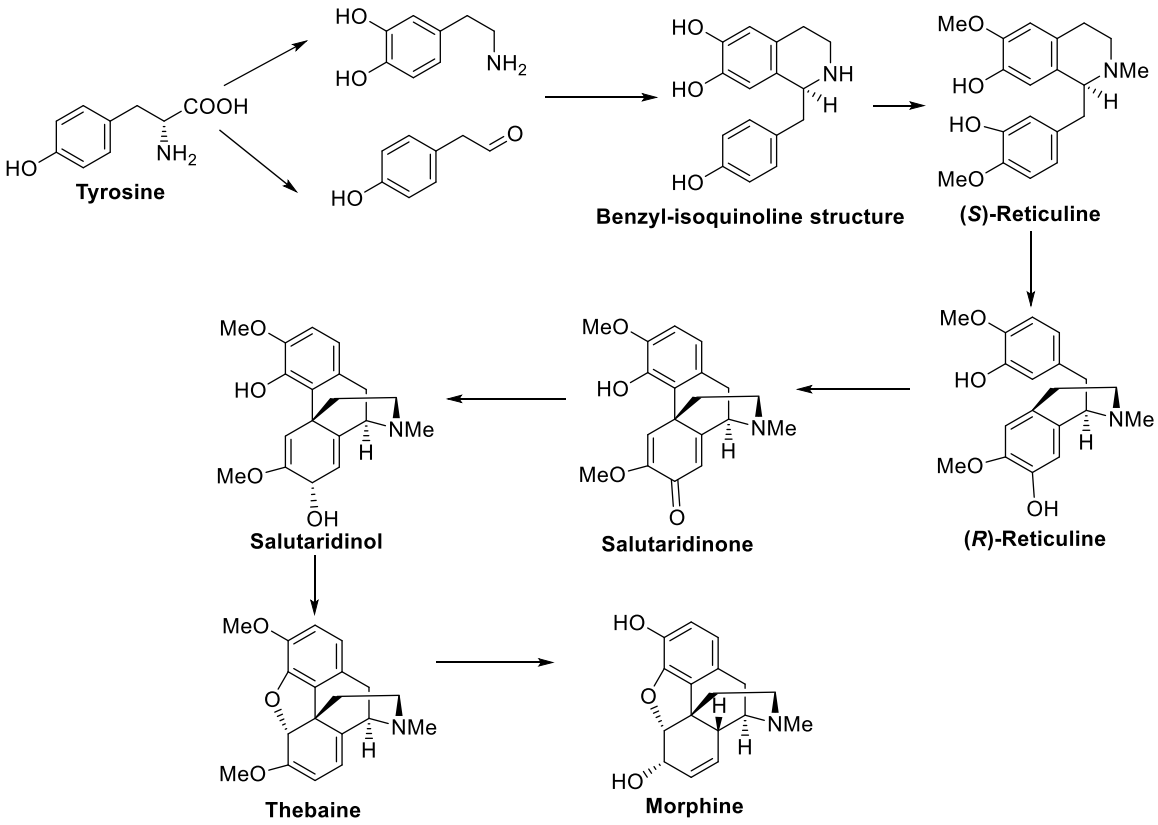


Scheme 1. *Biosynthetic pathway for nicotine*

Tyrosine- or phenylalanine-derived alkaloids

Morphine is an isoquinoline alkaloid which biosynthetic origin is from the amino acid, tyrosine. The steps involved in biosynthesis of morphine are described in Scheme 2. The first step is the formation of both the isoquinoline and the benzyl rings from **tyrosine**. This is followed by transformation of the benzylisoquinoline structure into **(*S*)-reticuline**. Isomerization of the (*S*)-enantiomer produced the **(*R*)-reticuline** followed by the phenol-coupling step and the transformation to **salutaridone**. The

ether bridge of morphine was formed from an elimination reaction involving **salutaridinol**. The process progressed through **thebaine**, **codeine** and finally **morphine**.



***Scheme** 2. The biosynthetic pathway for morphine*

Tryptophan-derived alkaloids

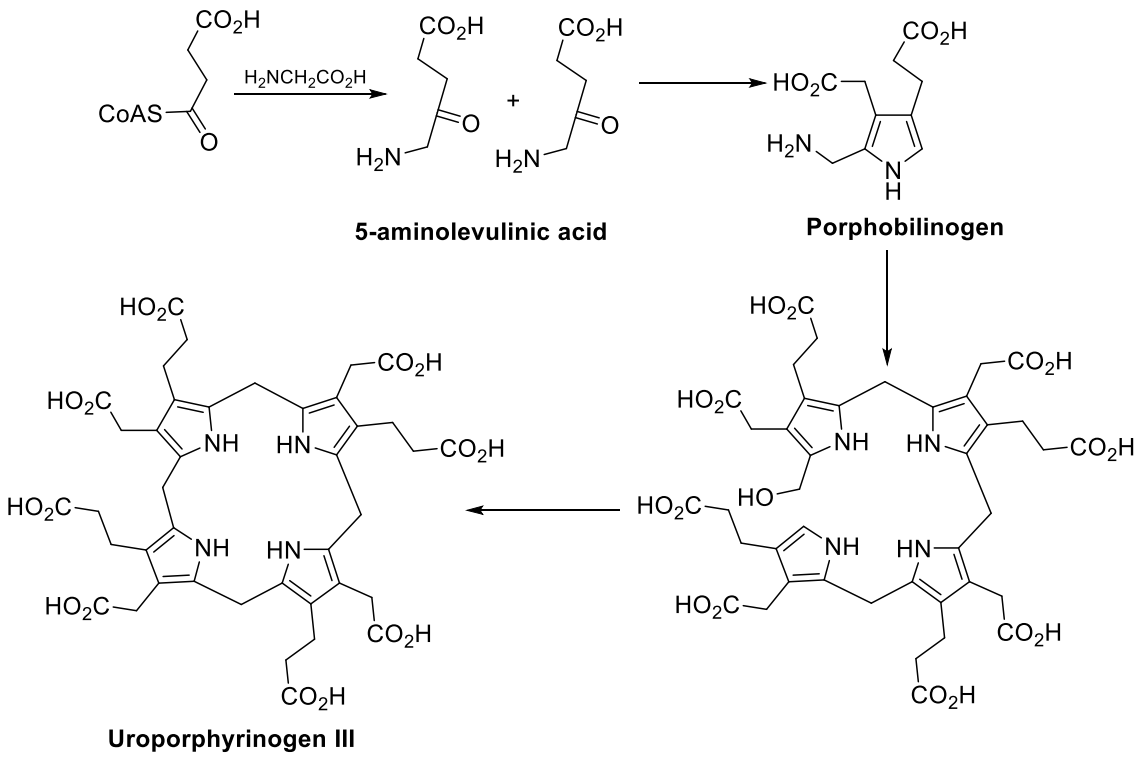
The indole alkaloids including reserpine, strychnine and vincaleucoblastine are derived from the amino acid tryptophan and the monoterpenoid secolaginin a C_{10} unit.

Chlorophyll biosynthesis

There are three main locations of pigments within the cell: (1) plastids, (2) vacuoles, and (3) cell walls. The chemistry of the pigments varies with the location. **Chlorophyll** is a porphyrin that constitutes the primary photoreceptor pigment for the process of photosynthesis in plants. It is produced in the chloroplasts and is responsible for the green appearance of leaves and stems, aerial and prop roots, many kinds of floral bracts, and green fruits before they ripen. The chlorophyll molecule is made up of four pyrrole rings, made from aliphatic amino acids that are ligated to form a **tetrapyrrole** ring with a magnesium atom in its centre. Apart from chlorophyll, other biological pigments referred to as “life pigments” including haem the active core of haemoglobin,

vitamin B_{12} and cytochromes contain the tetrapyrrole nucleus with various substituents and different central metals complexed with the pyrroles to form coordination compounds. All these “pigments of life” have a common biosynthetic origin known as **uroporphyrinogen III**. Following its formation, the uroporphyrinogen III undergoes substantial biosynthetic transformation that results in the individual biological pigment. Scheme 3 shows the biosynthetic pathway of uroporphyrinogen III.

In this process, two molecules of **5-aminolevulinic acid** were condensed to produce **porphobilinogen (PGB)**. The enzyme, **deaminase**, linearly combine four units of PGB in a head-to-tail manner through loss of the amino group to give a tetrapyrrole. The enzyme, **cosynthetase**, cyclizes the linear tetrapyrrole and rearranges the ring system to give uroporphyrinogen III.



Scheme 3. *The biosynthetic pathway for uroporphyrinogen III*

Ring IV is esterified with a hydrophobic long-chain **phytol** molecule ($C_{20}H_{39}$) made in the terpenoid pathway. For light harvesting, plants use two forms of chlorophyll- *a* and *b*. Chlorophyll *a* is in all plants and is the only chlorophyll at the reaction centres. It has a methyl group at C_3. Chlorophyll *b*, found in most plants, has a formyl group at this position and, like other accessory pigments, functions to absorb the energy from

wavelengths of light that differ from chlorophyll *a*. The biosynthesis of the chlorophylls is complex and has only recently been worked out in detail within the model plant angiosperm, *Arabidopsis thaliana*. It starts with the synthesis of L-glutamic acid 1-semialdehyde from L-glutamyl-tRNA through the action of the enzyme glutamyl-tRNA reductase.

This is then converted to δ-aminolevulinic acid through the enzymatic activity of glutamate 1-semialdehyde amino transferase. These are critical steps, because the porphyrin ring containing conjugated double bonds is assembled in the chloroplast from eight molecules of δ-aminolevulinic acid. Subsequent steps lead to the formation of protochlorophyllide, insertion of a Mg^{2+} ion in the centre of the tetrapyrrole ring, and addition of the phytol tail to form **chlorophyll *a*** and **chlorophyll *b***. Please note that it is not uncommon for each plant species to have more than one copy of a given gene.

In the chloroplasts, the chlorophyll pigments are bound to proteins of the photosynthetic membranes. (Stacks of thylakoid membranes inside chloroplasts are called **grana** stacks). These proteins, called chlorophyll *a*/*b* binding proteins, are arranged into large complexes with many other proteins, cytochromes, and quinones to form the photosynthetic electron-transport chain that, as its primary function, produces the ATP required to fix carbon dioxide. The pigment chlorophyll absorbs the energy of the sun and shuttles resulting free electrons to this all-important series of chemical events.

Chlorophyll absorbs photons of light energy from the sun or from artificial lamps (e.g., incandescent lamps, high-pressure lamps, light-emitting diodes) in the red and blue portions of the electromagnetic spectrum, with peaks of maximal absorption occurring at 660 and 450 nm, respectively. This is called its **absorption spectrum**. Absorption spectra are commonly used to characterise pigment types. Maximal rates of photosynthesis (measured by the rate of CO_2 uptake or O_2 evolution) also occur in the red and blue portions of the electromagnetic spectrum. This is called its **action spectrum**. When the action spectrum peaks, like that for photosynthesis, matches the absorption spectrum for a given pigment, like that for chlorophylls, one can deduce that the pigment is essential for the absorption of light for the particular process under consideration. Another way to prove pigment type is to find plants that lack the pigment of interest and determine which processes are functional. For example, albino mutants

and parasitic plants such as Indian pipe (*Monotropa uniflora*), which are devoid of chlorophyllpigments, cannot carry out photosynthesis. Also, please note that not all plants photosynthesise. **Parasitic plants** feed off the nutrients and sugars provided by their hosts.

Carotenoid biosynthesis

Plant carotenoids are responsible for the red, orange, and yellow pigments found in fruits and roots, including tomatoes, red peppers, pumpkins, and carrots. They can be seen in the petals of many flowers and are the primary pigments responsible for the fall colouration of deciduous trees. Carotenoids are synthesised in the terpenoid pathway as C_{40} tetraterpenes derived from the condensation of eight isoprene units starting with isopentenyl diphosphate. There are two basic types of carotenoids: (1) **carotenes** that contain no oxygen atoms and (2) **xanthophylls** that contain oxygen. At the centre of each carotenoid molecule, the linkage order is reversed, resulting in a molecule that is symmetrical. A set of double bonds in the molecule is responsible for the absorption of light in the visible portion of the spectrum. As mentioned above, this has an important impact on the absorption of a wider range of light wavelengths for use in photosynthesis. Consequently, in photosynthetic organisms, carotenoids are an integral structural component of photosynthetic antenna and reaction centre complexes, but they also protect against the harmful effects of photooxidation processes. Like chlorophyll, carotenoids are found in the thylakoids of green leaves and stems. In fruits and flowers, they are also found in plastids, but these plastids have structural differences and are referred to as **chromoplasts** to indicate that they contain pigments other than chlorophyll. β**-carotene** is the orange pigment in carrot (*Daucus carota*) roots, sweet potato (*Ipomoea batatus*) tuberous roots, pumpkin (*Cucurbita Pepo*) fruits, leaves of deciduous trees, and some flower petals. Zeaxanthin and violaxanthin are found in autumn-coloured leaves and flower petals and are responsible for the bright yellows that are sometimes seen. Colouration of flowers is very important to the survival success of the plants producing them. The colour of the flowers is one of the primary factors involved in attracting pollinators. β-carotene is important in the human diet because of its purported anticancer activity, its use as a food colouring, and it is an important source of **vitamin A** (an alicyclic alcohol), which is synthesised from β-carotene and other carotenoids. Vitamin A produced by animals is, in turn, converted to the pigment, **retinal**. This pigment is one of the essential

components in the light receptors of the eye that allow us to see. Carotenoid pigments can also function in fruit and seed dispersal by attracting animals, which, in turn, spread seeds.

Most fruits produce odour compounds, such as **monoterpenes**, to help attract these organisms, and the sugars produced and stored in the fruitsact as a positive reward. In ripening fruits, as in leaves turning colour in autumn, chlorophyll pigments gradually break down in chloroplast thylakoid membranes, revealing the carotenoid pigments that were masked by the chlorophyll pigments. During ripening, there is significant synthesis of new carotenoid pigments. In the case of ripening tomatoes and peppers, for example, the unripe fruits are characteristically bright green. As ripening progresses (triggered by the plant hormone, **ethylene**), various carotenoid pigments appear and, newly synthesised, account for the colour of the ripe fruits. **Lycopene** is the red pigment seen in mature tomato and red pepper fruits. Tomatoes (*Lycopersicon esculentum*) can have both red and yellow fruits depending on the genotype of the parent. In some peppers, ripe fruits are green (sold as sweet bell peppers) but turn red or yellow at maturity (*Capsicum frutescens* var, *grossum*). Other ripe peppers, often very “hot” to the taste (“hot” due to the presence of the alkaloid, **capsaicin**), may be green, orange, yellow, or red at maturity, depending again on the genotype of the parents. Similar types of colour changes occur in ripening cucurbit fruits (squash, gourds, and pumpkins in the Cucurbitaceae family) and in the fruits of eggplant (*Solanum melongena* var, *esculentum*).

Some plants, such as red maple (*Acer rubrum*) trees, produce red-coloured flowers or leaves, yet they are wind pollinated. It is obvious that these plants do not have to attract pollinators - so, why the colour? One interesting theory behind why they expend their energy to do this is that the pigments help to warm the flowers in early spring or the leaves during early spring or late fall. This extra heat would greatly aid seed development and photosynthetic processes in early spring, allowing the plant to get a head start on growth over other plants as well as providing a longer period during which to produce energy reserves in the fall. Red colouration in many plants is not due to carotenoids, but rather, to anthocyanin pigments found in the vacuoles of plant cells.

3.2 Other natural products of pharmaceutical importance

Prostaglandins, Thromboxanes & Leukotrienes

The members of this group of structurally related natural hormones have an extraordinary range of biological effects. They can lower gastric secretions, stimulate uterine contractions, lower blood pressure, influence blood clotting and induce asthma-like allergic responses. Because their genesis in body tissues is tied to the metabolism of the essential fatty acid arachidonic acid (5,8,11,14-eicosatetraenoic acid) they are classified as **eicosanoids**. Many properties of the common drug aspirin result from its effect on the cascade of reactions associated with these hormones. The metabolic pathways by which arachidonic acid is converted to the various eicosanoids are complex and will not be discussed here. A rough outline of some of the transformations that take place is provided below. It is helpful to view arachadonic acid in the coiled conformation shown in the rectangular box.

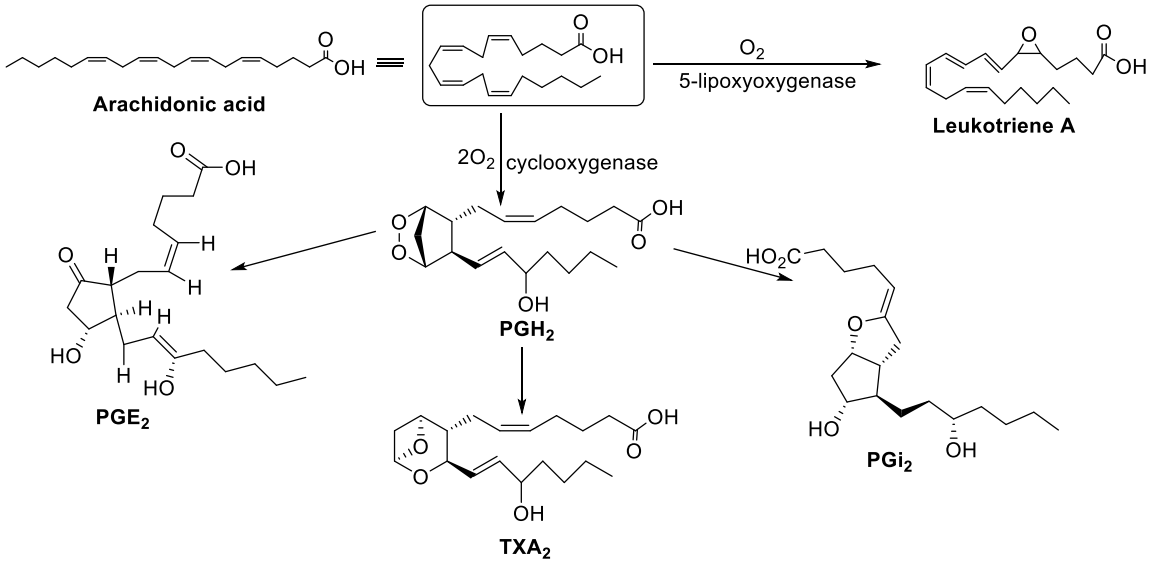


Figure 3: Rough outline of some of the metabolic pathways by which arachidonic acid is converted to the various eicosanoids:

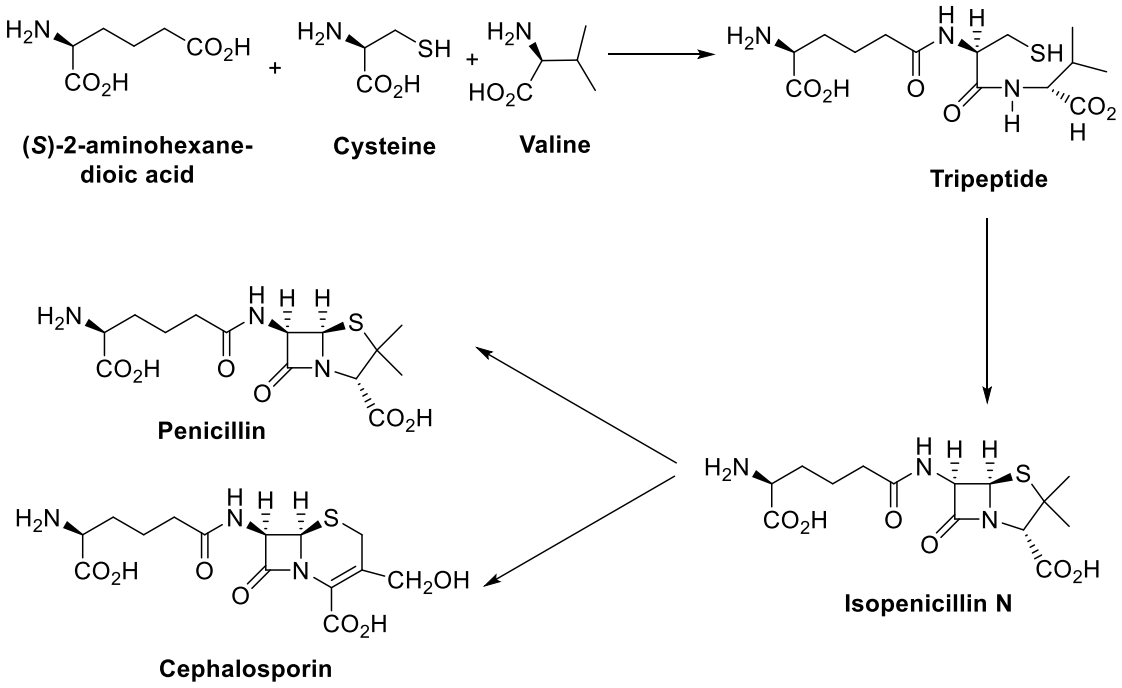
Leukotriene A is a precursor to other leukotriene derivatives by epoxide opening reactions. The prostaglandins are given systematic names that reflect their structure. The initially formed peroxide PGH_2 is a common intermediate to other prostaglandins, as well as thromboxanes such as TXA_2

Antibiotics

The alkaloids are not the only group of secondary metabolites that are derived from amino acids. Amino acids not only form the building blocks for the large peptides and

proteins but also for smaller peptides that are converted into the β-**lactam antibiotics** such as the penicillins and cephalosporins. The diketopiperazine antifungal agents produced by *Trichoderma* and *Gliocladium* species, such as gliotoxin**,** are also derived from amino acids.

Scheme 4 illustrates the biosynthesis of penicillins. The precursor of the penicillins is the tripeptide, **δ-(L-α-aminodipolyl)-L-cysteinyl-D-valine**, comprising (*S*)-2-aminohexanedioic acid, cysteine and valine. Oxidative cyclization of the tripeptide by the enzyme system called **isopenicillin N synthase** produced the **isopenicillin N**. The isopenicillin undergoes transformation to **penicillins** or conversion to **penicillin N** prior to ring expansion to give **cephalosporins**.



Scheme 4. *Biosynthetic pathway of penicillins and cephalosporins*

Vitamins

There are a number of natural products that are essential for life but which cannot be produced by the body. The recognition by Hopkins and others at the start of the 20th century that diseases such as scurvy, rickets and beriberi, which arose from deficiencies in the diet, might be associated with a requirement for particular compounds present in foods, led to the search for these essential factors. These natural products became known as vitamins. As each vitamin was isolated, it was identified by a letter of the alphabet. As their structures became known, these compounds acquired a trivial name.

A number of vitamins play a role as co-enzymes in the function of particular enzymes such as those involved in biological oxidation, reduction and carboxylation. This biological function of the vitamins places them in the class of primary metabolites. However, the elucidation of their structures and aspects of their chemistry and biosynthesis link them with secondary metabolites. In the period following 1920, bioassay-guided fractionation using test animals led to the isolation of various vitamins. They are present in their natural sources in low concentrations, and consequently very small amounts of material were available for structural studies. Nevertheless, the structures of many of the vitamins were established in the 1930s and confirmed by synthesis. Material then became available for biological studies and dietary supplements. The presence of the fat-soluble vitamin A in egg and in fish oils was established in 1913-1915, and its structure was elucidated by Karrer in 1931. The recognition that the disease beriberi, which was prevalent in South East Asia, was a deficiency disease and that it could be cured by an extract obtained from rice polishing, led Funk in 1911 to coin the name vitamin for these essential dietary factors. Vitamin **B1** (thiamine) was isolated in 1926 and in quantity by Williams in 1934. Its structure was established in 1936 and confirmed by synthesis. Other members of this group of vitamins include riboflavin (vitamin **B2),** pyridoxamine (vitamin **B6),** biotin**,** folic acid and vitamin **B12**. Vitamin BI2 was isolated in 1948 and its structure established by a combination of chemical work and X-ray crystallography in 1954. It is one of the most potent of the vitamins, being active in the treatment of pernicious anaemia at the level of micrograms. Vitamin C (ascorbic acid**)** is important in the prevention of scurvy. Its structure was established in 1932. Vitamin D, a steroid, has already been mentioned. Vitamin E, tocopherol**,** is an antioxidant and vitamin K, a quinone**,** is an antihaemorrhagic factor.

Flavonoids

The flavonoids have two benzene rings separated by a propane (propanoid) unit and are derived from flavone. The propanoid unit is illustrated in red colour and numbered I, 2 and 3 in the phenylpropanoid unit (Figure 4). The flavonoids are generally water-soluble compounds. The more conjugated compounds are often brightly coloured. They are generally found in plants as their glycosides, which can complicate structure determinations. Additional oxygen-containing heterocyclic rings and hydroxyl groups

distinguish the different classes within the group. These include the chalcones, flavones, flavonols, flavanones, anthocyanins, and isoflavones.

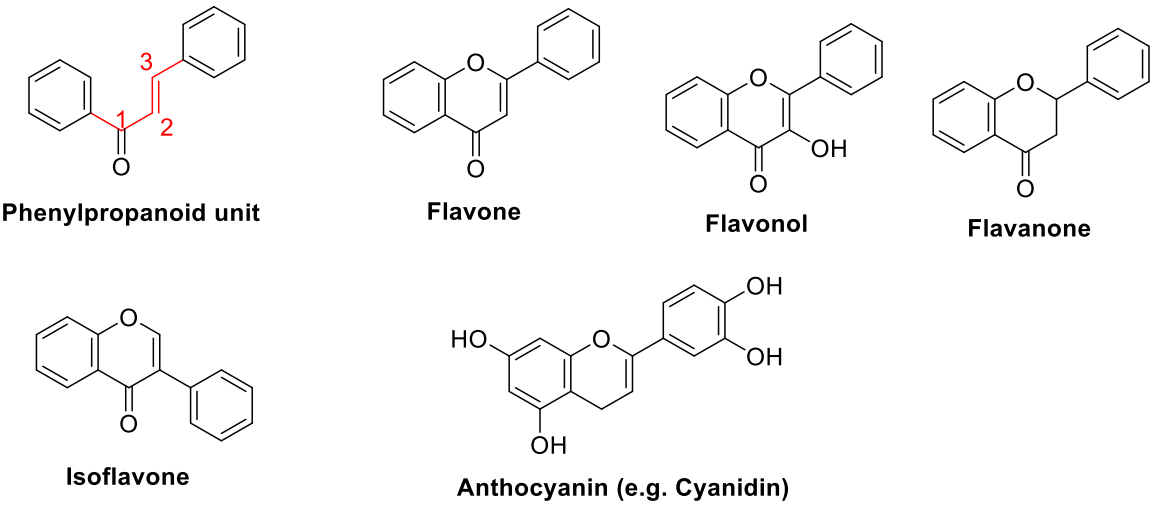


Figure 4: Showing the structures of the phenylpropanoid unit, flavone and some groups of flavonoids..

Other common flavonoid groups include aurones, xanthones, and condensed tannins. The catechins and leucoanthocyanidins are structurally similar and only rarely exist as their glycosides. They polymerise to form condensed tannins, which help give tea its colour. They also are sufficiently prevalent to darken the colour of streams and rivers in some woody areas, including the black waters of the Okefenokee Swamp in Georgia and the Suwannee River in Georgia and Florida. The flavanones and flavanonols are rare and normally exist as their glycosides. The flavones and flavonols are the most widely distributed of all the phenolics. The anthocyanins are the common red and rare blue pigments of flower petals and can make up as much as 30% of the dry weight of some flowers. The red pigment of beet (*Beta vulgaris*) is anthocyanin. The anthocyanins exist typically as glycosides. Flavanones often coexist in plants with their corresponding flavones (e.g., hesperidin and diosmin in the bark of *Zanthoxylum avicenna*). The flavone, acacetin, isolated from black locust (*Robinia pseudoacacia*), shows anti-inflammatory activity. Galangin, a flavonol from galanga root (*Alpina officinarum*), showed antibacterial activity against antibiotic-resistant strains of *Staphylococcus aureus*.

Isoflavones possess a rearranged flavonoid skeleton. A variety of structural modifications of this skeleton lead to a large class of compounds that includes isoflavones, isoflavanones, and rotenone. The isoflavonoid compounds are common

constituents of the legume family Fabaceae (formerly called Leguminos family). These compounds displayed estrogenic, insecticidal, and antifungal activity. Some are potent fish poisons. Thus, for example, the isoflavones biochanin A from red clover (*Trifolium pratense*), genistein from soybean (*Glycine max*), and coumestrol from alfalfa (*Medicago sativa*) are phytoestrogens, in addition to exhibiting antifungal activity. The isoflavanone rotenone is the principal insecticidal constituent of the Flavonoid classes. piscicidal plants *Derris elliptica* and *Lonchocarpus nicou*. It is a powerful inhibitor of mitochondrial electron transport. The chalcones, such as butein, lack the pyran ring found in flavonoids, although this is often subject to pH-controlled equilibria. The chalcone is more fully conjugated and normally brightly coloured.

Phlorizin is a strong inhibitor of apple seedling growth. The aurones are golden yellow pigments that are common in certain flowers. Sulfuretin is an aurone pigment responsible for the yellow colour of certain species of the aster family (Asteraceae), for example, cosmos (*Cosmos sulphureus*) and dahlia (*Dahlia variabilis*). Many of these phenols come from familiar sources. The condensed biflavonoids santalins A and B are the major pigments of red sandalwood (*Pterocarpus santalinus*). The flowers of the hawthorn tree provide hyperoside, one of the principal flavonoids from this source (*Crataegus laevigata*). Neohesperidin is responsible for the bitter taste of orange peels (*Citrus aurantium*), while the dihydrochalcone derivative is one of the sweetest-tasting chemicals known. Quercetin, a flavonoid present in numerous plants, has antioxidant activity. It is currently popular at health food stores, although any benefits are speculative. Silybin, one of the silymarins, a mixture of various flavanone derivatives (flavonolignans), is present in the fruit of the milk thistle (*Silybum marianum*). It is used to treat several liver disorders. Similarly, silymarin is the active antihepatotoxic complex used for the treatment of liver damage, and it increases the rate of synthesis of ribosomal ribonucleic acids. It is also used to prevent skin cancer. The secoiridoid glucoside centapicrin is an ultrabitter (bitterness value ca. 4,000,000) secoiridoid glycoside from the century plant (*Centaurium erythraea*). The isoflavones genistein and daidzein are found in high concentrations in kudzu (*Pueraria montana*), soybeans (*Glycine max*), as well as several other legumes. Both genistein and daidzein have anticancer activity.

In-text Question

Prostaglandins have ------ as their precursor.

Answer: *Arachidonic acid*

4.0 SELF-ASSESSMENT EXERCISE

1. List four natural products of pharmaceutical importance.
2. Give a brief outline of the biosynthetic pathway of chlorophyll
3. Give two examples of alkaloid produced from each group of the following amino acids:
    a) Lysine or ornithine,
    b) tyrosine or phenylalanine
    c) tryptophan

Answer:

1. *Prostaglandins, antibiotics, vitamins, flavonoids*
2. *See Section 3.1*
3. *Lysine or ornithine – Nicotine and hygrine*
   tyrosine or phenylalanine – Morphine and papaverine
   tryptophan – reserpine and strychnine

5.0 CONCLUSION

Plants produce a vast array of natural products, many of which have evolved to confer selective advantages on the host plant in ecological interactions. These natural products are synthesised through a diverse network of biochemical pathways, most of which belong to secondary metabolism. The elucidation of these biochemical pathways has been a challenging undertaking. It requires the use of advanced tools from the disciplines of analytical chemistry, biochemistry, molecular biology, and plant physiology. Before the advent of genomics, biochemical and genetic approaches were the two approaches employed in the study of plant natural product biosynthesis. In the genomics era, various genomic approaches, including transcriptomics, proteomics, and metabolomics, have become available. Like the study of general plant biology, the study of plant natural products in the genomics age is undergoing a paradigm shift from reductionist analysis to global analysis. The staggering amount of information that can be generated by these techniques has thus required the integration of computer and mathematical sciences to allow for the efficient manipulation of the data. The biosynthesis of plant natural products in several plant species, such as *A. thaliana*, rice, and *Medicago truncatula*, which is a close relative of alfalfa, is currently being

investigated at the global level and in a high-throughput mode. In addition to facilitating the investigation of natural products biosynthesis, the employment of various genomic approaches to the study will lead to the generation of large data sets, which can serve as a basis for using systems biology to understand the biological function of plant natural products. The elucidation of plant natural product biosynthesis will provide novel information for understanding the biology, ecology, and evolution of plants. Such investigations may also provide tools for predictive metabolic engineering to improve plant traits that are determined by natural products.

6.0 SUMMARY

Primary metabolites are natural products that are found in all cells, but secondary metabolites are natural products that are restricted in their occurrence. Secondary metabolites have been studied because of their biological activity, or for chemosystematic or ecological reasons. Secondary metabolites may be classified as polyketides, terpenoids and steroids, phenylpropanoid (C6- C3) compounds, alkaloids and carbohydrates, based on their biosynthetic building blocks. Acetyl co-enzyme A, isopentenyl pyrophosphate and shikimic acid are the building blocks for the polyketides, terpenoids and phenylpropanoid compounds, respectively.

Alkaloids may be classified in terms of the nitrogen-containing ring system which may reflect the amino acids (lysine, ornithine, tyrosine and tryptophan) which are involved in their biosynthesis.

7.0 REFERENCES/FURTHER READING

1. Leland, J. C. (2006). *Natural Products from Plants.* (2nd ed.). Boca Raton: CRC Press Taylor & Francis Group.

2. “Natural Products.” (www.cem.msu.edu/~reusch/VirtualText/biomol.htm)

- A Collection of Information on Important Classes of Natural Products.