Phytochemistry II

for

Second Year Students

By

Staff Memebers of Pharmacognosy

Faculty of Pharmacy

Menoufia University

Preface

The use of plants to provide humans with medicines goes back to the earliest stages of civilization. As early as 1600 BC, the ancient Egyptians compiled a list of more than 700 medicinal plants, the active ingredients of many of which are now known. Many drugs such as aspirin, morphine, digoxin and taxol were originally obtained from plants, and are still in use today. In order to use such plant in medicine, their components are needed to be identified and then biologically screened for their medicinal activities. These components are sometimes purified to order to increase their efficacy, and decrease the accompanied side effects. The traditional way of studying natural products includes fractionation of a crude mixture or extract, separation and isolation of the individual components using chromatographic techniques and structure elucidation using various spectroscopic methods (UV, IR, NMR, MS). In order to commercialize the isolated natural product, it should be evaluated and standardized.

The overall aim of this course is to illustrate the fundamental knowledge about neutraceuticals, aromatherapy, volatile oils, structure elucidation and chromatographic application on various natural products. The course emphasises on the development of the student intellectual skills through analyzing crude drugs qualitatively and quantitatively and structure elucidation of various classes of natural products.

The Authors

Course Specifications for Phytochemisry II

A- Basic Information:

- **Programme on which the course is given:** B. Pharm. Sci.
- **Department responsible for offering the course:** Department of Pharmacognosy
- **Department responsible for teaching the course:** Department of Pharmacognosy
- **Academic year:** Second Year- Second Semester.
- **Course title:** Phytochemistry II
- **Contact hours (Credit hours):** Lecture : 3 (3) , Practical : 2 (1), Total : 5 (3+1)
- **Course Coordinator:** Prof. Dalia Hemdan and Dr. Mohamed Salem

B- Professional Information

The course aim and intended learning outcomes are based on that mentioned in the programme specification with more course-related specific details.

1- Overall Aims of Course:

The aim of the course is to be able to understand the basic concepts of neutraceuticals, aromatherapy, plant active constituents, separation and structure elucidation techniques and the bases of phytochemistry especially in the composition, chemistry and separation of volatile oils.

2- Intended learning Outcomes of Course (ILO's):

a- Knowledge and Understanding:

By the end of the course the student should be able to demonstrate knowledge and understanding of:

a1- The different methods of preparation of volatile oils from their natural sources.

a2- The methods of identification of volatile oils.

a3- The various techniques used in separation and structure elucidation of natural products

b- Intellectual Skills:

By the end of the course the student should be able to:

b1- Categorize the different chemical classes of volatile oils.

b2- Predict the use of natural constituents for the production of synthetic analogues.

b3- Predict the structure of small molecules.

c- Professional and Practical Skills:

By the end of the course the student should be able to:

c1- Practice the isolation of different volatile constituents from plant origin.

c2- Analyze different types of volatile oil constituents.

c3- Apply spectroscopy in identification different natural products.

d- General and Transferable Skills:

By the end of the course the student should be able to:

d1- Work effectively as a part of a team and as an individual.

d2- Perform effectively in a creative way and time management abilities

3- Contents:

Topics	Lecture	Practical
Neutraceuticals: Basic concepts	3	0
Neutraceuticals: Classes and applications	3	0
Volatile oils: Definitions, method of preparation, distribution; chemistry.	6	2
Aromatherapy	3	0
Different classes of volatile oils: Hydrocarbons.	3	2
Oxygenated Compounds: Alcohols and Esters.	3	2
Ketones and Aldehydes; Oxides, Peroxides and phenols.	3	2
Resin and resin combination; Bitters.	3	0
Sturcture elucidation: UV, IR.	3	2
Sturcture elucidation: NMR and MS.	6	2
Practical exam	0	2
Total	**36**	**14**

4- Teaching and Learning Methods:

4.1. Lectures : ☒

4.2. Practical : ☒

5- Student Assessment

5.1. Methods:

5.1. Practical exam

5.2. Periodicals and final written exam

5.3. Oral exam

5.2. Weight of Assessments

Practical exam	100
Periodicals	50
Final written exam	250
Oral exam	100
Total	**(500 Marks)**

6. **List of References:**

1- Course Notes:

- Phytochemistry 2, approved by Pharmacognosy Department (2019).

2- Essential Books (Text Books):

1. Evans W. C. "Trease and Evans Pharmacognosy" 16th ed., Saunders Elsevier. Edinburg, London.
2. Rotblatt M. R. and Ziment I. Evidence-Based Herbal Medicine. Hanley & Belfus, Inc./ Philadelphia.
3. WHO monographs on selected medicinal plants volume I. and volume II. World health Organization, Geneva.
4. Spectroscopic Identification of Organic Compounds; Silverstein, R.; Webster, F. and Kiemle, D., Wiley and Sons.

3- Recommended Books:

1. Pharmacognosy and Pharmacobiotechnology; Robbers, J.E., Speedie, M.K. and Tyler, V.E.
2. Structural Elucidation of Natural Products; MS; Djerassi, C. and Budzikiewicz,H. and Williams, D.H.; Holden-Day USA.

4- Periodicals and websites:

- Web sites: Wikipedia, the free encyclopedia and other related botanical and natural medicinal plants web sites.
- Journals: Phytochemistry, Journal of Natural Products, Plants Medica, Ethnopharmacology

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Course Coordinators: Prof. Dr. Dalia Hemdan, Dr. Mohamed Salem

Head of department: Prof. Dr. Dalia Hemdan

Contents

Chapter I

NUTRACEUTICALS

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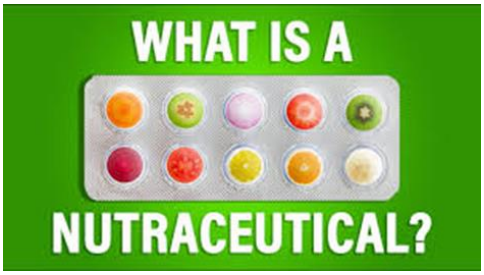
Definition

Nutraceutical can be defined as

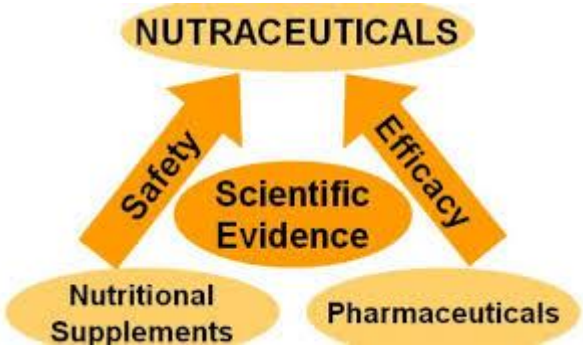
“ A food or part of food or nutrient, that provides health benefits, including the prevention and treatment of a disease.”



In the US, the term “nutraceutical” products are regulated as drugs, food ingredients and dietary supplements. The term is not defined the same in different countries, but is usually defined as a product isolated from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical product may be defined as a substance, which has physiological benefit or provides protection against chronic diseases.

Nutraceuticals may be used to improve health, delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of the body.

Nutraseuticals, in contrast to pharmaceuticals, are substances, which usually have or patent protection. Both pharmaceutical and nutraceutical compounds might be used to cure or prevent diseases, but only pharmaceutical compounds have governmental sanction.



History

The word **"nutraceutical"** is a portmanteau of the words "nutrition" and "pharmaceutical", was coined in 1989 by Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine. Indians, Egyptians, Chinese, and Sumerians are just a few civilizations that have used food as medicine. **“Let food be thy medicine.”** is a common misquotation attributed to Hippocrates, who is considered by some to be the father of Western medicine.

The modern nutraceutical market began to develop in Japan during the 1980s. In contrast to the natural herbs and spices used as folk medicine for centuries throughout Asia, the nutraceutical industry has grown alongside the expansion and exploration of modern technology **(Table 1)**.

Table 1: Global market value of nutraceutical sector and current status.

Global market	Nutraceutical market value	Current status
The US market	US $50.4 billion in 2010	Since the nutraceutical sector is catching up heights, the efforts are turning to be fruitful for maintaining natural health and increased consumer demand
The European market	US $35 billion in 2010	The foundation of nutraceutical industry lays on innovation and rise in research and development. Germany, Netherlands, and Sweden have emerged as the key nutraceutical innovation hubs in Europe
The Japan market	US $27.7 billion in 2010	Functional foods with approved health claim and foods which may provide health benefits without health claim are approved by Japanese Government
The Southeast Asia market	US $10.96 billion in 2009	The marketing curve is rising due to its emergence at international level. The market share figure of 7% in 2008 depicts the high growth potential in this segment
The China market	US $ 15 billion in 2008	The market enjoys the successful industrial growth due to the diversification of their products and increased consumer demand

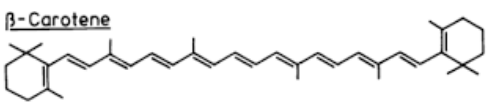
Classification and categorization of nutraceuticals

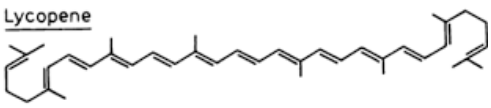
Nutraceuticals are products derived from food sources that are purported to provide extra health benefits, in addition to the basic nutritional value found in foods. Depending on the jurisdiction, products may claim to prevent chronic diseases, improve health, delay the aging process, increase life expectancy, or support the structure or function of the body.

A. Traditional nutraceuticals

The category consists of the food which does not undergo any manual changes. The components are natural and are having some potential which are actively involved in health benefits.

Lycopene, a constituent of tomatoes is an example of this category.





B. Non-traditional nutraceuticals

Boosting of nutritional content by addition of nutrients, dietary components for improvement of quality of nutrition comprise this category of nutraceuticals.

Beta carotene enriched rice is an example of this class.

C. Fortified nutraceuticals

Fortification of food components is the process of addition of micronutrients (essential trace elements and vitamins) to food for enhancing the effectiveness and nutritional value. Its example includes **milk fortified with cholecalciferol** used in Vitamin D deficiency.

D. Recombinant nutraceuticals

It involves the application of biotechnology and genetic engineering in the production of energy providing foods such as **yoghurt** and **cheese** or extraction of bioactive components by enzymatic or fermentation technology.

Gold kiwifruit is genetically modified for a high level of ascorbic acid, Carotenoids, and Lutein and Zeaxanthin.

E. Potential and established nutraceuticals

Potential nutraceuticals hold an assurance of medicinal benefits. These nutraceuticals have became established medicines only after sufficient data demonstration and clinical testing for their efficacy and safety. All nutraceuticals are potential nutraceuticals but all potential nutraceuticals are not established ones.

F. Phytochemicals

These are the chemical constituents of plants with distinct biological action. These are been reported to have active components which exerts their effects toward the metabolism and biochemical reactions in living beings and thus, provide health benefits.

G. Herbals

The herbs possessing medicinal values to be implicated in treatment and prevention of ailments are been included in the class. Botanical products may

consist of fresh plant used or any part such as dried leaf, fruit, stem, seeds, roots, or concentrated extract.

H. Functional foods

Functional foods are fortified or enriched during processing and then marketed as providing some benefit to consumers.

Health Canada defines functional foods as **"ordinary food that has components or ingredients added to give it a specific medical or physiological benefit, other than a purely nutritional effect.".**

In Japan, all functional foods must meet three established requirements: foods should be (1) present in their naturally occurring form, rather than a capsule, tablet, or powder;

(2) consumed in the diet as often as daily; and

(3) should regulate a biological process in hopes of preventing or controlling disease.

- **The class of functional food includes many further subclassed such as cereals, legumes, and fermented food.** The examples are kidney beans, split beans, chickpeas, lentils, and soybeans. These have been explored to have profound antioxidant and protective effect against cardiovascular diseases and diabetes.

- In addition, **chocolate** has also been found to be a subclass of functional food which is a richest source of proteins, calcium, iron, magnesium, and riboflavin.

- **Citrus fruits** are another type of functional food which have already been reported to produce therapeutic effects as anticancer, antiviral, antioxidant agents, and further have potential to stimulate immune system.
- Further classification includes **honey**, which is a natural sweetener and believed to possess nutritional and medicinal value. It is composed of

monosaccharide fructose, glucose, enzyme diastase, amino acids, vitamins, minerals, and aroma compounds. Its biological actions are reported in case of Type 2 diabetes, obesity and also to provide infant nutrition. It also has shown the positive results for improvement of renal functions, as antioxidant and as antimicrobial agent.

- **Colostrum** is another functional food which is referred to first milk secreted in parturition. It contains lactoprotein and lactalbumin due to which it is different from milk secreted later. It is rich in antibodies which provide passive immunity to a newborn in addition to proteins, immunoglobulins, and growth factors. Recent demonstrations reported its role in the treatment of autoimmune disorders.

- Extending further, **meat products** can also be included under the class of functional food. Proteins derived from soyabeans have been employed in comminuted meat products as meat replacements.

I. Dietary supplements and dietary fibers

In the United States, the Dietary Supplement Health and Education Act (DSHEA) of 1994 defined the term: **“A dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet.**

The "dietary ingredients" in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates, and may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

Dietary supplements do not have to be approved by the U.S. Food and Drug Administration (FDA) before marketing, but companies must register their

manufacturing facilities with the FDA and follow current good manufacturing practices (cGMPs).

With a few well-defined exceptions, dietary supplements may only be marketed to support the structure or function of the body, and may not claim to treat a disease or condition, and must include a label that says: **"These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease."** The exceptions are when the FDA has reviewed and approved a health claim.

Dietary fibers and high fiber products are of great interest because of significant health benefits. According to American Association of Cereal Chemists (AACC), it is defined as edible part of plant or carbohydrate analogous which is resistant to digestion and absorption in the small intestine.
These products normalize the intestinal transit time. Its sources include brown rice, banana, cereals, oats, dry beans, and legumes.

J. Probiotics and prebiotics

Probiotic category includes the live microbial food ingredients which are advantageous to health. Their action includes adhesion to gastrointestinal tract at specific sites and their survival lead to elimination of pathogens.

Prebiotic category includes selectively fermented ingredients or a fiber that promote changes in gastrointestinal microflora and its activity providing good effects to the health of host.

- **Probiotics:** These are live bacteria found in certain foods or supplements. They can provide numerous health benefits.
- **Prebiotics:** These substances come from types of carbs (mostly fiber) that humans can't digest. The beneficial bacteria in your gut eat this fiber.

Applications of nutraceuticals in disease management

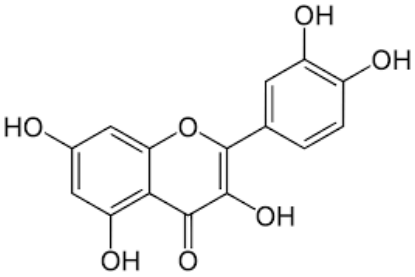
A. Allergy and nutraceuticals

Allergy is a hypersensitivity disorder of the immune system. An allergic reaction usually occurs when a person's immune system reacts to normally harmless substances.

Allergic reactions are distinctive because of excessive activation of certain white blood cells called mast cells and basophils by a type of antibody called immunoglobulin E.

This reaction results in an inflammatory response which can range from uncomfortable to dangerous.

Quercetin is a plant flavonoid with antihistamine and has anti-inflammatory properties. It protects low-density lipoprotein (LDL-C) from becoming damaged, especially to blood vessels. LDL-C is an underlying cause of heart disease and quercetin acts as an antioxidant and scavenges free radicals. Diabetic patients are at higher risk of blood vessel damage from oxidative stress. Therefore, quercetin is beneficial in these patients, too.

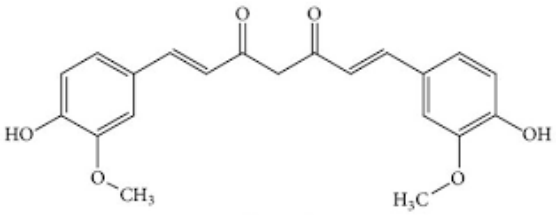


B. Alzheimer's disease and nutraceuticals

Alzheimer's disease (AD) is the most common form of dementia. There is no cure for the disease and eventually leads to death. Most often, AD is diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer's can occur much earlier. There were 26.6 million sufferers worldwide in 2006 and is predicted to affect 1 in 85 people globally by 2050.

Women are more affected in comparison to men, at a ratio of almost 2:1. Several lines of evidence suggest that oxidative stress might be related to a number of neurodegenerative disorders including AD.

Nutraceutical antioxidants such as c**urcumin, lutein, lycopene, turmerin and β-carotene** may exert positive effects on specific diseases by combating oxidative stress. The growing trends in nutraceutical usage are due to the belief that these compounds are able to postpone the development of dementias such as AD.



Curcumin

There are several recently published papers showing the positive effects of different nutriceutical plants such as ***Zizyphus jujube*, *Lavandula officinalis*** on AD, learning or memory.

C. Cardiovascular diseases and nutraceuticals

Worldwide, the prevalence of CVD and the researches in this area is increasing. CVD is a term which is used for disorders of the heart and blood vessels and includes coronary heart disease (heart attack), peripheral vascular diseases, cerebrovascular disease (stroke), hypertension, heart failure, and so on.

Majority of the CVD are preventable. Many studies have reported a protective role for a diet rich in vegetables and fruits against CVD. Nutraceuticals in the form of **vitamins, minerals, antioxidants, dietary fibers and omega-3 polyunsaturated fatty acids (*n*−3 PUFAs)** together with physical exercise are recommended for prevention and treatment of CVD. The molecules such as polyphenols alter cellular metabolism and signaling, which is believed to reduce arterial disease.

- **Flavonoids** are widely distributed in vegetables, onion, endives, cruciferous, grapefruits, apples, cherries, pomegranate, berries, black grapes, and red wine, and are available as flavones, flavanones and flavonols, playing a major role in prevention and curing the CVD. Flavonoids block the

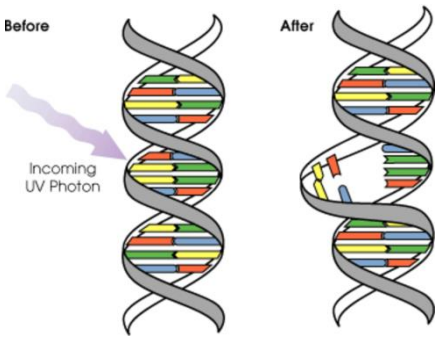
angiotensin-converting enzyme, block the cyclooxygenase enzymes that break down prostaglandins, and prevent platelet aggregation. They also protect the vascular system that carries oxygen and nutrients to cells.

  - Orange juice containing pulp is rich in flavonoids. Hesperidin is a flavanone glycoside which is classified as a citrus bioflavonoid. Citrus sinensis and tangelos are the richest dietary sources of hesperidin. The peel and membranous parts of lemons and oranges have the highest hesperidin concentrations. Hesperidin is used for the treatment of venous insufficiency and hemorrhoids.

- **Anthocyanins**, tannins (proanthocyanidins), tetrahydro-β-carbolines, stilbenes, dietary indoleamines, serotonin and melatonin, in plant foods are hypothesized to impose health benefits.
- **The rhizome of *Zingiber officinalis*** is a common condiment for various foods and beverages. It has a long history of medicinal use and has a positive effect on CVD. Ginger has potent antioxidant and antiinflammatory activities and recently it has been recommended for various diseases including hypertension and palpitation. This plant has a good protective effect on toxicity of synthetic drugs, too.
- **Phytosterols** compete with dietary cholesterol by blocking the uptake as well as facilitating its excretion from the body. Hence, they have the potential to reduce the morbidity and mortality of CVD. Phytosterols occur in most plant species and although green and yellow vegetables contain significant amounts of sterols, their seeds concentrate them.
  - **Buckwheat seeds** possess phytosterols, flavonoids, flavones, proteins and thiamin-binding proteins, etc., Buckwheat proteins lower blood cholesterol and hypertension. Dietary fibers have also cholesterol-lowering property with beneficial effects in prevention and alleviation of CVD and diabetes.
- **Fatty acids of the omega-3 series (*n*−3 fatty acids)** present in fish are dietary components affecting plasma lipids and the CVD, like arrhythmias. Octacosanol, present in whole grains, fruits and leaves of many plants, has lipid lowering property, with no side-effects.

D. Cancer and nutraceuticals

Cancer has emerged as a major public health problem in developing countries. According to the World Cancer Report the cancer rates are increasing and it would be 15 million new cases in the year 2020 that is, a rise in 50%. A healthy lifestyle and diet can help in prevention of cancer.



- **Carotenoids** are a group of phytochemicals responsible for different colors of the foods. They have antioxidant activities and effective on cancer prevention. Recent interest in carotenoids has focused on the role of lycopene in human health, especially in cancer disease.
    - β-carotene has antioxidant activity and prevents cancer and other diseases. Among the carotenes, **β-carotene** has the most antioxidant activity. **Alpha-carotene** possesses 50–54% of the antioxidant activity of β-carotene, whereas epsilon carotene has 42–50% of the antioxidant activity.
    - Because of the unsaturated nature of **lycopene** it is considered to be a potent antioxidant and a singlet oxygen quencher. Lycopene concentrates in the prostate, testes, skin and adrenal where it protects against cancer.
    - **Lycopene** is one of the major carotenoids and is found exclusively in tomatoes, guava, pink grapefruit, water melon and papaya.
    - **Lycopene** contained vegetables and fruits exert cancer-protective effect via a decrease in oxidative stress and damage to DNA.
    - The linkage between carotenoids and prevention of cancer and CAD, heightened the importance of vegetable and fruits in human diet.
- Plants rich in daidzein, biochanin, isoflavones and genistein, also inhibit prostate cancer cell growth.
- Ginseng is an example of an antiinflammatory molecule that targets many of the key players in the inflammation-to-cancer sequence.
- Citrus fruit flavonoids are able to protect against cancer by acting as antioxidants.

- The polyphenolic phytochemicals exemplified by epigallocatechin gallate from tea, curcumin from curry and soya isoflavones possess cancer chemopreventive properties.
- Saponins are reported to possess antimutagenic and antitumor activities and might lower the risk of human cancers, by preventing cancer cells from growing. Saponins are phytochemicals which can be found in peas, soybeans, and some herbs with names indicating foaming properties such as soapberry, soapwort and soapbark. They are also present in tomatoes, potatoes, alfalfa, spinach, and clover. Commercial saponins are extracted mainly from *Yucca schidigera* and *Quillaja saponaria*
- Tannins also scavenge harmful free radicals and detoxify carcinogens. Tannins present in grapes, lentils, tea, blackberries, blueberries and cranberries is a proven anticarcinogen is used in alternative medicine and to prevent cancer.
- Pectin is a soluble fiber found in apples has been shown to prevent prostate cancer metastasis by inhibiting the cancer cells from adhering to other cells in the body.
- Glucosinolates and their hydrolysis products, including indoles and isothiocyanates, and high intake of cruciferous vegetables has been associated with lower risk of colorectal and lung cancer.
- The sulfur compounds, in garlic have been found to boost the immune system and reduce atherogenesis and platelet stickiness and cancer.

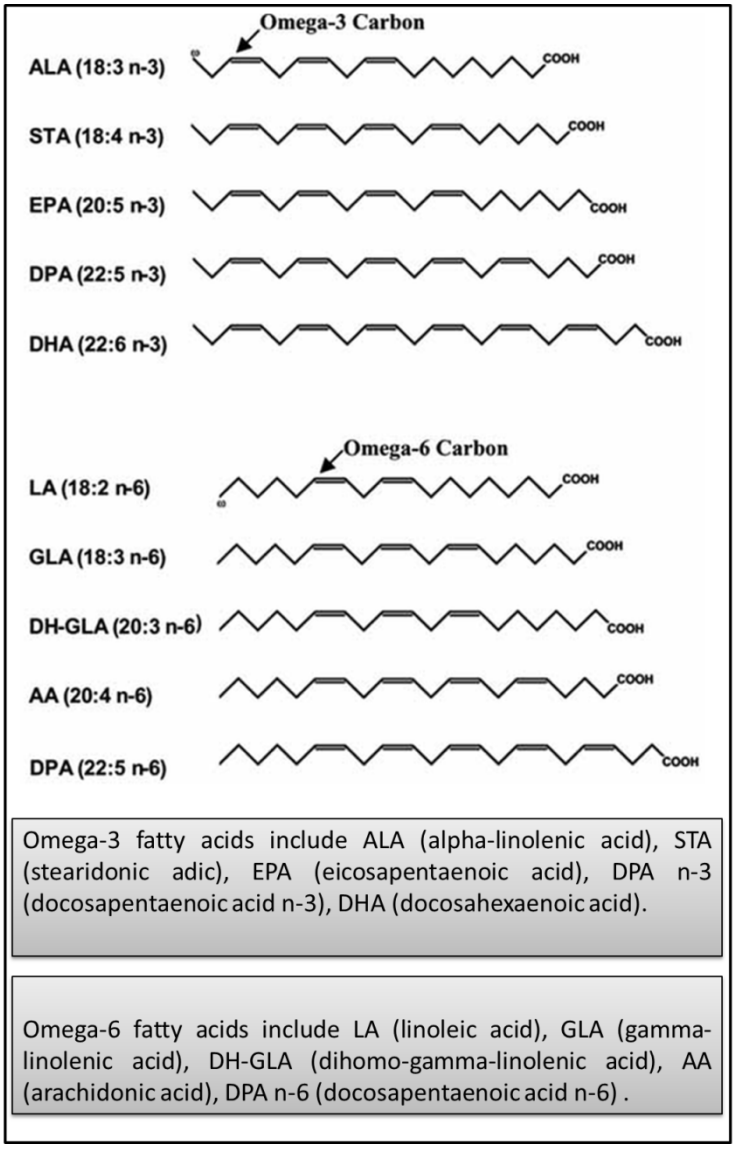
E. Diabetes and nutraceuticals

The most common form of diabetes is type 2 diabetes with 95% prevalence and is associated with obesity. Although various drugs for prevention and treatment of diabetes have been introduced, however, globally the total number of people with diabetes with various causes is increasing.

- **Isoflavones**, are phytoestrogens which have structural/functional similarities to human estrogen. Soy isoflavones have been studied most and their consumption have been associated with lower incidence and mortality rate of type II diabetes, heart disease, osteoporosis and certain cancers.
- **Omega-3 fatty acids** have been suggested to reduce glucose tolerance in patients predisposed to diabetes. For the synthesis of a long chain *n*−3 fatty

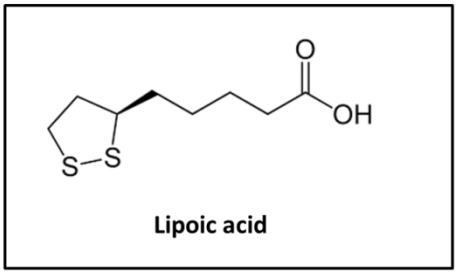
acids, insulin is required; the heart may thus be particularly susceptible to their depletion in diabetes. Ethyl esters of *n*−3 fatty acids may be potential beneficial in diabetic patients.

- **Lipoic acid** is an antioxidant which is used for the treatment of diabetic neuropathy and seems to be effective as a long-term dietary supplement for protection of diabetics from complications.
- Dietary fibers from **psyllium** have been used extensively both as pharmacological supplements, food ingredients, in processed food to aid weight reduction, for glucose control in diabetic patients and to reduce lipid levels in hyperlipidemia.
- A lot of plants extracts such as **Toucrium polium, cinnamon and bitter melon** have been shown to prevent or treat diabetes.

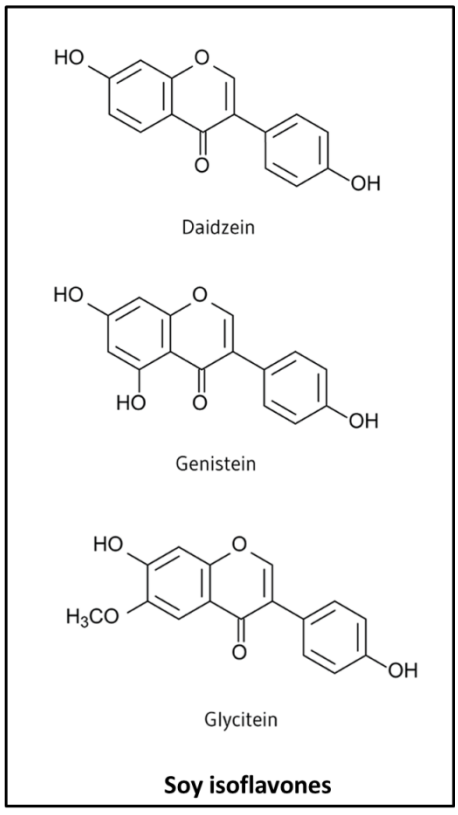


Omega-3 fatty acids include ALA (alpha-linolenic acid), STA (stearidonic adic), EPA (eicosapentaenoic acid), DPA n-3 (docosapentaenoic acid n-3), DHA (docosahexaenoic acid).

Omega-6 fatty acids include LA (linoleic acid), GLA (gamma-linolenic acid), DH-GLA (dihomo-gamma-linolenic acid), AA (arachidonic acid), DPA n-6 (docosapentaenoic acid n-6) .



Lipoic acid



Soy isoflavones

Miscellaneous complications and nutraceuticals

Angiogenesis is an enzymatic process that is generally down-regulated in healthy individuals. Antiangiogenic compounds are selective against newly formed blood vessels while sparing existing ones may not lead to side effects even after prolonged exposure.

Antiangiogenic compounds may prevent diseases involving degenerative process such as multiple sclerosis, arthritis, osteoporosis, diabetes, cancer, AD and Parkinson's diseases.

Some bioactive compounds such as curcumin, flavins, isoflavones and catechins, resveratrol, proanthocyanidins, flavonoids, Saponins, terpenes, Chitin, chitosan, Vitamins B3 and D3, Fatty acids, peptides and amino acids are potentially effective angiogenic compounds.

Moringa oleifera Lam has an impressive range of medicinal uses and is a good source various amino acids and phenolics, protein, vitamins, β-sitosterol, caffeoylquinic acid, kaempferol and β-carotene with high nutritional and therapeutic values. Various parts of this plant like leaves, seed, bark, fruit, roots, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antiinflammatory, diuretic, antihypertensive, antidiabetic, cholesterol lowering, antiulcer, antispasmodic, antioxidant, hepatoprotective, antibacterial, and antifungal activities.

Toxicity potential of nutraceuticals

A large number of people believe that nutraceuticals, especially medicinal plants, are important remedies to address health issues with no side-effects. This belief has been raised from the fact that they have been used for a long period without serious toxicities. Although this is true for a wide variety of nutraceuticals and they generally have less side effects in comparison to pharmaceuticals, but conventional medicine is considered that if a drug is to be effective, inevitably, it will have toxic or side-effects.

The medical establishment considers herbal medicines as drugs, and as such, they must have side effects. Therefore, they need to be prepared with correct ingredients and use with caution, too.

People consume thousands of species of plants and other nutraceuticals to meet their basic nutritional needs, but only a limited number of them have received

significant safety studies. Many remain poorly understood and largely undeveloped, and their wild relatives are threatened with extinction and in need of conservation attention. Stewardship of these valuable plant resources will require rigorous science combined with an approach that respects and values traditional knowledge systems.

Antitoxicity of nutraceuticals

Most of the synthetic drugs possess toxicity properties, and nutraceutical compounds, particularly herbal nutraceuticals have been investigated for their potential in combating the toxic effects of toxins and other medications.

Although the toxicology of drugs is complex, there is great evidence for involvement of oxidative stress in the toxicity of a wide variety of drugs.

Most of plants possess antioxidant activity and other than various specific ways to combat toxins and synthetic drugs, they generally may reduce their toxicity by reduction of oxidative stress.

Kidney and liver are two organs which more than others are involved in toxic effects of other drugs as well as toxins.

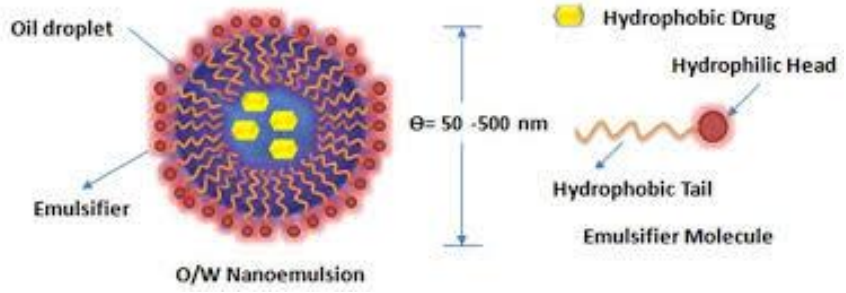
In this regard there are a wide variety of studies investigating the protective activities of nutraceuticals, especially medicinal plants against toxins and other drugs and promising results have been achieved.

Advancement in drug delivery systems with medicated herbs

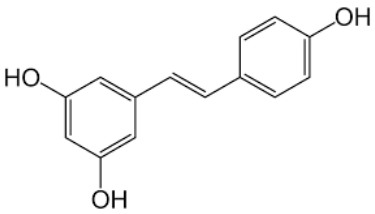
The increasing preferences of consumers to eat healthy food products and the nutraceuticals showing up to be favorable in preventing as well as curing many diseases impelled scientists and researchers to look for efficient delivery systems. The use of novel drug delivery system to deal with the efficacy issues of the products is drawing more and more attention of the researchers.

A. Nanoemulsions

Nanoemulsion is the nano-sized formulation in which two immiscible liquids are mixed to form a single phase, thermodynamically stable isotropic system with the help of an emulsifying agent. The droplet size ranges from 20 to 200 nm.

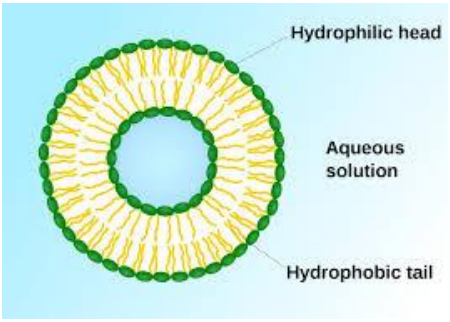


- **Resveratrol** which is a natural compound found in red grape skin, peanuts, and blueberries has been found to possess powerful antioxidant properties. However, the problem with the compound is the poor bioavailability.



    - Therefore, to overcome the problem and enhance the effect, it has been encapsulated in the nanoemulsion formed with spontaneous emulsification method which has resulted in better retention and enhanced properties of the system.
- Similar researchers have proven **curcumin** to be effective in the form of nanoemulsion for the treatment of inflammation in mice by the inhibition of inflammatory mediator.

B. Liposomes

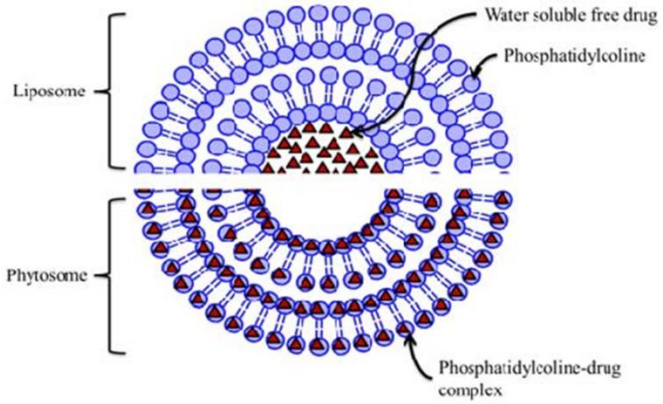


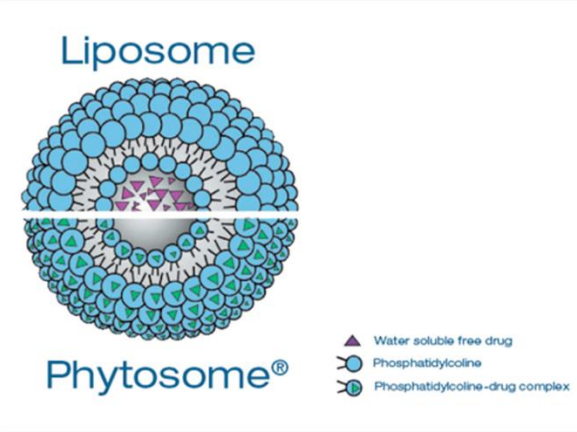
Liposomes are the spherical vesicles composed of phospholipids consisting lipid bilayer. These are spherical in shape and can be formulated using cholesterol and natural phospholipids.

- Liposomes are also preferred to be an advanced delivery system for nutraceutical products. Intranasal **quercetin** liposomes is one of the examples which have been reported to enhance the penetration of quercetin through blood brain barriers and increase the therapeutic anticancer efficacy of the product.
- Similarly, buccal liposomal formulation of **silymarin** has also been proved to offer hepatoprotective effect with enhanced bioavailability of the product resulting into better therapeutic response.
- The antigout topical liposomal preparation of **colchicine** has also been proven very effective in the treatment of gout.

C. Phytosomes

Phytosomes are the complex of phospholipids and the biologically active ingredients.



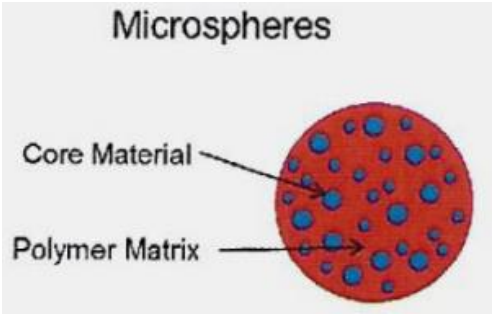


- Oral formulation of **Ginseng** phytosomes, prepared using phospholipid complexation has been found to overcome the problem related to the low solubility of Ginseng and results in the increased absorption in the body which enhances the therapeutic effect of Ginseng as an immunomodulator.

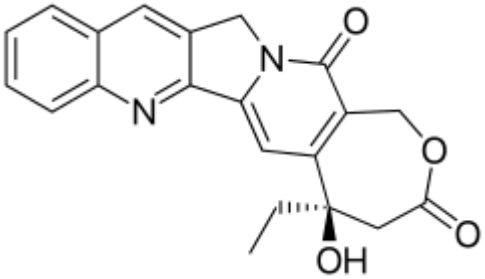
- Oral phytosomal preparation of **Hawthorn** (Flavonoid) having cardioprotective and antihypertensive properties has also been reported to offer enhanced efficacy.
- **Quercetin**, possessing the properties of an anticancer as well as an antioxidant compound was also subjected to the oral preparation of Quercetin phytosomes providing better therapeutic efficacy of the drug.
- Furthermore, **curcumin** phytosomal oral preparation, using curcumin-phospholipid complexation method has also been researched on, and it has offered elevated bioavailability and increased antioxidant activity.

D. Microspheres

Microspheres are the spherical vesicular particles falling within the diameter range of 1-1000 µm. Due to their small size, microspheres can be ingested or injected, can be adjusted to any desired release profile and can also exhibit site-specific as well as organ targeted drug delivery.

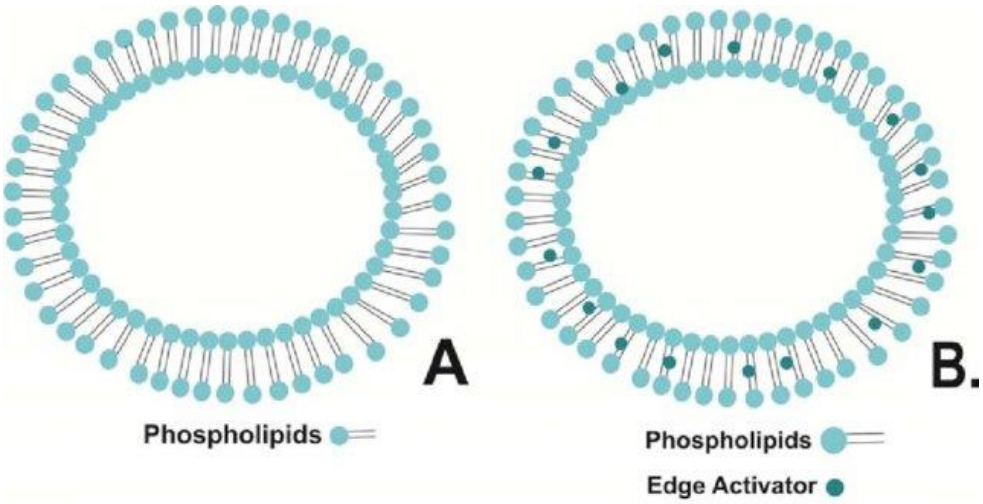


- Intravenous preparation of **camptothecin** (natural product) loaded microspheres, formulated using oil-in-water evaporation method has been reported to provide prolonged anticancer effect.



E. Transfersomes

Transfersomes are also known as the **ultradeformable vesicles** consisting of an aqueous phase as a core surrounded by the lipid bilayer complex which makes the formulation selfoptimizing and self-regulating. Therefore, the transfersomes are capable of crossing several transport barriers conveniently and acts as the carrier in the non-invasive techniques of delivery.



Structural Representation of A. Liposomes, B.transfersomes,

- The researchers have proven topical preparation of **capsaicin** transfersomes to offer enhanced skin penetration with improved analgesic effect.
- **Colchicine** transfersomal formulation has also been studied to provide better penetration and thus advanced treatment of gout.
- Similarly, in vitro preparation of **vincristine** transfersomes exhibits increased entrapment efficiency and improved skin permeation and results into the better therapeutic effect of the anticancer drug.
- Transdermal formulation of **curcumin** transfersomes has been also reported to deliver enhanced entrapment efficiency with intensified permeation through the skin which improvises the anti-inflammatory effect of curcumin on the body.

Chapter II

AROMATHERAPY & VOLATILE OILS

VOLATILE OILS

Definition

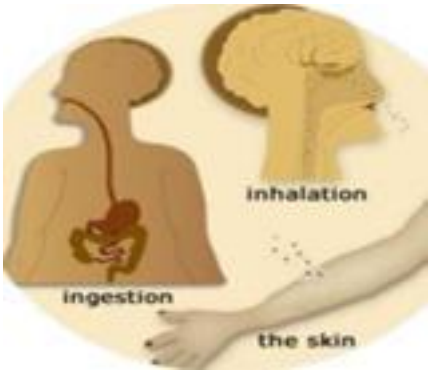
Volatile oils: Complex liquid mixtures of odoriferous principals of varying chemical composition, which easily evaporate when exposed to air at room temperature, and which are used for either their specific therapeutic activity or their aroma.

- **"Volatile oil"** is a term used to designate the odoriferous principals obtained mainly from different plant organs, and rarely from animal sources.
- They are described as **"volatile"** or **"etherial"** oils to indicate that they easily evaporate on exposure to air at room temperature (volatile, from the Latin *"volare"* i.e. to fly).
- They are also known as **"essential oils"** after the Latin word *"essentia",* which means a liquid easily changed to a gas and most probably because they represent the "essences" or odoriferous principals of the plants.
- They are generally mixtures formed of hydrocarbons and their oxygenated derivatives. They differ entirely from **"fixed"** oils in both chemical and physical properties.

Historical overview of volatile oils

- The ancient Egyptians, in the embalming process, extensively used the antibacterial properties of essential oils and resins. The use of aromatic essences and their application in massage was also very common in Roman baths and within the Roman culture.
- However, after the demise of the Roman Empire the use of aromatics in Europe declined until the returning Crusaders reawakened the art of perfuming by the fragrant scents of the Orient.
- Resins rich in volatile oils (e.g. oleoresins) were widely used as incenses in temples, churches and mosques, from antiquity and still until now.
- Inhalation of aromatics, either as tranquilizers (e.g. incenses in case of irritability) or stimulants (e.g. onions in case of fainting), is common in folk medicine.

Aromatherapy



- "Aromatherapy" is a branch of complementary medicine, which depends on the use of aromatic plants, their extracts, mainly their essential oils to promote health, beauty and vitality.

- Essential oils can be massaged into the skin (the most effective way of application) inhaled, added to bath water or as compresses or poultices. Since they may cause damage to the sensitive mucous membranes and skin, none can be taken internally nor used undiluted externally.

- Massage is believed to stimulate the healing processes of the body by increasing blood flow in the skin and at the same time the pungent aromas stimulate the "limbic system", and trigger the emotional centers in the brain.

- In aromatic baths, essential oils act as lubricants to the skin, and their either stimulating or calming activity will assist the healing or relaxation processes, respectively.

- Other routes to achieve aromatherapy include the use of burners, diffusers and sprayers, thus resulting in decongestion of mucous membranes and exerting an antibacterial effect.

- **Examples of essential oils used in aromatherapy:**
  1. Sedatives (e.g. sandal wood, lavender and chamomile oils),
  2. Stimulants (e.g. jasmine and peppermint oils), and
  3. Adaptogens (e.g. rosewood and geranium oils) that adapt their action to balance the systems of the body depending on individual needs, therefore, may produce either a calming or stimulant effect.

Aromatherapy and Essential Oil Diffusers

Diffusion is the process of dispersing essential oils so that their aroma fills a room or an area with the natural fragrance. From the simple to the elaborate, many different methods exist for diffusing essential oils into a room. Three easy methods exist which can be done with things you probably already have in your household. In addition, there are numerous diffusers and diffusing devices available for purchase from aromatherapy vendors.

- **Simple Tissue Diffusion**
  - Place 3-4 drops of essential oil on a tissue. Place the tissue near you. As movement occurs in the room (i.e. as you move or as someone walks by), you will notice the aroma.
  - **Advantages:**
    This method can be used anywhere and is quickly transportable.
  - **Disadvantages:**
    This method does not emit much aroma into a room.

- **Steam Diffusion**
  - Boil 2 cups of water. Pour the water into a bowl and add up to 10 drops of oil to the water. Use fewer drops if you are using an oil that may cause irritation to your mucous membranes (i.e. cinnamon, eucalyptus, rosemary, pine, thyme, cajuput, etc.). Use of energizing or relaxing oils can make this method useful any time of day or night. The steam will heat the oils and cause them to evaporate quickly into the room.

- **Advantages:** This method will quickly diffuse the oils into a room.
- **Disadvantages:** The aroma is not exceptionally long-lasting. Additionally, the heat may alter or destroy certain constituents of the oils and thus the therapeutic benefit may not be as optimal as using cold-air diffusion methods.

- **Candle Diffusion**
  - Light a candle and allow it to burn for about 5 minutes. Extinguish the candle, place 1 drop of essential oil in the melted wax (not on the wick!) and then relight the candle. Essential oils are highly flammable, so great care must be used.

  - **Advantages:** This method can be used most anywhere that a candle may be used.
  - **Disadvantages:** Essential oils are flammable, so great care must be used. The aroma is not long-lasting. The heat may alter or destroy certain constituents of the oils and thus the therapeutic benefit may not be as optimal as using cold-air diffusion methods.

Herbal Essential Oil Recipes

Examples:

Healing Recipes:

Congestion Chest Rub

2 drops Peppermint

4 drops Eucalyptus

4 drops Lavender

10 drops olive oil

Cleaning Recipes:

Rosemary Disinfectant Spray

6 drops Rosemary

6 drops Grapefruit

4 drops Lemon

2 oz purified water

Blends:

Lifting Spirit

4 drops Ylang Ylang

4 drops Clary Sage

2 drops Bergamot

Examples of Essential Oils and their uses in aromatherapy

Each essential oil has very specific properties and qualities, and this site has each on its own page, to give you all the relevant information.

- **Peppermint Essential Oil**
  - This cooling and refreshing essential oil is used in aromatherapy to stimulate the mind, increase mental agility and to increase focus, while cooling the skin, reducing redness and calming irritation and itchiness. It furthermore helps to ease spastic colon, migraine, headaches, sinus and chest congestion and boosts the digestive system.
  - **Oil properties** - Peppermint oil has a fresh, sharp, menthol smell, is clear to pale yellow in color and watery in viscosity.
  - **Therapeutic properties** - The therapeutic properties of peppermint oil are analgesic, anesthetic, antiseptic, antigalactagogue, antiphlogistic, antispasmodic, astringent, carminative, cephalic, cholagogue, cordial, decongestant,

emmenagogue, expectorant, febrifuge, hepatic, nervine, stimulant, stomachic, sudorific, vasoconstrictor and vermifuge.

- Peppermint oil blends particularly well with benzoin, eucalyptus, lavender, marjoram, lemon and rosemary.

- **Frankincense Essential Oil**
  - It is one of the firm favorites in aromatherapy. This essential oil has a wonderfully calming effect on the mind and helps to create inner peace, while helping to sooth the respiratory and urinary tract and relieve pain associated with rheumatism and muscular aches, while having a rejuvenating, balancing and healing action on the skin.
  - **Oil properties** - Frankincense has a woody, spicy, haunting smell, a little bit camphoric, but very pleasant. It is pale yellow-green in color.
  - **Therapeutic properties** - The therapeutic properties of frankincense oil are antiseptic, astringent, carminative, cicatrisant, cytophylactic, digestive, diuretic, emmenagogue, expectorant, sedative, tonic, uterine, vulnerary and expectorant.
  - Frankincense oil blends well with other oils, such as benzoin, sandalwood, lavender, myrrh, pine, orange, bergamot and lemon.
- **Lavender Essential Oil**
  - Lavender oil is one of the most favorite essential oils. It has wonderful qualities and also smells great. It is a calming, relaxing oil, which combats stress and crisis, while the antiseptic properties helps with cold, flu and other ailments. It is excellent for asthma and migraines. Apart from that it supports female health and on the skin it has a healing effect, while preventing scarring and balancing the skin.
  - **Oil properties** - Lavender oil has a light fresh aroma, is clear in color and watery in viscosity.
  - Lavender oil blends particularly well with cedarwood, clary sage, geranium, pine, nutmeg and all the citrus oils.
- **Eucalyptus Essential Oil**
  - In aromatherapy, this essential oil helps to clear the mind and focus concentration, while reducing swelling in the mucus

membranes. It is very valuable in fighting respiratory problems, fighting inflammation and sore muscles, rheumatism, headaches and nervous exhaustion, while boosting wound and ulcer healing and soothing skin eruptions.

- **Oil properties** - Eucalyptus has a clear, sharp, fresh and very distinctive smell, is pale yellow in color and watery in viscosity.
- Eucalyptus oil blends particularly well with benzoin, thyme, lavender, lemongrass, lemon and pine.

- **Orange (Sweet) Essential Oil**
  - This essential oil is used in aromatherapy to create the feeling of happiness and warmth, while calming nervous digestive problems. It deals very well with colds and flu, eliminates toxins and stimulates the lymphatic system, while supporting collagen formation in the skin.
  - **Oil properties** - Sweet orange oil has a sweet, fresh and tangy smell, is yellow to orange in color and watery in viscosity.
  - Orange oil is used in many Curacao type liqueurs and for the flavoring of food, drink and confectionery and when added to furniture polish, helps to protect against damage from insects.
  - Orange oil blends particularly well with black pepper, cinnamon, cloves, ginger, frankincense, sandalwood and vetiver.
- **Lemon Essential Oil**
  - This clean smelling citrus essential oil is not only good for helping you make decisions and to improve your concentration, but cuts down on acidity in the body - thereby assisting the digestion, as well as with rheumatism, arthritis and gout, while also sorting out cellulite, abscesses, boils, carbuncles and acne.
  - **Oil properties** - Lemon oil has a sharp, fresh smell, is pale greenish-yellow in color and is watery in viscosity.
  - The shelf life of lemon oil is only 8-10 months, if it is to be used in aromatherapy, but can still be used in fragrance therapies after this time, such as vapor therapy.
  - Lemon oil blends particularly well with lavender, rose, sandalwood, benzoin, eucalyptus, geranium, fennel, juniper, neroli and elemi.

- **Rosemary Essential Oil**
    - This crisp and clean smelling essential oil is great for stimulating the brain, improving memory and mental clarity, while helping with a variety of congested respiratory tract problems, stiff muscles, coldness as well as boosting the liver and gall bladder. It is also used for improving hair and scalp health.
    - **Oil properties** - Rosemary oil has a clear, powerful refreshing herbal smell, is clear in color and watery in viscosity.
    - Rosemary oil blends particularly well with Cedarwood, Citronella, Geranium, Lavender, Lemongrass and Peppermint.

Natural Sources of essential oils

Essential oils occur almost exclusively in higher plants, a limited number is obtained from animal sources.

A-Animal sources:

Commercially available animal-derived essences are musk, musk-like products (such as civet and castoreum) and ambergris. These products are secretions produced by the animal to act either as attractants or protectants.

1. **Attractants**: These are secreted in order to attract the other sex as in the case of musk and musk-like products (civet and castoreum):
   - Musk is derived from the male musk deer.
   - Civet from both the male and female civet cat.
   - Castoreum is derived from different types of Russian and Canadian beavers called castors.
2. **Protectants**: These are produced as a defensive mechanism to protect the animal against injury, as in case of ambergris that is obtained from the sperm whale.

Animal-derived fragrant constituents are usually macrocyclic compounds of high molecular weight and are used, in minute amounts, as fixatives in high-grade perfumes.

B-Botanical sources:

- There are about 17,500 aromatic species. The genera producing volatile oils are distributed in a limited number of families (over 90) of the phanerogams.

- Among families rich in volatile oils are the following: Pinaceae, Lauraceae, Rutaceae, Myrtaceae, Zingiberaceae, Apiaceae (Umbellifereae), Lamiaceae (Labiateae), and Asteraceae (Compositeae).

Clove (Myrtaceae)

Mentha (Labiateae)

Anise (Umbellifereae)

German chamomile (Compositeae)

- **Volatile oils may accumulate in all types of vegetable organs, such as:**
  - Flowers, e.g. rose, bergamot, tuberose, and jasmine.
  - Leaves, e.g. citronella, eucalyptus, and laurel.
  - Barks, e.g. cinnamon and cassia.
  - Woods, e.g. rosewood and sandalwood.
  - Roots, e.g. vetiver.
  - Rhizomes, e.g. turmeric and ginger.
  - Fruits, e.g. anise, star anise, and allspice.
  - Seeds, e.g. nutmeg and cardamon .

- All the organs of a given species may contain essential oil, but the composition of the oil usually varies with the site of its production in the plant e.g.
  1. **Cinnamon oil** obtained from the bark of Cinnamomum zeylanicum is rich in cinnamaldehyde, while that from the leaf contains mainly eugenol.
  2. **The bitter orange tree** (Citrus auriantium ssp. auriantium) produces three different types of oils:
     - "Bitter orange oil": from the fresh pericarp of the fruit (or zest),
     - "Neroli oil": from the flowers, and
     - "Petit grain oil": from the leaves, twigs and unripe fruits. These three essential oils have different composition and consequently differ in aroma.

Production, Distribution and Function in plant tissues

- Volatile oils may be present free or in combination with:
  1. Sugars forming glycoside e.g. Amygdalin
  2. Gums forming oleogum e.g. Gum acacia
  3. Resins forming oleoresin e.g. Ginger- Capsicum
  4. Gums and resins to form oleogumresin e.g. Myrrh

- They usually accumulate in **specialized histological structures**, which are often **located** either **on** or **near the surface** of **the plant** such as:
  1. **Oil cells**: in family Lauraceae and Zingiberaceae.
  2. **Glandular hairs**: in family Lamiaceae (Labiateae).
  3. **Secretory canals** (tubes): in family Apiaceae (Umbellifereae).
  4. **Oil cavities** (glands): in family Rutaceae and Pinaceae.

Glandular hairs (F: Lamiaceae).

Oil cavities or glands (F: Rutaceae).

Oil cells (F: Zingeberaceae).

Secretory canals or tubes (F: Apiaceae).

- **Physiologically** volatile oils are considered as:
  1. Waste products of metabolism
  2. Energy producers in case of deficiency from CO_2 assimilation
  3. H^+ donors in certain metabolic reactions
  4. Solvents for wound healing resins

- Their presence at the outer layers of plant organs facilitates their action as:
  1. **Protectants against predators**: such as insects and fungi thus acting as insect repellents and antifungal.
  2. **Pollinators**: attracting insects, thus playing an effective role during cross-pollination.

Physical properties

- Volatile oils have a number of **common physical properties** despite of their variable composition.

  1. They are **colorless**, pleasant smelling liquids at ambient temperature, but they are also **volatile**.
  2. They can be **steam distilled**.
  3. They have a **high refractive index** and **most** of them are **optically active**.
  4. Their **density is mostly lower than that of water** except for few ones.
  5. They are **immiscible with water**, but sufficiently soluble to impart a distinct fragrance to water (aromatic waters).
  6. They are soluble in common organic solvents and lipids (**liposoluble**).
  7. They may darken in color when exposed to light (due to **resinification**).

On cooling, volatile oils separate into a **liquid** fraction, **oleoptene,** generally formed of hydrocarbons and a **solid** one, **stearoptene**, consisting of one or more solid oxygenated compounds (**stearoptenes were previously collectively called "camphors").**

Chemical composition

- Practically, all volatile oils are **complex and variable mixtures** of constituents. Many types of **hydrocarbons** and **oxygenated compounds** such as alcohols, ketones, aldehydes, ethers, oxides, phenols and esters are found. Consequently, more than one constituent may possess physiological activity and they collectively give the odor of the oil.

- Few oils may consist of **one main component** such as the volatile oil of mustard (93% allylisothiocyanate) and oil of clove (85% eugenol).

- Constituents of volatile oils belong to two main groups of distinct biogenetic origin, the **terpenoids** (derived from acetate) and the **aromatic compounds** (derived from phenylpropane), therefore, also called **phenylpropanoids**.

- Some essential oils contain **degradation products** of non-volatile constituents (e.g. furfural a degradation product of carbohydrates).

Factors influencing the chemical composition of volatile oils

1. **The geographical origin:**

   Thyme (*Thymus vulgaris* L) is **morphologically homogeneous** when obtained from different localities but it has **different chemotypes**: samples obtained from different locations in France have **thymol**, **carvacrol** or **geraniol** as the major component, while that obtained from Spain has **cineole**.

2. **The stage of maturity:**

   For a given species, the proportions of the different constituents of an essential oil may vary greatly throughout its development e.g. the level of **linalol** is 50% higher in the **ripe coriander fruits** than in the **unripe** fruits.

3. **The environmental factors:**

Environmental factors exert a direct influence, especially on species that have superficial histological storage structures (e.g. glandular hairs of the Lamiaceae); oils of deeper localization have more constant quality.

These factors include temperature, relative humidity, and total duration of daylight, as well as, wind patterns.

Examples are:

- In some ***Citrus***, the higher the temperature, the higher the yield of the oil;
- In the **peppermint**, long days and temperate nights lead to higher yields of oil and an increase in the menthofuran level, whereas cold nights favor the formation of menthol.

4. **The cultivation practices:**

These include fertilization and watering and they also greatly influence both the yield and quality of the final product.

5. **The preparation method:**

- Most volatile oil constituents are **thermolabile** and, therefore, **the product obtained by steam distillation is often different from the mixture of constituents present in the secretory structures (natural oil).**
- During distillation, the water, the acidity and the temperature may induce hydrolysis of esters and rearrangements, isomerizations, racemizations, oxidations etc….

Characterization of volatile from fixed oils

Fixed oils constitute another type of oils derived from plants. They, sometimes occur together with volatile oils in the same plant organ (e.g. seeds). **Table (1)** summarizes the main points of differentiation between these 2 types of oils.

Table (1) Characteristics of volatile and fixed oils

Property	Volatile oil	Fixed oil
Volatilization at ordinary temperature	Volatile	Non-volatile
Steam Distillation	Distillable	Non-distillable
Solubility	Soluble in organic solvents (ether, $CHCl_3$) and alcohol of different strengths	Limited solubility in organic solvents, almost insoluble in alcohol
Stain on filter paper	Transient	Permanent and greasy
Response to long exposure to air and light	Resinification	Rancidity
Composition	Different types of hydrocarbons and oxygenated compounds	Glyceryl esters of fatty acids
Nutritive value	None	Nutritive
Soap formation	Negative	Positive

The medicinal importance

The following are the fundamental **pharmacological activities** attributed to volatile oils:

1. Some oils exert an antiseptic effect **against** various **pathogenic bacteria**, including strains that are usually resistant to antibiotics. Some are also active **against fungi** responsible of mycosis as well as yeast (e.g. in candidiasis).
2. Several oils are found efficient in decreasing or suppressing **gastrointestinal spasms**, so they are used as **spasmolytic and sedative properties** e.g. chamomile, clove, mint and thyme.
3. Other oils can stimulate gastric secretions and therefore, described as **digestive** and **stomachic**.
4. Certain oils cause an **increase in capillary blood flow,** rubefaction, a sensation of heat, and in some cases local anesthetic activity e.g. oil of **turpentine**, **eucalyptus** and **wintergreen** etc…. They are used as ingredients in many ointments, creams or gels designed to relieve arthritic or muscular pains.

5. **Eucalyptus** and **pine** stimulate mucous cells and increase the motility of the ciliated epithelium of the bronchi and thus are used as **expectorants**.

6. **Juniper oil** enhances the renal excretion of water by a direct local effect (**diuretics**).

Other pharmacological effects attributed to volatile oils are choleretic, healing, antidepressant, calming and sedative.

Commercial uses

1. Various crude drugs are powdered and used as **spices and condiments** in food seasoning to impart aroma and flavor or to act as preservatives.
2. They are used as **flavoring agents** in **food** (e.g. beverages, soups, bakery products, confectionery) and **pharmaceutical industries** especially medicines used for infants.
3. They are used in all types of **perfume industries** such as those of cosmetics, soaps, deodorizers, household cleaners, polishes and insecticides.

Methods of preparation of essential oils

Selection of the suitable method

The major **factors influencing the selection of the suitable method for preparation** of volatile oils are:

1. The **condition of the plant material** (moisture content i.e. dry or fresh and the degree of comminution i.e. intact, crushed or powdered)
2. The **localization of the oil in the plant** (superficial or deep)
3. The **amount of the oil**
4. The **nature of the oil constituents**

- Fresh material should be collected before sunrise.
- Dried material should be comminuted just before distillation to avoid volatilization.
- Even charging (packing) is necessary.
- Plants should be wetted before subjecting to saturated steam distillation.

Methods used for volatile oils extraction

I- Distillation methods:

1. Water distillation.
2. Water and Steam distillation.
3. Direct Steam distillation.

II- Scarification and Expression methods:

1. Sponge method.
2. Ecuelle a piquer method.
3. Expression of raspings.

III- Solvent extraction methods including the use of:

1. Non-volatile solvents e.g enfleurage method.
2. Volatile solvents

IV- Enzymatic hydrolysis of glycosides.

I. Distillation methods

Principle

The boiling range of most volatile oil constituents lies between 150-300 ºC. In order to avoid decomposition at such high temperatures, volatile oils are distilled in the presence of water. The process is therefore, carried at temperatures below 100 ºC as stated by **Dalton's law** of partial pressure: "When two immiscible liquids are heated together, they will boil at a temperature below the boiling point of either one".

The choice of the method used is limited by the condition of the plant material (e.g. fresh or dry, coarsely or finely powdered).

Three types of distillation methods could be distinguished according to the degree of contact between the plant material and water. These are:

1- Water distillation.

2- Water and Steam distillation.

3- Direct Steam distillation.

Description of the distillation apparatus:

A distillation apparatus is basically formed of 3 parts:

1- The body of the still (distillation flask): made of copper lined with Lead- free tin or stainless steel (the use of Fe is avoided as any Fe may catalyse hydrolytic & oxidative decomposition of Constituents).

2- The condensing system (condenser): designed to provide proper cooling to avoid reflux of the distillate.

3- The receiver (collecting flask): specifically designed to allow separation of the oily layer from water in the distillate.

Different types of apparatuses and receivers used in hydrodistillation of volatile oils are represented in **Figures (1 and 2)**

Precautions in handling the plant material

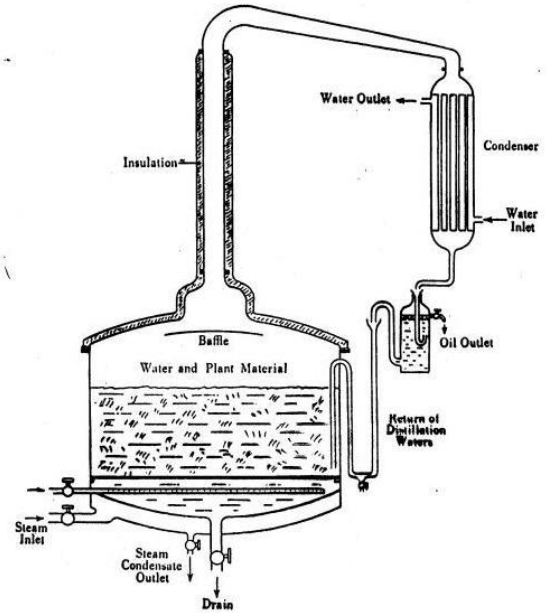
1. **Comminution** should be carried **just before distillation** to prevent loss by volatilization.
2. **Dried plant material** should be **wet** before packing if subjected to saturated steam distillation.
3. **Packing** or charging should be **even** in order to facilitate "hydrodiffusion".

- **Hydrodiffusion** is the process by which water or steam penetrates the plant tissues.
- The **highest the rate of hydrodiffusion**, the lowest the rate of hydrolysis and decomposition of the constituents, therefore **coarse comminution** and **even packing** are recommended.

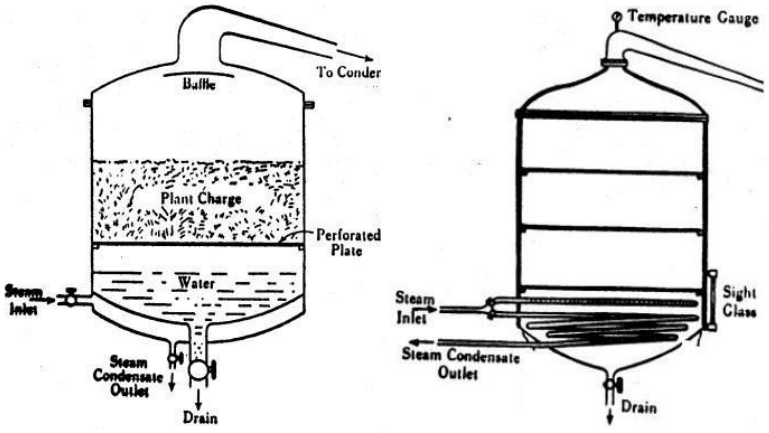
Influence of distillation on the quality of the final product

It is important to notice that distilled oils are actually considered as **"artifacts"** and not exactly a reproduction of the oils present in the plants due to the following reasons:

1. Insufficient distillation time (shorter) may result into fractionation of the oil.
2. High boiling point constituents are mostly not carried over by steam (this is may be avoided by **addition of glycerol** to water in the still to raise the boiling point).
3. Hydrolytic products (e.g. lower alcohols and acids) are mostly water-soluble and not carried over by steam.
4. Steam volatile impurities such as amines and furfural may contaminate the final product.
5. Sensitive constituents could be affected by boiling water e.g.
   - Esters are hydrolyzed.
   - Tertiary alcohols are dehydrated to hydrocarbons.
   - Unsaturated terpenes are polymerized.



Still for water distillation



Still for water and steam distillation

Still for direct steam distillation

Fig.1. Different types of distillation apparatuses

A. Simple steam distillation:

- This method, also known as "**water distillation**", is based on **immersing the plant material** (intact or crushed) directly **in a still filled with water**, which is then boiled.

- The heterogeneous vapors (water vapor and volatile oil) are condensed on a cold surface, and are collected in special receivers.

- The **oil** separates from **water** based on the **difference in density and immiscibility.**

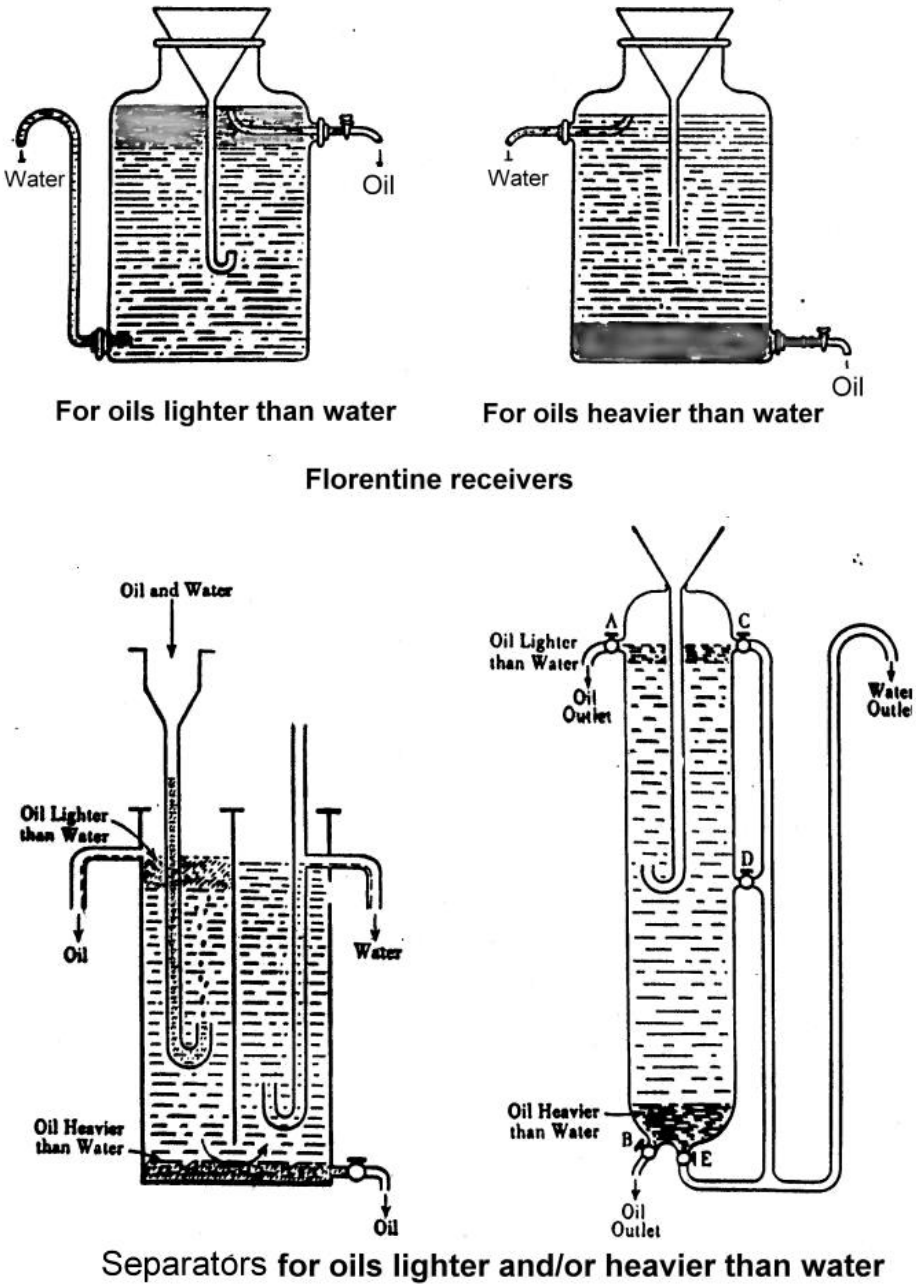


Fig. 2. Different types of oil and water separators

- This method allows "**cohobation**": the return of the aromatic water (water saturated with volatile oil) to the body of the still to be redistilled in order to improve the yield of oil.

B. Saturated steam distillation:

In the techniques based on the use of **saturated steam** (also known as **"direct steam distillation"**), **the plant does not come in contact with the water:** steam is injected through the plant material placed on perforated trays. This process is usually adopted in order to:

1. Shorten the duration of distillation.
2. Limit the alteration of the constituents.
3. Conserve energy.

The **steam pressure and temperature could be modified** according to the oil composition:

- **Lower pressure and temperature** are necessary for **thermolabile constituents** (oil rich in esters).

- **Higher pressure and temperature** are used in case of oils rich in **high boiling point constituents** (sesquiterpenoids)

A modification of the technique is **the use of water to generate steam inside the still** while the plant material is placed on perforated trays and is penetrated by the water vapors. This method is also known as **"water-and-steam distillation".**

The main differences between distillation methods used for preparation of volatile oils, their advantages and disadvantages are summarized in **Table (2)**.

Rectification of hydrodistilled oils (Purification)

1. Removal of bad smelling, irritating and / or colored impurities is carried by:
   - Redistillation in steam.
   - Dry distillation under reduced pressure.
2. Removal of water and moisture is done by filtration over anhydrous sodium sulfate.

Table (2): Distillation methods

	Water distillation	Saturated steam distillation	
		Direct Steam Distillation	**Water and Steam Distillation**
Plant material	Dried plant material. Petals.	Fresh materials containing sufficient moisture.	Dried & fresh, especially herbs & leaves
Mode of charging	Plant material is completely dipped in H_2O.	H_2O is completely absent. Steam is forced through the plant material.	H_2O is present in the still but steam alone is in contact with the plant material **N. B.**: Dried material should be wet.
Hydrodiffusion	Better when material moves freely in H_2O.	Better when charging is even.	Better when charging is even.
Steam pressure	About atmospheric	Can be modified according to the plant condition & nature of oil.	About atmospheric
Temperature	About 100ºC	Can be modified according to the plant condition & nature of oil.	About 100ºC
Rate of distillation	Relatively low	High	Fairly good
Yield of oil	Relatively low , since: ▪ Esters may be hydrolyzed. ▪ Water soluble as well as high boiling point constituents remain in the still.	The best if no lumping or channeling.	Better since hydrolysis is diminished
Commercial preparations	▪ Oil of turpentine. ▪ Oil of rose.	Oil of peppermint.	▪ Oils of cloves and cinnamon. ▪ Oil of citronella.
Advantages	▪ Low priced & portable stills. ▪ Could be carried near the production area & so, transportation fees are not required.	▪ Stills, more durable & suitable for large-scale production. ▪ Suitable for oils rich in esters & those rich in high boiling point constituents.	Hydrolysis is prevented since no excessive wetting of material.
Disadvantages	▪ Often burning of plant. ▪ Cohobation must be carried. ▪ Not used for oils rich in saponifiable, water-soluble & those rich in high boiling point constituents.	▪ Powders could not be used since easy channeling usually occurs. ▪ Wet steam is usually preferred to superheated steam to avoid plant drying.	Hydrodiffusion could be hindered by fine comminution, uneven charging or excessive wetting resulting in low yield.

II. Scarification and Expression Methods

Principle

These are **mechanical** procedures carried at **room temperature** and based on puncturing and/ or squeezing of the plant material to liberate the oil, which is collected.

Applications

They are used for preparation of heat sensitive oils which are present in large amounts in outer peels of fruits e.g. epicarps of *Citrus* fruits (Rutaceae), such as orange, lemon and bergamot.

Preparation and Purification of the oil

The peel (or zest) of *Citrus* fruits consists of two distinct layers:

- An **outer colored zone** rich in waxes and pigments and containing the oil glands,
- An **inner white zone** formed of pectin and cellulose.

The classical process includes:

1. **Squeezing of the peel** under a **stream of water** yielding an **emulsion** formed of essential oil, water, pectin, cellulose, pigments and traces of waxes.
2. **Removal of water**, pectin and cellulose by centrifugation.
3. **Removal of waxes** by strong cooling (chilling) followed by filtration or decantation.

A. Sponge Method

- This is based on **squeezing the removed peels** to collect the oil and is used in Sicily for preparation of orange oil as follows:

1. Fruits are washed, cut into halves and fleshy parts removed.
2. Peels are soaked in water, turned inside out and pressed between a convex projection and a sponge.
3. The saturated sponge is periodically squeezed in a vessel and the emulsion obtained centrifuged and cooled.

- The tissue of the **sponge serves for** both:

Collection of the oil, and

1. **Filtration** of the product from any particles of the inner white zone of the peel.

B. Ecuelle-à-piquer method

- This is based on **puncturing** (scarifying) **the surface of whole fruits** and thus allowing exudation of the oil from the outer colored zone of their peels. The method is applied, in France, for preparation of lemon oil.

- The **instrument** used is funnel-shaped, consisting of a shallow bowl with a tubular projection at the center. The bowl-like part bears, internally, numerous pins, long enough to scarify the oil glands and release the oil.

- The **tubular part** serves both as:
  1. A **handle** to rotate the instrument.
  2. A **receiver** to collect the oil.

Ecuelle-á-piquer

C. Expression of rasping

- This is based on removing the **outer layer of the peel** with a grater, collecting the rasping in special bags (horsehair bags) followed by strong **pressing**.
- The produced oil is collected in large vessels, left to stand, decanted and filtered.

D. Machine processes

New machine techniques based on the same principle have been designed to replace the above three traditional methods A, B and C.

For example, whole fruits are charged into machines in which the peels are removed, sprayed with water, squeezed or pressed and the oil collected through wool filters.

III. Extraction methods

These include a group of methods that are usually applied for preparation of delicate flower oils, such as jasmine, violet, tuberose, narcissus etc… which contain either:

- **Small amounts** of oils, easily lost in the large volume of distillation water or ,
- Oils, which decompose by heat (i.e. formed of **thermolabile** constituents).

According to the **nature of the solvent** used, they are **subclassified** into:

- Non-volatile solvent extraction methods.
- Volatile solvent extraction methods.
- Supercritical fluid extraction.

A. Non-volatile solvent extraction

These processes are used for preparation of natural flower oils producing the finest perfumes. They are based on the **liposolubility** of the fragrant components of plants. Lipids of high degree of purity are used.

Lipid selection

The lipids or non-volatile solvents used in this process include:

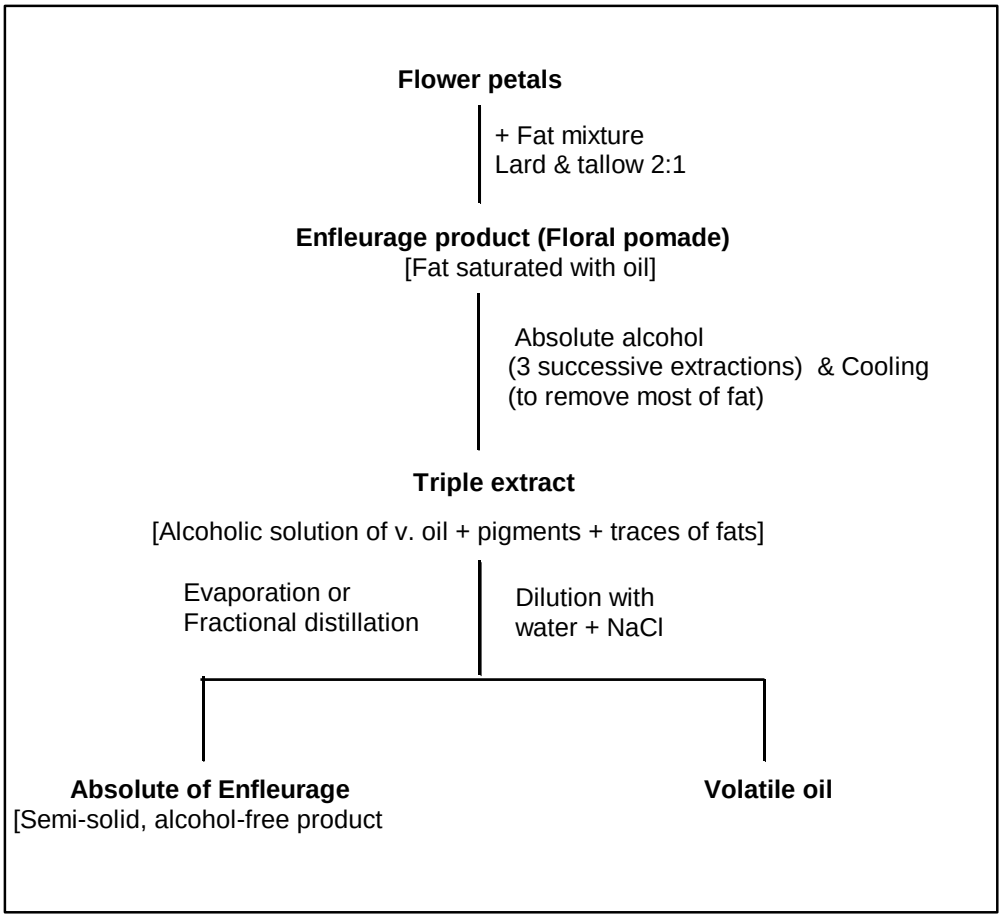
- **Fats** such as **lard and tallow**, generally a mixture of 2:1 is used to avoid melting during extraction (tallow being harder and of higher m. p. than lard).
- **Fixed oils** such as **olive oil**.

"Enfleurage" process

This technique is applied in the South of France, in the city of Grasse, for the preparation of **oil of jasmine**.

- The **equipment** consists of a great number of **glass plates** closely arranged in a wooden frame (or chassis).
- The **procedure**, summarized in **scheme 1**, involves:

1. Spreading the pure mixture of fat (lard / tallow 2: 1) on each of the surface of the glass plates.
2. Covering the top of each plate with flowers or petals, so that each layer of plant material is enclosed between an upper and a lower layer of fat.
3. Replacing old flowers by fresh ones periodically, and repeating the process until the fat is saturated with the oil or until a certain concentration of the oil is reached.
4. Removing the last charge of flowers from the fat (or **Defleurage**) e.g. for jasmine after 70 days.
5. Scraping the oil-saturated fat layers, warming the combined fat and filtering through gauze followed by cooling to yield the "**Enfleurage product**", sometimes called "floral pomade"
6. The "**Enfleurage product**" could be marketed as such or after special treatments in the form of "**Triple extract**", "**Absolute of enfleurage**" or the separated "**Volatile oil**".



B. Volatile solvent-extraction [Preparation of "concrete" and "absolute"]

Most modern commercial methods are accomplished by extraction with volatile solvents.

Preparation of "floral concretes"

- The procedure involves the treatment of the flowers or petals as follows:

1. Extraction by either percolation or maceration at room temperature, or by continuous hot extraction in a **Soxhlet apparatus** at constant temperature not exceeding 50º C (generally at reduced pressure).
2. Removal of the solvent by distillation under reduced pressure.

- The "**floral concrete**" of jasmine obtained by this procedure is a semi-solid product with yellowish-orange color.
- The "**floral concrete**" consists of the **fragrant constituents** together with the liposoluble **impurities** such as fats, waxes, albuminous matter and pigments.

Floral concrete = Fragrant constituents + Fats + Waxes + Albuminous matter + Fat soluble pigments

Preparation of "floral absolutes"

These are **more expensive** and **purified** products **than** the **corresponding concretes**, from which they are prepared as follows:

1. Repeated extraction with absolute alcohol thus **extracting most of the oxygenated constituents of the oil**.
2. Chilling (strong cooling) followed by decantation after each extraction in order **to remove waxes, albuminous matter and fat-soluble pigments**.
3. The process is stopped when no turbidity is observed in the mixed alcoholic extract.

- The resulting "absolutes" are, therefore, **richer in oxygenated constituents**, which are in most cases responsible for the odor of the oil.
- Thus, they can be used in much **smaller concentrations** than the corresponding concretes in the perfume industry.

Drawbacks of the solvent extraction methods

The main drawbacks of solvent extraction techniques are:

1. The **lack of selectivity**: many lipophilic substances are obtained in the concretes including phospholipids, carotenoids, waxes, coumarins and others.
2. The **toxicity** of the solvents.
3. The problems of **residues** in the final product.

C. Supercritical fluid extraction [Extraction by supercritical gases]

This technique has been previously discussed in the extraction section (see under Introduction and General Methods, p 14). Despite of its high cost, it is ideal for preparation of volatile oils where:

- The products obtained are close in composition to the natural oils present in the plant material.
- The oil components are not subjected to hydrolysis or deterioration.

Applications, advantages and disadvantages of distillation, expression and solvent extraction methods are summarized in **Table (3).**

Table (3): Applications, advantages and disadvantages of the major techniques used for preparation of volatile oils from their natural sources.

Process	Applications	Advantages	Disadvantages
Distillation	Suitable for dried and fresh material, rich in volatile oils with constituents mostly unaffected by heat	Cheapest commercial method (as regards, apparatus, solvents and source of heat)	The use of high temperature and the presence of water may affect the constituents.
Scarification and expression	Suitable for preparation of oils present in large amounts in outer peels of fruits and rich in heat-sensitive constituents.	Carried at room temperature, and yields oils with more natural odors.	Expensive due to need of high number of workers.
Extraction	Useful for fresh materials with heat-sensitive oils present in small amounts.	Carried at room or low temperatures, and yields oils with more natural odors	Expensive due to use of solvent and/or high number of workers.

IV. Methods based on enzymatic hydrolysis of glycosides

Principle

The process includes the following steps:

- Subjecting the plant material to **hydrolysis** (generally enzymatic) to liberate the **volatile aglycones** from the parent odorless glycosides.
- **Separation** of the **volatile aglycones** by either distillation or extraction.
- **Purification** of the products

N.B.: Plant material **rich in fixed oils**, such as seeds, should be subjected to pressing before hydrolysis to separate most of the fixed oil.

Glycosides with volatile aglycones appear to be widely distributed, Examples of these types of **glycosides and their hydrolytic products** are listed in **Table (4)**.

Table (4): Examples of glycosides of volatile substances

Plant name	**Non-volatile Glycoside**	**Volatile aglycone**	**Other hydrolytic products**	**Hydrolytic enzyme**
Gaultheria procumbens **(Ericaceae) &** Betula lenta **(Betulaceae)**	Gaultherin & / or Monotropin	Methyl salicylate Methyl salicylate	Primeverose (Xylose + glucose) Glucose	Gaultherase Gaultherase
Geum urbanum **(Rosaceae)**	Gein	Eugenol	Glucose	β-Glucosidase
Brassica nigra **(Brassicaceae or Crucifereae**)	Sinigrin	Allyl isothiocyanate	Glucose +Potassium acid sulfate	Myrosin
Vanilla planifolia **(Orchidaceae)**	Glucovanillin	Vanillin	Glucose	β-Glucosidase
Amygdala amara **(Prunus amygdalus, Rosaceae)**	Amygdalin	Benzaldelhyde	Gentiobiose (2 glucose units) +HCN	Emulsin

These **volatile aglycones** are frequently known as the **"essential oils"** of the plants from which they are obtained, e.g.

- **Methyl salicylate** is known as **oil of wintergreen**
- **Allyl isothiocyanate** as **volatile oil of black mustard**
- **Benzaldehyde** as **volatile oil of bitter almond**

Storage of volatile oils

- Deterioration of essential oils during storage is attributed to changes in their constituents.
- Reactions such as oxidation, polymerization, hydrolysis and interaction of functional groups should be avoided.
- Factors, such as elevation of temperature, oxygen, moisture, light and traces of metals, enhance deterioration.

- **Precautions before and during storage:**

1. Before storage the oil should be freed from any metallic impurities (that may act as catalysts for decomposition reactions).
2. The oils should be dried over anhydrous sodium sulfate to get rid of any traces of moisture.
3. Containers used for storage should be dark colored, completely filled and tightly closed.
4. The oils should be kept, away from light, at low temperature and, if necessary under an inert atmosphere such as CO_2 or N_2 gases.

Chemistry of volatile oils constituents

- The composition of volatile oils is quite complex.
- They include a great variability of constituents that belong mainly to the following groups:
    1. **Terpenoids** (mainly mono- and sesquiterpenoids, derived from acetate).
    2. **Phenyl propanoids** (C_6-C_3, aromatic, derived from shikimic acid).
    3. **Aliphatic compounds** (acyclic, straight chain compounds that are generally degradation products of fatty acids or may be terpenoids).
    4. A number of **miscellaneous compounds** mainly **organo-nitrogen** and **organo-sulfur** compounds.
- Each group includes both **oxygenated** and **non-oxygenated** (or hydrocarbons) members.
- The **oxygenated compounds** are, in most cases, those **responsible for the characteristic odor of the oil**.

Removal of terpenoid hydrocarbons

- Oils rich in terpenoid hydrocarbons are liable to rapid deterioration on storage through **oxidation** and **polymerization** to yield **bad smelling** (generally with turpentine-like odor) and **resinified** products.
- The process of elimination of **terpenoid hydrocarbons** could be considered as a **specific procedure for rectification.** Thus a considerable amount of the **terpenoid hydrocarbons** could be removed by any of the following methods to produce **"terpeneless-oils":**

    1. **Fractional distillation** under reduced pressure; hydrocarbons have lower boiling points than oxygenated compounds and therefore, distill first and are discarded.
    2. **Column chromatography** on silica gel, by eluting hydrocarbons with **n-hexane** then oxygenated compounds with **absolute alcohol**.
    3. **Selective extraction** of the oxygenated components with **dilute alcohol** followed by distillation.

Terpeneless oils are more expensive than **natural oils**, and are characterized by being:

1. Richer in oxygenated compounds.
2. More soluble in low-strength alcohols.
3. Employed in smaller quantities to give the same strength of odor.
4. More stable being less liable to deterioration

Volatile oil isolates

- A volatile oil isolate is **a single chemical substance** isolated from the oil. Isolates may be oxygenated or non-oxygenated (i.e. hydrocarbons).

- **Isolates** are **different** from: **stearoptenes** and **oleoptenes,** which are generally mixtures of several components (rarely single), and from **terpeneless oils** that are mixtures, mostly, formed of oxygenated compounds**.**

Terpenoids

- **Terpenoids** and **steroids** are formed from common precursors, although through different pathways.
- They constitute the largest known group of secondary metabolites.
- They yield **isoprene** as final product of **destructive distillation** (i.e. pyrolysis).
- They were collectively called **"terpenes"**, however, the suffix **–oid** is more logical, the **–ene** suffix should be restricted to the unsaturated hydrocarbons of the class.

Distribution

- Most terpenoids are specific to the vegetable kingdom, but some occur in animals such as the sesquiterpenoid insect pheromones and the juvenile hormones, as well as, the diterpenes of certain marine organisms (e.g. *Spongiae*).
- The volatile low molecular weight terpenoids are the major components of essential oils and oleoresins.
- Plant steroids are represented by different groups of secondary metabolites including cardenolides, steroidal alkamines, saponins and phytosterols.

Biogenesis

- Since1887, isoprene, a five carbon-atom unit, was suggested to be the building unit of all terpenoids. Later on, Ruzicka, in 1953, postulated the following **theoretical biogenetic rule**: "Each group of terpenes arises from the head-to-tail condensation of a variable number of isoprene units".
- **Isoprene** is also known as **isopentene** or **2 methyl-buta-1: 3-diene**.
- **Biogenesis of Isoprene:** The isoprene units arise from **acetate**, via **mevalonate**, and are branched five-carbon units containing two unsaturated bonds.
  - **Triterpenoids (C_{30}, and steroids**) arise from **squalene**, a hydrocarbon produced by the reductive coupling of **two FPP** units (2 x $C_{15} = C_{30}$).
  - **Carotenoids (C_{40})** arise from **phytoene**, a hydrocarbon produced by the reductive coupling of **two GGPP** units (2 x $C_{20} = C_{40}$).
  - **Polyprenols or polyisoprenoids** (rubber and related compounds) are formed of great number of C_5 units **$(C_5H_8)n$.**

- Natural **hemiterpenoids** (C_5) are of rare occurrence (e.g. certain volatile hydrocarbons, free and glycosilated alcohols).
- **Meroterpenoids** are secondary metabolites of **mixed origin**, **their structure** involves **an isoprene moiety**, as in the case of **polyphenols** (e.g. the coumarin, bergapten; the isoflavonoid, rotenone; and, the naphthoquinone, shikonin), some **alkaloids** (e.g. in the ergoline nucleus of ergot alkaloids).

▪ A theoretical approach to represent the coupling of isoprene units in a head-to-tail manner to yield different types of mono- and sesquiterpenoids, and consequently of other regular terpenoids, is shown in **Figure (3)**.

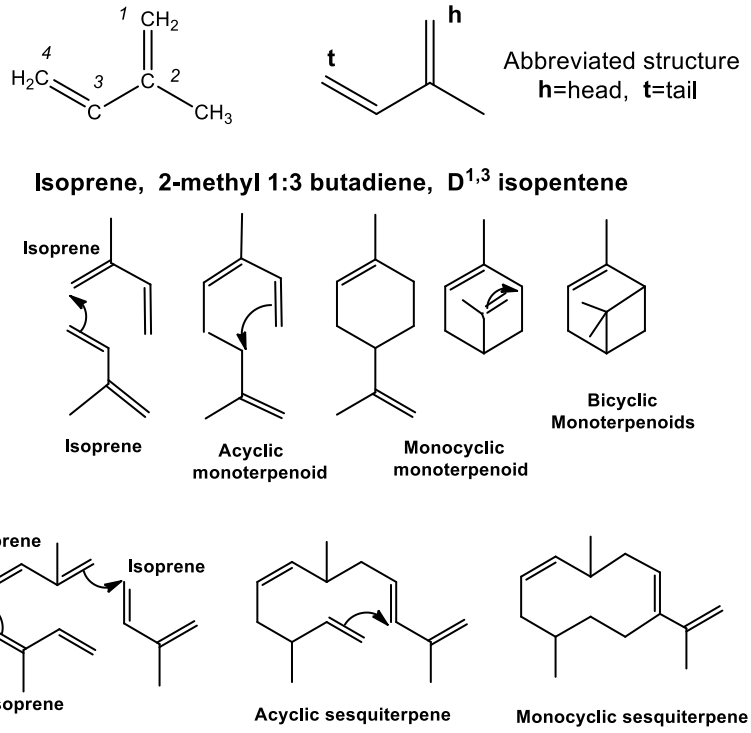


Figure (3) :Coupling of isoprene units to yield different types of mono- and sesquiterpenoids - A theoretical approach.

Terpenoids in essential oils

Thousands of terpenoids have been identified in essential oils.

- These are mainly either **mono- or sesquiterpenoids** (beside certain C_{13} and C_{14} compounds that are **terpene degradation products**).
- They are **volatile** and of relatively low molecular weight.
- They may be **acyclic** (i.e. aliphatic) or **alicyclic** (i.e. contain one or more non-aromatic ring-structures).
- They are generally represented by abbreviated structures in which as many Hs and Cs are removed, only double bonds and functional groups are written in full details.
- They are often **optically active**, the proportion of the two enantiomers usually differ with the plant species e.g. d-linalol is major in coriander, l-linalol in lavender and dl-linalol in passion fruit oil.

Monoterpenoids

- They constitute the most abundant class of the essential oil constituents.
- The **hydrocarbons** of the series are represented by the empirical formula **$C_{10}H_{16}$**.
- They are variable in structure being **acyclic** or **alicyclic** (mainly mono-and bicyclic) compounds.
- The **hydrocarbons** are always present and sometimes amount to 90% of the constituents of the oil such as in the *Citrus* and turpentine oils.
- **Oxygenated monoterpenoids** may be alcohols, aldehydes, ketones, esters, ethers and peroxides.

Sesquiterpenoids

- Different types of sesquiterpenoids have been identified in the **high boiling point** fractions of essential oils (250-280^{o}C).
- They usually are **viscous liquids** or may be **crystalline**.
- They show structural variability such as the monoterpenes. The most common are the hydrocarbons, alcohols and ketones of the series.
- The **hydrocarbons** of this group are represented by the empirical formula **$C_{15}H_{24}$**
- The elongation of the chain (5 carbons more than monoterpenes) increases the number of possibilities for cyclization.
- They may be **acyclic**, **monocyclic** or **polycyclic**.
- Over 100 different skeletons have been described, some with ring size ranging from 4, 7, 8, 10 and 11 carbon atoms.
    - **Azulenes**, **$C_{15}H_{18}$**, are mostly discussed under **sesquiterpenoids because they have the same number of C atoms, and distill in the same boiling range.**
    - However, **they** possess **aromatic properties** due to **high conjugation** and are **highly colored** (Generally blue, green or violet) e.g. Chamazulene.

Phenyl propanoids

- These compounds are of less common occurrence than the terpenoids.
- They contain the C_6 phenyl ring to which is attached a C_3 propane side chain (**C_6 - C_3**).
- Many of the phenyl propanoids found in volatile oils are allyl-and propenyl phenols (e.g. eugenol), or phenol ethers (e.g., anethole, safrole, apiole), and sometimes aldehydes such as cinnamaldehyde.
- In some cases the propane side chain is reduced to give a **C_6-C_1** structure such as in vanillin, methyl salicylate and methyl anthranilate.
- Lactones derived from cinnamic acid (i.e. coumarins) can be steam distilled and, therefore found in certain oils, especially those from Rutaceae.

- Certain **aromatic C_{10} compounds** such as p-cymene, thymol and carvacrol are sometimes described under monoterpenoids.

Examples of phenyl propanoids are shown in **Figure (4)**.

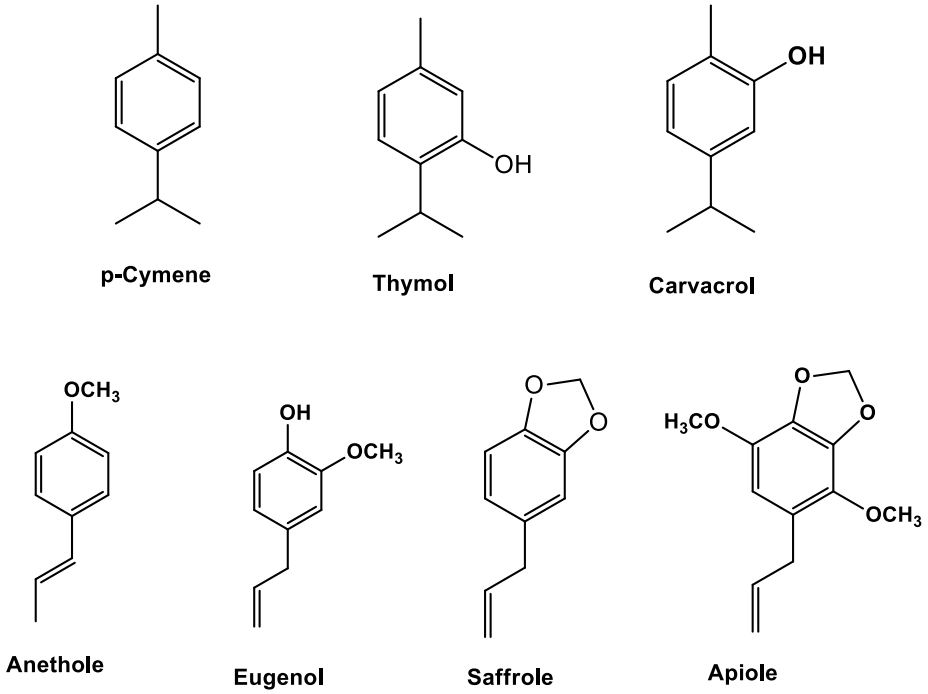


Figure (4): Examples of phenyl propanoids in volatile oils

Hydrocarbons in volatile oils

Hydrocarbons or non-oxygenated constituents detected in volatile oils belong mainly to the following groups:

A. Acyclic monoterpenoids (e.g. myrcene and ocimene) and **sesquiterpenoids** (e.g. farnesene and sesquicitronellene)**.**

B. Alicyclic mono-and sesquiterpenoids, e.g.

- **Monocyclic monoterpenoids**: limonene, terpinene and phellandrene.
- **Bicyclic monoterpenoids**: sabinene and α-pinene.
- **Monocyclic sesquiterpenoids**: zingiberene.
- **Bicyclic sesquiterpenoids**: cadinene (azulenes are sometimes described under this group despite of their aromatic characters).
- **Tricyclic sesquiterpenoids**: santalene.

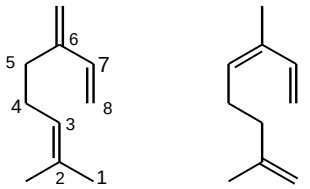
C. Aromatic hydrocarbons e.g. p-cymene and azulenes

A- Acyclic terpenoid hydrocarbons

Monoterpenoids

Myrcene [2-methyl-6-methylene $\Delta^{2:7}$ octadiene]

Ocimene [2,6-dimethyl $\Delta^{1:5:7}$ octatriene]



Myrcene ***Ocimene***

Source

- **Myrcene** occurs in oils of hops (*Humulus lupulus*, Cannabinaceae), bay (*Myrcia acris*, Myrtaceae) and lemon grass (or citronella, *Cympobogon nardus* and other *Cympobogon* spp. , Gramineae) and in oil of turpentine.
- **Ocimene** is present in large amounts in oils of *Ocimum basilicum* and *Ocimum gratissimum* (Lamiaceae) and other *Ocimum* spp.

Isolation

- The distilled oils of bay (for myrcene), and *Ocimum* (for ocimene) are rich in **phenolic** compounds. These are **removed as water-soluble phenates by treatment with NaOH**.
- The **non-phenolic fraction** is then subjected to **fractional distillation** to obtain the individual hydrocarbons.

Test for differentiation

Myrcene and Ocimene, on oxidation, using either $KMnO_4$/acetone or $KMnO_4$ in alkaline medium, give different acids, which can be transformed to lead salts that have different crystal shapes.

- **Myrcene** yields succinic acid that is transformed to the **needle-shaped** lead succinate.
- **Ocimene** on oxidation yields a mixture of low molecular weight acids, which give crystalline **rhomboid-shaped** lead salts.

Biological activity

- **Myrecene** has antibacterial activity against staphylococcus aerus
- **Myrecene** also showed cytotoxic acticity against human hepatocellular carcinoma (HepG2), colon cancer (HCT116) and breast (MCF7).

B- Alicyclic terpenoid hydrocarbons

These represent the major group of terpenoid hydrocarbons detected in essential oils and belong mainly to the groups of:

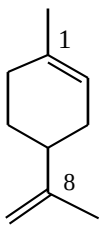
- Mono- and bicyclic monoterpenoids .
- Mono-, bi-, and tricyclic sesquiterpenoids.

I. Monocyclic monoterpenoid hydrocarbons

- The most common of the group are the **p-menthadiene** derivatives (e.g. limonene, dipentene, terpinenes, terpinolenes and phellandrenes).
- The **m-menthadiene** derivatives are of rare occurrence and are mostly degradation products formed during hydrodistillation (e.g sylvestrene and carvestrene).

Para-menthadiene derivatives

Limonene and **Dipentene** ($\Delta^{1:8}$ p-menthadiene)



Limonene & Dipentene

Source

Limonene occurs in d-, l- and dl forms.

- The ***d* (+) form** is widely distributed especially in **citrus oils** e.g. oils of **orange (90%)**, lime, grapefruit, bitter orange, mandarin, bergamot and neroli; it occurs also in **umbelliferous oils** of **dill**, **fennel** and **celery (20%).**
- The ***l* (-) form** is less common, present in oil of **turpentine**, star anise , peppermint , spearmint and cajuput .
- The ***dl* form** (racemic) is known as "**Dipentene**" and occurs in oils of lemon grass, pepper, nutmeg, neroli, fennel and turpentine. It is most probably formed by the action of **heat** in **oils rich** in **limonene** and/or **α-pinene**.

Isolation

Limonene could be isolated from orange oil by:

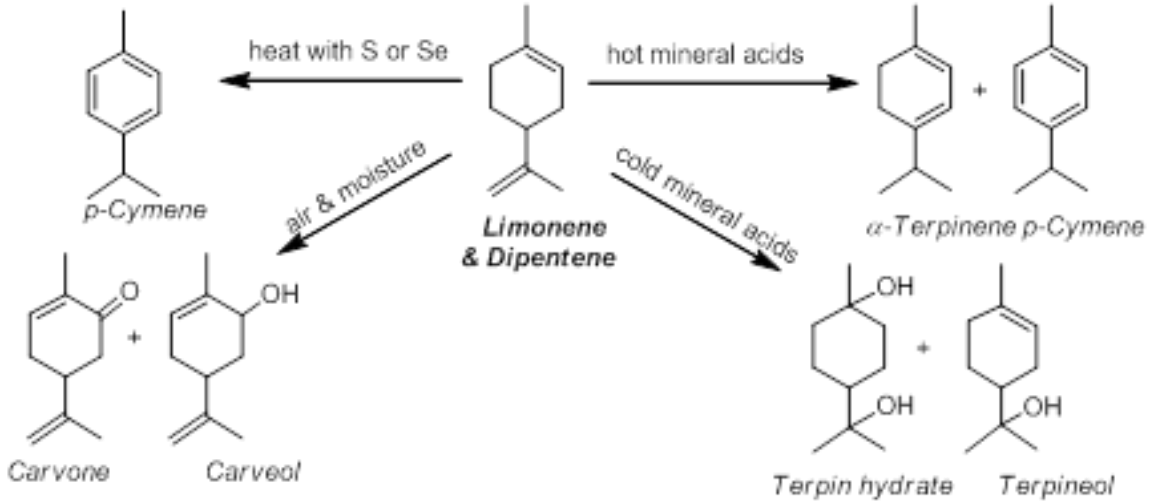
- Fractional distillation of the oil, and collection of the fraction boiling at 176ºC.
- Formation of haloderivatives, e.g. tetrabromide (m.p.104-105ºC) followed by regeneration by treatment with Zn / HAc (debromination).

Properties

- It is an optically active liquid.
- It has an **orange-like** odor.

Stability

- It is quite stable when stored away from light and air.
- Both d-and l- limonene may yield dipentene (dl) by the action of heat or acids.
- **Effect of mineral acids:** Limonene and dipentene yield different products on treatment with mineral acids, according to the dilution of the acid and the conditions of the reaction.



- **Heating with mineral acids** results in **isomerization** and/or **dehydrogenation** to yield other p-menthane derivatives e.g. terpenene and/or p-cymene.
- **Treatment with cold dilute mineral acids** results in **hydration** with formation of mono- and/or dihydric alcohols (terpineol and/or terpin hydrate). Careful hydration yields only terpineol.

- **Heating** of limonene in presence of **S or Se** results in **dehydrogenation** to the aromatic hydrocarbon p-cymene.
- **Limonene on exposure to air and moisture** is transformed, by **auto-oxidation**, to a mixture of **carvone** and **carveol**. This explains the **caraway-like** odor of badly stored orange oils.

  Similarly, **all terpenoid hydrocarbons** are greatly **affected** by exposure to **moisture**, **air**, **heat**, **acids** and **S** or **Se** yielding different types of hydrocarbons or oxygenated compounds.

Uses and biological activity:

Limonene and **dipentene** are widely used as:

- Flavoring agents for cosmetics, soaps and pharmaceuticals.
- Substitutes for preparation of orange oil.
- **Limonene** has antioxidant and chemopreventive activity.
- **Limonene** also possessed anxiolytic effects in a mouse maze model were comparable to diazepam.
- It has anti-inflammatory effects in models of osteoarthritis and asthma.

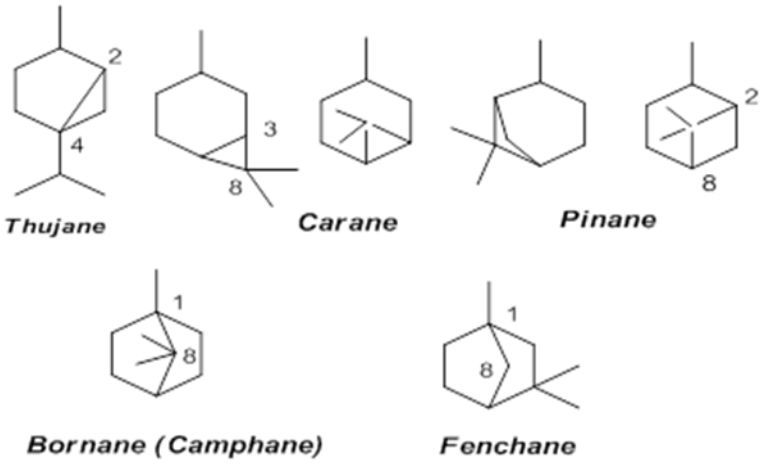
ll. Bicyclic Monoterpenoid Hydrocarbons

Characters

- Their structures show two fused 3-, 4-, 5- or 6-membered rings and one double bond.
- They are conveniently classified into 5 major groups according to the parent saturated hydrocarbons from which they are derived viz., thujane, carane, pinane, camphane and fenchane.
- They have a tendency to undergo:
  - Molecular rearrangement (e.g. from pinane to camphane),
  - Shifting of the double bond,
  - Oxidation, hydrogenation and dehydrogenation,
  - Opening of the rings on treatment with acids to yield monocyclic monoterpenoids.

Nomenclature

They are named after cyclohexane with indication of the site of ring fusion, or after the parent saturated hydrocarbons



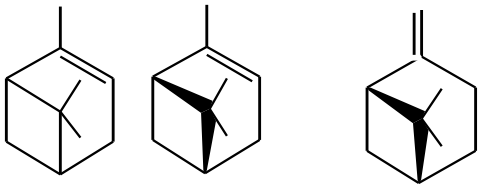
Thujane *Carane* *Pinane*

Bornane (Camphane) *Fenchane*

Kind of rings	Saturated hydrocarbon	Site of ring fusion
3- + 6-membered rings	Thujane & Carane	C_2 and C_4
4- + 6-membered rings	Pinane	C_2 and C_8
5- + 6-membered rings	Camphane or Bornane	C_1 and C_8

Pinane derivatives

α- and *β*-Pinenes



(+) α–pinene ***(+) β–pinene***

Source

- ***α*-Pinene** is widely distributed in volatile oils.
- It is the main constituent of **oil of turpentine** and most coniferous oils and is also present in oils of lemon, coriander, American peppermint and cumin.

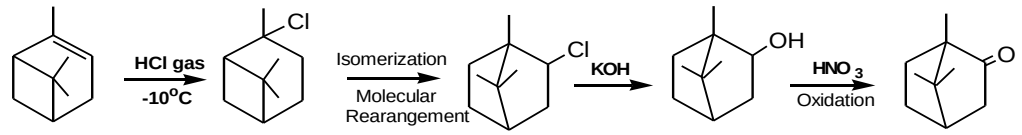
Isolation

It is obtained from oil of turpentine by either:

- **Fractional distillation** of oil of turpentine, and collection of the fraction containing α-pinene, or
- **Precipitation** as its **crystalline nitroso-chloride** (with **Tilden's reagent**), from which it is regenerated by treatment with aniline.

Properties

- **α-Pinene** is a liquid that easily polymerizes on exposure to air.
- It is optically active:
  - The ***l*-form** is the main component of **French oil of turpentine**.
  - The ***d*-form** is that of **American oil of turpentine**.
- It is used as **starting material for semi-synthesis of different compounds** including camphor, borneol and terpineol.



α-Pinene — *Pinene hydrochloride* — *Bornyl chloride* — *Borneol* — *Camphor*

Semi-synthesis of borneol and camphor form α-pinene

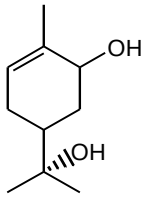
- Molecular rearrangement occurs from pinane to camphane structure.
- Any trace of chloride in a provided sample of borneol or camphor thus indicates that it is obtained by synthesis (HCl being used in the preparation).
- Synthetic borneol and camphor are racemic.

Uses

- It serves as raw material for manufacture of borneol, camphor, terpineol, insecticides and plasticizers.
- It is used for Rheumatism as a liniment and is best known by Aromatherapists
- A tonic of the mucus membranes of the respiratory system.
- Pinene is also important for its pleasant fragrance and is believed to have diuretic properties.

Derivatives of pharmaceutical importance

Sobrerol® or **pinol hydrate**, used as **mucolytic** in cough mixtures is formed by **auto-oxidation of α-pinene** in the presence of water and especially in sunlight.



Pinol hydrate

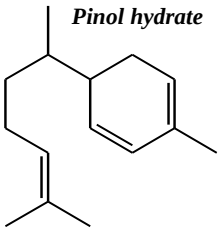
III. Monocyclic sesquiterpenoid hydrocarbons

Zingiberene

Source

Zingiberene is the main constituent of ginger oil

(*Zingiber officinalis*, Zingiberaceae)



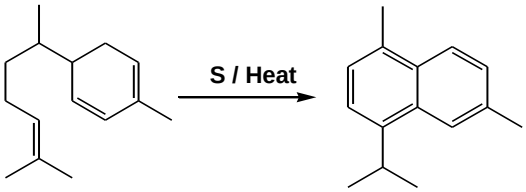
Zingiberene

Isolation

It is obtained from ginger oil by **fractional distillation under vacuum** (due to its high boiling point).

Properties

- It is a colorless oily liquid that easily resinifies on storing.
- When **dehydrogenated with sulfur**, the aromatic naphthalene hydrocarbon, **cadalene**, is obtained (cyclization and dehydrogenation)



Zingiberene ***Cadalene***

Biological activity:

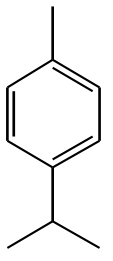
- Help fight infections caused by viruses.
- Protect against stomach ulcers.
- Ease the pain and discomfort caused by stomach gas (as a carminative herbal compound)

C. Aromatic Hydrocarbons

Para-cymene (or **Cymol)**

Source

- This compound is **widely spread** in essential oils such as those of lemon, sage, thyme, origanum, lavender, nutmeg, cinnamon etc…



p-Cymene

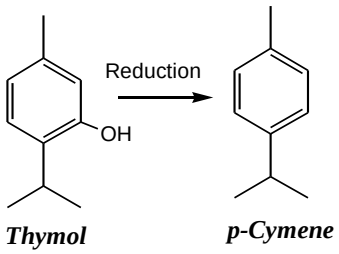
- It is **probably** an **artifact** formed **during hydrodistillation** by cyclization, dehydrogenation or reduction of monoterpenoids.

Isolation

- The distilled oil is subjected to fractional distillation.
- **The fraction containing p-cymene contains a mixture of cineole and terpenoid hydrocarbons of boiling points close to p-cymene.**
- **The terpenoid hydrocarbons are removed by oxidation with cold dilute $KMnO_4$ (p-cymene is stable under these conditions).**
- **Cineole is removed by formation of its crystalline hydrobromide.**

Semi-synthesis

- **From monoterpenoids,** which can easily be transformed to p-cymene e.g.
  - **Cyclic monoterpenoid hydrocarbons** e.g. limonene, pinene and terpenene yield p-cymene on dehydrogenation by heating in presence of S or Se.
  - **Oxygenated monoterpenoids** e.g. carvone and citral yield p-cymene on dehydrogenation and / or cyclization.
- **From thymol** by reduction.



Thymol ***p-Cymene***

Uses

It is commonly used for perfuming soaps and all kinds of technical preparations and is an important constituent in imitation essential oils.

Biological activity

- **p-Cymene** has antifungal, antiviral, antimicrobial, insecticide effect on the Aedes aegypti, antinociceptive and anti-inflammatory.
- **p-Cymene** showed an effect on leukocyte chemotactic behavior, inhibiting rolling and adherent leukocytes modulated by cytokines, responsible for regulating these effects. Thus p-Cymene could be considered a promising drug as an anti-inflammatory agent in the processes that take place in leukocyte infiltration, such as rheumatoid arthritis

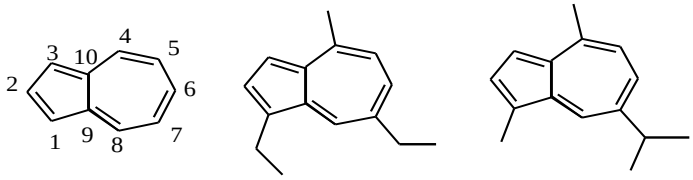
Azulene derivatives

Properties

- These are **colored** hydrocarbons (**blue**, **violet** or **green**); present in oils of cubebs , valerian, galbanum , geranium and chamomile etc …
- They mostly occur as **colorless precursors** called **pro-azulenes** (generally sesquiterpenoid lactones), which are decomposed by physical or chemical means, such as heating or treatment with acids, to give colored azulenes.
- They are named after the plants in which they occur e.g. **chamazulene** from chamomile,**vetivazulene** from vetiver oil, **guaiazulene** from oil of guaiacum, etc…
- Most are crystalline with melting points ranging from 30-100ºC.

Chemical structure

- They are **highly conjugated** compounds (therefore, **colored**) having certain aromatic properties.
- The parent hydrocarbon is **azulene**, $\mathbf{C_{10}H_8}$. It is formed of a five- and a seven- membered ring that are fused together.
- The naturally occurring azulenes are substituted with alkyl groups and are $\mathbf{C_{15}}$ compounds. In this respect, they are closely related to sesquiterpenoids.
- They are represented by the empirical formula $\mathbf{C_{15}H_{18}}$.

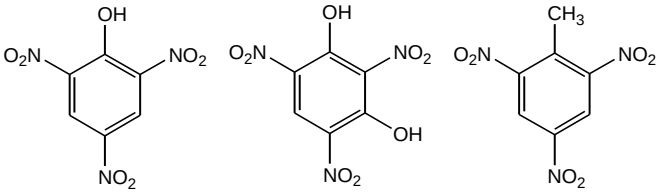


Azulene ***Chamazulene*** ***Guaiazulene***

Isolation and identification

These are carried through adduct formation or derivatization. Azulenes form a large number of adducts with:

- **Strong mineral acids** e.g. concentrated H_3PO_4 and H_2SO_4, which can be **decomposed with water** with recovery of the parent azulene derivative.
- **Ferrocyanic acid** $[H_4Fe(CN)_6]$, they give the corresponding ferrocyanates, which are **decomposed with water** .
- **Nitrocompounds** e.g. picric, styphnic and tortylic acids, they form derivatives with sharp melting points that can be **decomposed by NaOH**



Picric acid ***Styphnic acid*** ***Tortylic acid***

Pharmacological action and uses

They exert an **anti-inflammatory** effect and were believed to possess anti-ulcerative activities.

Guaiazulene (1,4-dimethyl-7-isopropyl azulene)

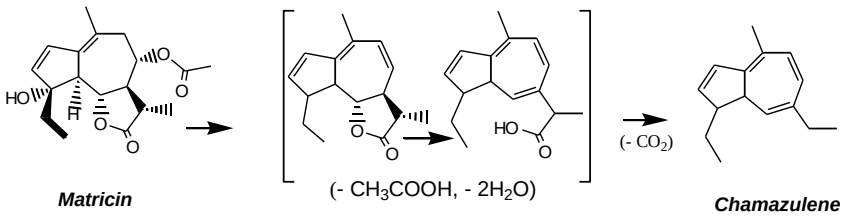
Source

It is a constituent of **geranium oil** and could be, also, obtained from **dehydrogenated guaiacum wood oil**.

Chamazulene (4-methyl-1, 7-diethyl or 1-methyl-4, 7-diethyl azulene)

Source

- It occurs in the **hydrodistilled oil of chamomile** (1-15%) and is responsible of its blue color.
- It arises from the decomposition of the sesquiterpenoid lactone, **matricin** (prochamazulene).



Matricin ***Chamazulene***

Uses

- Chamazulene is widely used in cosmetic preparations, as **anti-inflammatory** and **mild antiulcerative**.
- The volatile oil of chamomile is used in **GIT** disturbances and has **spasmolytic** properties.
- The **ulcer protective properties** are mainly **attributed to** its **bisabolol** and **bisabolol oxide** content.

Oxygenated Compounds

Alcohols in volatile oils

- Alcohols are usually present in essential oils together with their esters.
- They are mostly **terpenoids** or **aromatic** in nature, some arise from fatty acid degradation (e.g. hexenol).
- They may be produced as artifacts during hydrodistillation due to hydrolysis of the corresponding esters.

Classification

- They may be classified according to the type of alcoholic group present into **primary** (RCH_2OH), **secondary** (R_2CHOH) and **tertiary** (R_3COH) alcohols.

- They can be classified according to their carbon skeleton into **aliphatic**, **alicyclic** and **aromatic** alcohols.

- **Aliphatic alcohols** detected in essential oils are either:
  - **Saturated** e.g. methyl and ethyl alcohol (1ry alcohols), or
  - **Unsaturated** (generally acyclic **mono**- and **sesquiterpenoids**) such as the **primary** alcohols: citronellol, geraniol, nerol and farnesol, and the **tertiary** alcohols: linalol and nerolidol.

- **Alicyclic terpenoid alcohols** are represented by:
  - **Monocyclic monoterpenoids** such as menthol (2ry alcohol) and terpineol (3ry alcohol).
  - **Bicyclic monoterpenoids** such as borneol and isoborneol (2ry alcohols).
  - **Bicyclic sesquiterpenoids** such as carotol (3ry alcohol).
  - **Tricyclic sesquiterpenoids** e.g. santalol (1ry alcohol).

- **Aromatic alcohols** of common occurrence are the primary alcohols: benzyl, phenyl ethyl and cinnamyl alcohols.

Isolation of terpenoid alcohols

They can be isolated from the corresponding oils by one of the following methods:

- Fractional distillation.
- Counter current extraction.
- Chromatography.
- Derivatization.

Determination of alcohol content in volatile oils

I. General method

- This is based on **acetylation** of the oil sample using acetic anhydride, and **determination of the ester value of the oil before and after acetylation**. The alcohol content is then obtained by calculation.
- The method is suitable for determination of **primary** and **secondary** alcohols.
- The major **drawbacks** of the method are:
  1. **Tertiary alcohols** do not react quantitatively as they are **easily dehydrated**.
  2. Certain aldehydes, ketones and phenols are converted to compounds that can be acetylated.

II. Determination of primary alcohols.

- Oils containing **primary alcohols** are refluxed with **phthalic anhydride at 100 ºC**, to yield the corresponding acid phthalates. The **excess phthalic anhydride** is back titrated against **standard alkali**.
- **Secondary alcohols** react under **more drastic conditions**.

III. Determination of tertiary alcohols.

- **Modification of the acetylation method:**

  Tertiary alcohols undergo partial or complete break down and **dehydration** when treated with acetic anhydride. This is overcome by **dilution of the reaction mixture with xylene** or **oil of turpentine** to decrease the dehydrating effect of acetic anhydride.

- **Dehydration method:**

  This is carried by catalytic dehydration of tertiary alcohols using $ZnCl_2$ or I_2, followed by determination of the amount of water released from the reaction, which is equivalent to the amount of tertiary alcohol present

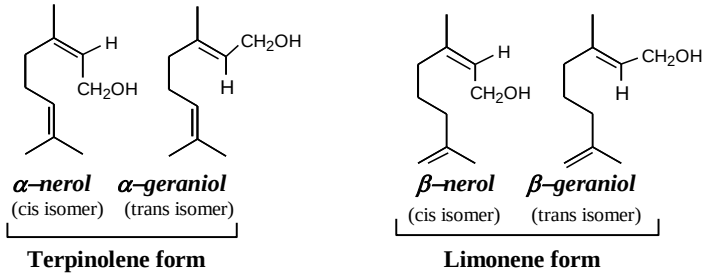
IV. Special method for determination of citronellol (formylation)

- **Most** of the **terpene alcohols** are **dehydrated** by **strong formic acid (100%)** i.e. they are not esterified.
- On the other hand, **only citronellol** resists dehydration and is quantitatively **converted** to the corresponding **formate**.

A. Acyclic Terpenoid Alcohols

The most common are the **monoterpenoids**: citronellol, geraniol, nerol and linalol, and the **sesquiterpenoids**: farnesol and nerolidol.

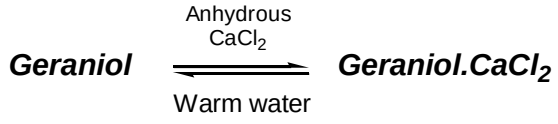
Geraniol and **Nerol**



Source

- **Geraniol and its esters** are present in oils of palmerosa (95 %), **geranium** (40 - 50 %), citronella (30 - 40 %) and **rose** etc…
- **Nerol and its esters** are obtained from oils of neroli, petitgrain, bergamot, and generally occur together with geraniol and its esters.

Isolation

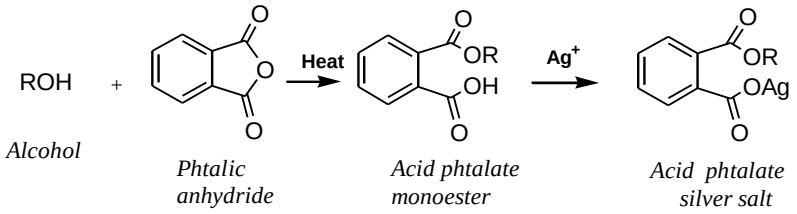
- **Geraniol** is isolated through formation of the crystalline calcium chloride derivative ($2C_{10}H_{18}O.\ CaCl_2$) as follows:
  - Addition of **anhydrous $CaCl_2$** to the oil containing geraniol.
  - Extraction with organic solvent (ether, benzene or chloroform) [to remove constituents other than geraniol].
  - Washing of the deposited adduct with **warm water** [to decompose the complex and regenerate geraniol].
  - Steam distillation of the liberated geraniol.
  - 

- **Nerol** is difficult to be obtained in a pure form.

Separation of geraniol from other alcohols

Geraniol is usually present in oils together with the closely related **nerol** and **citronellol** from which it can be separated through formation of crystalline derivatives such as:

- The **calcium chloride additive compound** that is separated and geraniol regenerated by warming with water.
- The **acid phthalate silver salts that** allow the separation of geraniol from **citronellol** through fractional crystallization of the derivatives produced.



Properties

- Both **nerol** and **geraniol** are colorless, optically **inactive** (c.f. citronellol and linalol) liquids with **rose-like odor** (similar to citronellol).

- Due to the **higher degree of unsaturation** (two double bonds), **geraniol** and **nerol** are **reactive** and consequently **more susceptible to degradation.**

- They are sensitive to **mineral acids** and **dehydrating agents**:
  - In presence of **cold dilute mineral acids** (or any traces of acidity), they are converted to the **monocyclic alcohols** terpineol and/or terpin hydrate.

$$\textit{Geraniol} \xrightarrow{\text{Cold dilute mineral acids}} \textit{Terpin hydrate and / or terpineol}$$

  - **Dehydration** by either treatment with **concentrated mineral acids** (e.g. HCl gas, HCOOH and H_3PO_4), or **heating with K bisulfate** results in production of a mixture of isomeric **p-menthadiene hydrocarbons** (e.g. limonene and / or terpinene).

$$\textit{Geraniol} \xrightarrow{\text{Heat with dehydrating agent}} \textit{Dipentene and / or other terpenoid hydrocarbons}$$

Uses

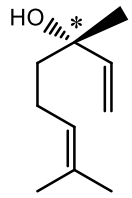
- **Geraniol** and its esters are extensively used in perfume, cosmetic and soap industries.

Biological activity

- **Geraniol** was evidenced to have therapeutic and preventive effects on differenttypes of cancer, such as breast, lung, colon, prostate, pancreatic and hepatic cancer.
- **Geraniol** also sensitizes tumour cells to the commonly used chemotherapy agents.

Linalool

Source



(+)- Linalool

Linalool is a common component of essential oils. It occurs either free (in d- and l- isomers) or in the form of esters, especially the acetate.

- ***d*- form** : oils of bois de rose (rose wood oil), nutmeg , sweet orange etc…
- ***l*- form** : oils of **lavender** , bergamot , neroli , lemon , rose etc…
- **Linalyl acetate**: oils of lavender, bergamot etc….

Properties

- It is an **optically active** liquid with a characteristic **lavender-like odor**.
- Being a tertiary alcohol, it is easily dehydrated to yield monoterpenoid hydrocarbons.
- **Treatment with acids** gives different products according to the reaction conditions and the strength of the acid:
  - **Traces of acids** → isomerization to **geraniol** (1ry alcohol).
  - Treatment with **cold dilute H_2SO_4** → cyclization and hydration to give **terpin hydrate.**
  - Heating with **glacial acetic acid** → mixture of the **acyclic** and **monocyclic monoterpenoid alcohols**, geraniol, nerol and terpineol.
  - Treatment with **chromic acid** → isomerization followed by oxidation to the aldehyde, **citral**.
  - Treatment with **halogen acids** yields the corresponding **halides** that can be used for identification (e.g. linalyl chloride and linalyl bromide).

The rapid **degradation** of linalool is due to the **presence of two double bonds and a tertiary alcoholic group.**

Uses and biological activity

- **Linalool and its acetate ester** are widely used in perfume, cosmetics, soap and flavor industries to impart a **lavender-like odor**.
- **linalool** and the corresponding **acetate** play a major role in the anti-inflammatory activity.
- **linalool** possesses anticonvulsant effect which can be attributed to both an inhibition of potassium-stimulated glutamate release in cortical synaptosomes, and antagonism of NMDA receptors. This effect deserves further investigation of using linalool as a strategy for antiepileptic-drug development.

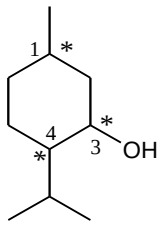
B. Alicyclic terpenoid alcohols

I. Monocyclic monoterpenoid alcohols

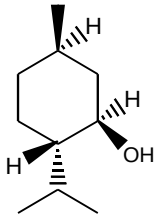
Menthol (Para-menthane-3-ol)

Structure

- The structure of menthol show **3 centers of asymmetry**, the compound is **optically active**.
- It has **4 racemates** (menthol, isomenthol, neomenthol and isoneomenthol) and $2^3 = 8$ optically active isomers (i.e. the d- and l- forms of each racemate).



Menthol



(-)- Menthol

Source

The most common isomer is **l-menthol** which occurs in large amounts in **oil of peppermint** (*Mentha piperita,* Labiatae or Lamiaceae) 60 - 65 %, and in **Japanese mint oil** (*Mentha arvensis*) up to 75 -90 %.

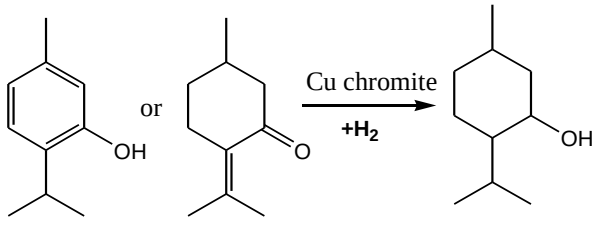
Isolation

It is obtained from **Japanese mint oil** (75 - 90 %) by **successive cooling** and collection of the deposited crop of crystals, as follows:

- The oil is first cooled to + 15 ^oC then centrifuged to separate the deposited crystals (1^{st} crop).
- The process is repeated by cooling at + 5 ^oC then -10 ^oC and collecting the deposits after centrifugation (2^{nd} and 3^{rd} crops).
- The remaining oil contains about 40-50 % menthol and a large amount of menthone that hinders crystallization on further cooling. Therefore, most of the **menthone** present is **removed through formation of its oxime** and shaking with dilute H_2SO_4.
- The ketone-free oil is then cooled again to obtain the residual menthol.

Synthesis

The synthetic dl-menthol could be obtained by catalytic reduction of thymol or pulegone as follows:



Thymol ***Pulegone*** ***Menthol***

Properties

- **Menthol** occurs as hexagonal needle prisms (m.p. 42-43 ^{o}C). It has a powerful **peppermint-like odor** and a **cooling taste**.
- It is affected by dehydrating, oxidizing and reducing reagents:
  - **Dehydration** by $KHSO_4$ or $ZnCl_2$ $\rightarrow \Delta^3$p-menthene, which on dehydrogenation $\rightarrow$ p-cymene.
  - **Oxidation** with $K_2Cr_2O_7 \rightarrow$ menthone with loss of one center of asymmetry.
  - **Reduction** with HI $\rightarrow$ p-menthane.

Tests for identification

- Crystals + few drops concentrated **H_2SO_4** + few drops of **Vanillin/H_2SO_4** $\rightarrow$ **orange-yellow** color + few drops of **H_2O** $\rightarrow$ **violet** color.
- Crystals + glacial acetic acid + 3 drops of concentrated H_2SO_4 + 1 drop of concentrated $HNO_3 \rightarrow$ no green or bluish color is produced (c.f. thymol which gives a green color).

Pharmacological action and uses

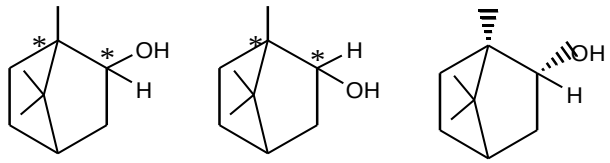
- **Local action**: menthol is used as **antipruritic**, **counter irritant**, mild **local anesthetic,** antibacterial, antifungal, anticancer and analgesic effects. In addition, menthol is one of the most effective terpenes used to enhance the dermal penetration of pharmaceuticals.

- **Systemic action**: It exerts a **depressant effect on the heart**, and is used as **carminative** and **gastric sedative**.

- Menthol is used as **flavoring agent** in mouthwashes and toothpastes, as well as, in candies, chewing gums and cigarettes.

II. Bicyclic monoterpenoid alcohols

Borneol and **isoborneol**



Borneol ***Isoborneol*** ***(-)-Borneol***

Source

- **Borneol** occurs as *d*- or *l*- isomer free or as ester mainly the acetate.
- **Isoborneol** is not a natural constituent but probably an artefact during preparation of oils rich in borneol.
- ***d*-Borneol** (or Borneo camphor) occurs in oils of *Dryobalanops camphora* and in that of *D. longifolia* , and also in oils of nutmeg , rosemary , lavender, olibanum etc…
- ***l*- Borneol** occurs in oils of citronella, coriander, valarian root and *Pinus pallustris.*
- **Bornyl acetate** is the main constituent of pine needle oils (up to 40 %).

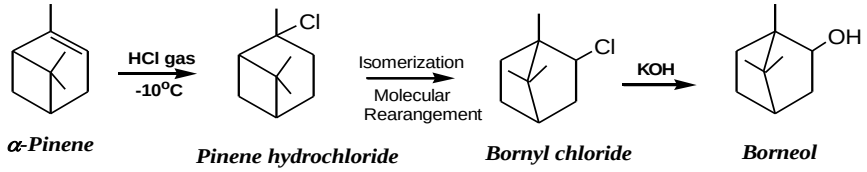
Isolation

- The distilled pine needle oil is saponified (hydrolysis of esters) then subjected to fractional distillation (removal of hydrocarbons).
- The fraction boiling between 205-215 ^{o}C is cooled where upon crystals of borneol are deposited.
- The remaining borneol is isolated as its acid phthalate.

Semi-synthesis

Borneol can be obtained by semi-synthesis from α- pinene and camphor.

- **From α-pinene:**



From camphor: By **reduction** in presence of catalyst, or by using sodium amalgam in alcohol.

Purification

Borneol usually occurs in combination with **isoborneol** and **camphor** from which it should be separated.

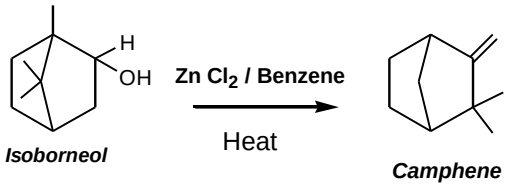
- **Purification from camphor**

This can be performed by one of the following methods:

1. Heating Borneol with phthalic or succinic anhydride for several hours to yield borneol acid phthalate or borneol acid succinate which when treated with aqueous NaOH are transformed to the corresponding Na salts that are soluble in water. Camphor is then extracted with organic solvents.
2. Camphor could be removed from the mixture through formation of its oxime which is separated by shaking with dilute H_2SO_4.

- **Purification from isoborneol**

  This depends on that **isoborneol is more easily dehydrated to camphene** hydrocarbon than borneol. The hydrocarbon is then removed from the reaction medium by fractional distillation.



Isoborneol ***Camphene***

Uses

- Borneol is used for scenting all kind of technical preparations such as room sprays, inhalants, soaps etc…

Biological activity

- Borneol and its different esters showed anti-proliferative activity against seven human tumor cell lines [breast (MCF7), ovarian (NCI-ADR/RES, OVCAR-03), renal (786-0), colon (HT-29) and leukemia (K-562)].
- Borneol also showed antinociceptive and anti-inflammatory activities in mice.

Derivatives of pharmaceutical importance

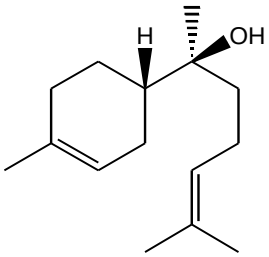
- ***d*-Bornyl isovalerate**: sedative.
- ***d*-Bornyl α- bromoisovalerate**: sedative and hypnotic.
- **Bornyl chloride**: antiseptic.
- **Bornyl salicylate**: counter irritant.

Separation of a mixture of α- pinene, borneol, and camphor

- **α-pinene** is separated from the mixture by **fractional distillation**, or column chromatography and elution with non polar solvent.
- **Camphor** is then separated from borneol and isoborneol by formation of its **oxime**.
- **Borneol** can be removed by formation of **its acid phthalate ester**.

III. Monocyclic sesquiterpenoid alcohols

α -Bisabolol



(-)α-Bisabolol

Source

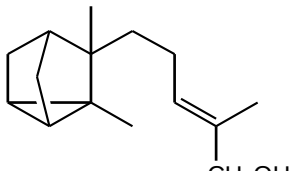
- Four optically active isomers of bisabolol are possible. **(-) α-Bisabolol** is the most common.
- It is **a monocyclic tertiary alcohol** that constitutes together with bisabolol oxides the major components (about 50%) of the volatile oil of the flowers of German chamomile (*Matricaria chamomilla*).

Pharmacological effect

The **bisabolol type constituents** were found **responsible** of the **ulcer-protective properties** of **chamomile**.

IV. Tricyclic sesquiterpenoid alcohols

α-Santalol



α–Santalol

Source

α-Santalol is the main constituent of East-Indian sandal wood oil

Isolation

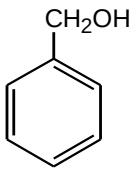
Sandal wood oil is first **treated with KOH** (to remove phenolic constituents), then subjected to repeated **fractional distillation** to get rid of admixed β-santalol which has a close boiling point.

Uses and Biological activity

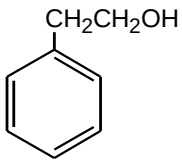
- Medicinal and odoriferous values of sandalwood are due to the santalol content of its essential oil.
- alpha-Santalol exerts antitumour and cancer preventive properties which involves cell death induction through apoptosis and cell cycle arrest in various cancer models.
- A marked decrease in inflammatory markers have also been shown with alpha-santalol administration in skin tissue models.
- It also showed antihyperglycemic and antioxidant activity in mice.

C. Aromatic alcohols

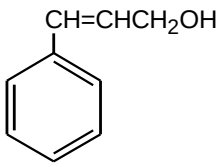
The aromatic alcohols of common occurrence in volatile oils are benzyl, phenyl ethyl, and cinnamyl alcohols.



Benzyl alcohol



Phenyl ethyl alcohol



Cinnamyl alcohol

Phenols and phenolic ethers in volatile oils

- Most of these compounds possess **powerful aromatic odors** and flavors, which make oils such as, clove, anise and sassafras etc … very popular.
- They are responsible for the **antiseptic** and **germicidal properties** of certain oils.
- They constitute the largest group of **phenyl propanoid** constituents detected in volatile oils.

Properties of phenols and phenolic ethers

- **Phenols** are **acidic**, **unstable** and **soluble in alkalis**.

1. They react with **Br_2** with liberation of HBr (substitution) and formation of crystalline **bromoderivatives**, which are used for identification.

2. They react with dilute **$FeCl_3$** solution giving highly **colored compounds**, **generally violet**.

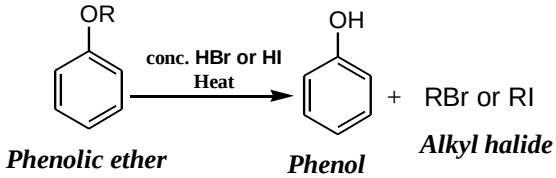
3. They condense with **phthalic anhydride** to yield **phthaleins**. They are colorless crystalline compounds insoluble in water, soluble in alkalis to form deep red solutions.

4. They form esters, as alcohols, with for e.g. acetic anhydride, phenyl isocyanate, benzoyl chloride, p–nitrobenzoyl chloride, etc…

- **Phenolic ethers** are **very stable**, **neutral** compounds, sparingly soluble in water and **insoluble in alkalis** (c.f. phenols).

1. They do not react with alkalis.

2. When treated with hot HBr or HI phenolic ethers are converted to the corresponding phenols with liberation of the alkyl halides.



3. They are identified by conversion to the corresponding phenols, or by complete **bromination**, **nitration** or **sulfonation** (similar to any aromatic compound).

Isomerisation and oxidation of phenols and phenolic ethers

Most of the commonly occurring compounds of this group contain a C_3 side chain, which is either an **allyl** or a **propenyl** group yielding two series of isomeric compounds **(Figure 8)**. Transformation of one isomer to the other or oxidation of either of the allyl or propenyl group allows the use of this group of compounds for synthesis of other more valuable odoriferous products.

- **Isomerisation** (or conversion) of an **allyl-substituted isomer** to the corresponding **propenyl-substituted** isomer is carried by **heating** the compound with **alcoholic alkalis**.

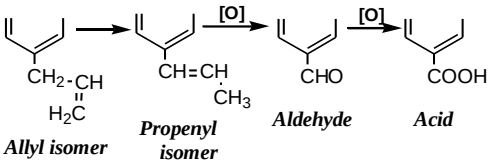
Allyl substituted isomer $\xrightarrow{\text{Heat / Alcoholic KOH}}$ ***Propenyl substituted isomer***

For example:

Eugenol	→	***Isoeugenol***
Safrole	→	***Isosaffrole***
Myristicin	→	***Isomyristicin***
Apiole	→	***Isoapiole***

- **Oxidation** of any allyl or propenyl substituted **phenol** or **phenolic ether** with a suitable oxidizing agent such as **$K_2Cr_2O_7$ / dilute H_2SO_4 or $KMnO_4$** yields the corresponding **aldehydes,** which on further oxidation are transformed to the corresponding **acids**.

Anethole	→	***Anisaldehyde***
Eugenol	→	***Vanillin***
Safrole	→	***Heliotropin***
Myristicin	→	***Myristicin aldehyd***
Apiole	→	***Apiole aldehyde***



General method of isolation of phenols

The isolation of phenols is based on that they are slightly acidic **and can form water-soluble salts, known as** "phenolates" **or** "phenates" **with dilute alkali solutions.**

This can be performed by:

1. Shaking the oil with dilute aqueous alkali e.g. 5% KOH.
2. Separation of the aqueous layer containing the phenate.
3. Acidifying the aqueous solution to regenerate the phenol, which is separated either through extraction with ether or steam distillation.

Salts of certain phenols such as **thymol** and **carvacrol** dissociate easily and therefore, they **could be distilled without acidification.**

Determination of phenols and phenolic ethers

Phenols

- Large amounts are determined through formation of phenates by treatment of the corresponding oils with alkalis.
- Phenates are soluble in water, the potassium salts are more soluble than the corresponding sodium salts.
- The non-phenolic portion separates as an upper layer, its volume is determined (cassia flask) and the amount of phenol is calculated by difference (% v / v).

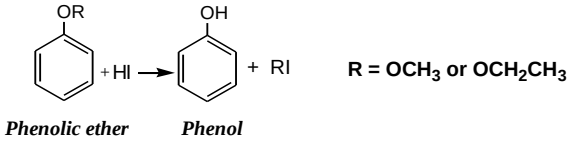
Disadvantages:

- Alkali-soluble compounds (e.g. acids) or water-soluble adulterants (e.g. alcohols) are calculated as phenols.
- The method is unsuitable for determination of small amounts.
- The phenolate solution can dissolve a part of the non-phenolic portion of the oil.

Phenolic ethers

Methyl and **ethyl ethers** are determined by using **Zeisel's method** that involves estimation of the amount of $-OCH_3$ or $-OCH_2CH_3$ groups that is equivalent to the amount of phenolic ether present.

- The phenolic ether-containing oil is treated with **hydrogen iodide** and is transformed to the corresponding phenol with release of an equivalent amount of **alkyl iodide**.
- The released alkyl (methyl or ethyl) iodide is received in a solution of **silver nitrate** in ethanol upon which an equivalent amount of silver iodide is precipitated, separated and weighed.
- The amount of silver iodide that precipitates is equivalent to the phenolic ether present.



Classification

Phenols and phenolic ethers of common occurrence in volatile oils are usually classified **according to the number of phenolic and/or ether groups** present in their molecules into: **Mono-, di-, tri- and tetrahydric** compounds.

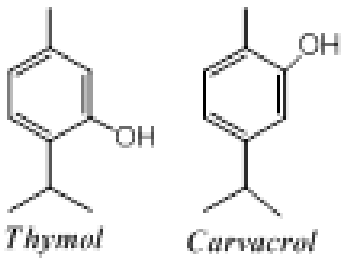
I. Monohydric phenols and phenolic ethers

Examples of this group are thymol, carvacrol, anisole, estragole (methyl chavicole) and anethole

Thymol (3-hydroxy *p*-cymene) and **Carvacrol** (2-hydroxy *p*-cymene)

Source

Thymol and carvacrol are the predominant constituents of several oils of family Lamiaceae (Labiatae). They occur in the oils of thyme (*Thymus vulgaris* and *T. zygis*), ajowan and several species of *Ocimum*.



Isolation

Thymol can be separated by any of the following methods:

1. Cooling of the oil upon which it crystallizes out.
2. Fractional distillation of the oil followed by cooling.
3. Extraction of the oil with dilute aqueous alkali, followed by separation of the aqueous layer, acidification and extraction with ether or steam distillation.

Properties

Thymol is a crystalline solid, with **thyme – like odor**.

- Alcoholic solution of thymol gives no color with alcoholic ferric chloride (c.f. carvacrol which gives a green color).
- **Thymol** reacts with **$FeCl_3$** only after addition of **concentrated H_2SO_4** or if dissolved in concentrated H_2SO_4. The reaction is used to detect traces of carvacrol in thymol.

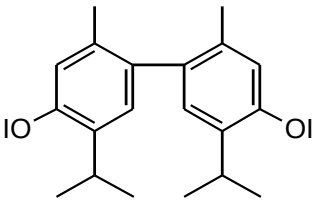
- Thymol + **10% NaOH,** heat on water bath a colorless or pale red solution is obtained that darkens on standing. On shaking with **$CHCl_3$** a **violet** color is produced in the chloroform layer. This test forms the base of a general method for colorimetric assay, general of phenols.

Uses

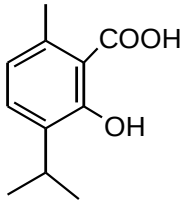
- **Disinfectant**, **antibacterial** used in antiseptic mixtures applied to mucous cavities such as gargles.
- **Local anaesthetic in tooth ache** (dental analgesic).
- **Gastro intestinal disinfectant** in fermentative gastritis, enteritis etc …
- **Anthelmintic** in case of infestation with nematodes.
- **Preservative** in food and drug industries (destroying molds).

Both the essential oil of **thyme** and **thymol** are ingredients of various proprietary drugs: antiseptic and healing ointments, syrups for the treatment of respiratory disorders, and preparations for inhalation. They are widely used in aromatherapy.

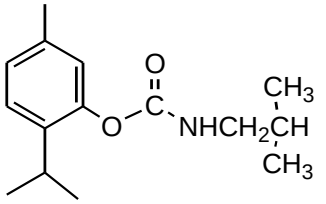
Thymol derivatives of pharmaceutical importance



Dithymol diiodide



Thymotic acid



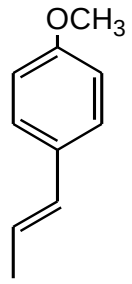
Thymol-N-isoamyl-carbamate

- **Thymol iodide** [Aristol®, Iodothymol®, Thymiode®]
  Thymol iodide is a mixture of iodine derivatives of thymol, principally dithymol diiodide, prepared by treating a solution of thymol in NaOH with I/KI solution. It is specially used for **dental antisepsis** as potent **anti-infective** and **antifungal**.
- **Thymotic acid**

  Similar to salicylate, this derivative acts as **potent analgesic**.
- **Thymol- N-isoamyl-carbamate** [Egressin®]

  This is an ester of thymol with isoamyl carbamic acid. It is used as effective **anthelmintic** in case of nematode infestation.

Anethole or **Anise camphor**



Anethole

Source

- Oils of fruits of family Apiaceae (Umbellifereae) especially **anise** (*Pimpinella anisum*, about 80%) and **fennel** (*Foeniculum vulgare).*
- Main constituent of oil of **star anise** (*Illicium verum,* Fam. Magnoliaceae).

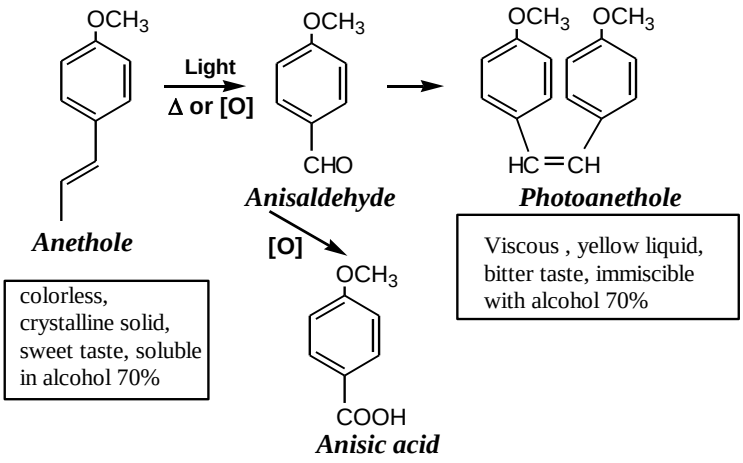
Isolation

1. **Fractional distillation** of the oil, followed by **cooling** the fraction containing anethole for crystallization.
2. From oils with high percentage of anethole, it may be separated by direct cooling

Properties

1. **Anethole** is a crystalline **solid** with a **sweet** taste and an odor characteristic of anise fruit, it is soluble in all proportions with organic solvents.
2. **Anethole** is greatly affected by exposure to light, heat or air. Its ability to crystallize is hindered. It acquires a viscid consistency, a yellow color and somewhat a **bitter taste**.

These changes are due to **oxidatio**n and **dimerization** to produce **photoanethole** that is an **alcohol-immiscible** liquid thus explaining the **turbidity** of aged anethole solution.

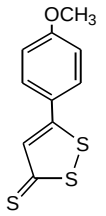


Uses

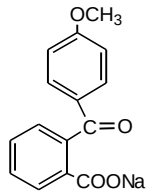
Anise-flavored oils (anise, fennel and star anise), anethole, and semi-synthetic products with anise-like odor are extensively used as **flavoring** agents in **pharmaceutical technology** and **beverages manufacture** for example:

- All kinds of food products especially confectionery, as well as alcoholic and non-alcoholic beverages.
- Pharmaceuticals and cosmetics such as dentrifices, mouth washes and gargles.
- **Anethole** showed an antinociceptive.
- **Anethole** can control some nonimmune acute inflammation-related disease, probably by an inhibitory action on production and/or release of prostaglandin (PGE2) and nitric oxide (NO).
- **Anethole** also has Acetylcholinesterase inhibition activity (with IC_{50} 0.135 mg/mL)

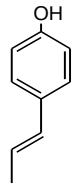
Anethole derivatives of pharmaceutical importance



Anethole trithione



o-(p-Anisoyl)benzoic acid sodium salt



p-Anol

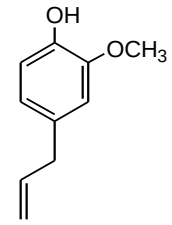
- **Anethole trithione** (3 p-Anisyl trithione, Heporal®, Mucimol®, Sulfarlem®, Tiotrifar®): this compound has a **very bitter** taste; it occurs as orange-colored prisms and is used as **choleretic.**

- **O-(p-Anisoyl) benzoic acid sodium salt**: this is characterized by being soluble in water and **150 times as sweet as cane sugar**, proposed as sweetening agent.

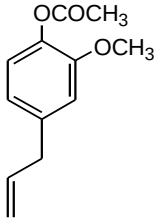
- **p-Anol** (p-propenyl phenol): this is a solid with weak clove-like odor, and is an **intermediate in synthesis of estrogens**.

II. Dihydric phenols and phenolic ethers

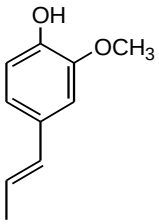
Eugenol (4-allyl, 2-methoxy phenol or 4-allyl guaiacol)



Eugenol



Acetyl eugenol



Isoeugenol

Source

- **Eugenol**, **isoeugenol** and **acetyl eugenol** are the main constituents of several oils obtained from plants belonging to family Myrtaceae and family Lauraceae e.g. clove (*Syzygium aromaticum*), cinnamon leaf and pimenta oils.
- Other sources are oils of *Ocimum gratissimum*, cinnamon bark (small quantities), Java citronella, nutmeg and sassafras.
- **Eugenol** is present in the **combined form** as the glucoside **gein** in *Geum urbanum.*

Isolation

Eugenol is isolated from oil of clove as follows:

1. Treat the oil with aqueous NaOH (3-5 %).
2. Extract the non-phenolic constituents with ether.
3. Acidify the alkaline solution (containing the phenates) and separate the liberated phenols by steam distillation or extraction with solvent e.g. ether.

Identification

Eugenol could be identified by any of the following:

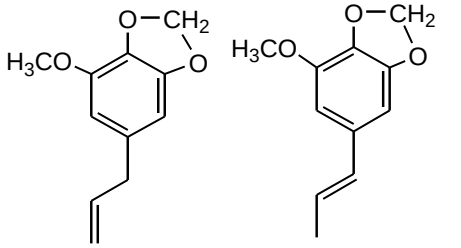
1. Color tests :
   - Eugenol + 2% **alcoholic $FeCl_3$** → **blue** color.
   - Eugenol + cold saturated aqueous $FeCl_3$ → greyish yellow turbidity.
3. By formation of sodium eugenate:
   1 drop of oil containing eugenol + 1 drop of 3% aqueous NaOH saturated with sodium bromide → crystal of sodium eugenate are formed on a cover glass and appeared under the microscope pear-like and arranged in rosettes

Uses

- As **flavoring agent** in pharmaceutical preparations as well as perfume, cosmetic and soap industries.
- It inhibits nerve conduction and is used as a local anesthetic in toothache remedies (**dental analgesic**).
- It acts as **anti-inflammatory** by inhibition of prostaglandin synthesis.
- It has also **bactericidal** activity and as insect attractant..
- As starting material for **preparation of high-grade vanillin** (by oxidation).

III. Trihydric phenolic ethers

Myristicin and **Isomyristicin**



Myristicin **(6-methoxy safrole)** ***Isomyristicin*** **(6-methoxy isosafrole)**

Source

- Myristicin is obtained from **oils of nutmeg** (*Myristica fragrans*, 5-12%), mace, French parsley and dill.
- **Isomyristicin** occurs in oils of dill herb and mace.

Isolation

Myristicin is isolated by fractional distillation from the corresponding oils.

Properties

- **Myristicin** is a **liquid** while **isomyristicin** is a **solid**.
- Myristicin is converted to isomyristicin by boiling with alcoholic KOH.

Uses

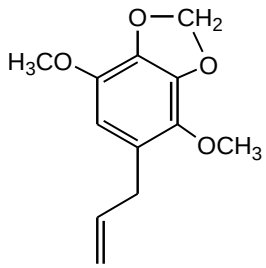
- Nutmeg is used as a **spice**.
- Ingestion of large quantities causes drowsiness, stupor and death. These **psychotropic properties** are attributed to **myristicin** that is transformed in the human body into an amphetamine derivative.

IV- Tetrahydric phenolic ether

Parsley Apiole (or Parsley camphor)

Source

Parsley apiole is the main constituent of parsley seed oil (*Petroselinum crispum*, 60-80%)



Parsley Apiole

3, methoxy myristicin
3, 6 dimethoxy safrole

Isolation

Apiole separates on cooling the oil and is recrystallised from alcohol and petroleum ether.

Properties

Apiole occurs as colorless crystals with faint parsley odor.

Identification

Apiole can be identified by:

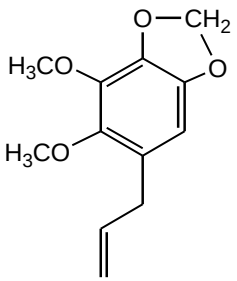
- Preparation of apiole tribromide m. p. 80ºC.
- Oxidation to apiole aldehyde m. p. 102 ºC or apiolic acid m. p. 173 ºC.

Dill Apiole (5, methoxy myristicin or 5, 6 dimethoxy safrole).

Source

This is obtained from the volatile oil of Indian dill fruits

(*Anethum sowa* up to 30 %).



Dill Apiole

5, methoxy myristicin
5, 6 dimethoxy safrole

Uses

- **Apiole** was formerly official and has been used as **emmenagogue** but was **deleted due to its toxicity**.
- Both **Parsley** and **Dill Apioles** exert a synergistic effect with insecticides.

Aldehydes and Ketones in Volatile Oils

Classification

Aldehydes and ketones, which occur in volatile oils, belong to the following chemical classes:

- **Aliphatic** (or acyclic including terpenoids).
- **Alicyclic** (mostly terpenoids),
- **Aromatic**.
- **Heterocyclic**.

Isolation

Carbonyl compounds, other than the water-soluble ones such as formaldehyde and acetaldehyde are isolated from the hydrodistilled oils by any of the following methods:

1. Fractional distillation of the oil.
2. Preparation of derivatives e.g. bisulfite compounds and regeneration by heating with dilute acid or alkalis.
3. Crystallization by cooling if the compound is a solid present in high amounts.

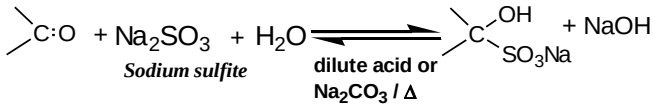
Determination

The methods used for determination are classified into adsorption and condensation methods:

A. Adsorption methods

Neutral sulfite method

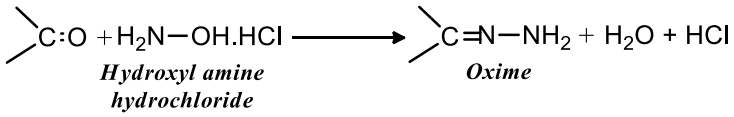
The oil sample is treated with neutral sodium sulfite solution, and the liberated NaOH is titrated against strong HCl using phenol phthalein as indicator.



B. Condensation methods

i. Hydroxylamine method:

An **excess of hydroxylamine hydrochloride** is added to a known weight of the oil. The **released HCl** is **titrated against standard alcoholic KOH** using either methyl red (in case of aldehydes) or dimethyl yellow (in case of ketones) as indicator.



Advantages over the neutral sulfite method:

1. Smaller amounts of oil are required.
2. Reactions with aldehydes need about 15 minutes.

3. Water-soluble adulterants do not interfere.
4. Applicable to oils containing small amounts of aldehydes and ketones even in the presence of large amounts of acids.

This method is generally applicable to **all ketones**, and especially useful for those which could not be analyzed by the bisulfite method such as **menthone** and **pulegone**.

Disadvantages:

1. The calculation involves the molecular weights of the compounds (% w/v). Therefore, any carbonyl compound of lower molecular weight added as adulterant will give high results.
2. The carbonyl portion could not be separated from the non-carbonyl one.
3. All carbonyl compounds are calculated in terms of one component.

Aldehydes

A. Terpenoid aldehydes

- The most important terpenoid aldehydes found in volatile oils are of the **acyclic** type (aliphatic) such as: citronellal and citral a and b.
- **Monocyclic** and **bicyclic** ones are of rare occurrence e.g. phellandral, perillaldehyde, safranal and myrtenal.

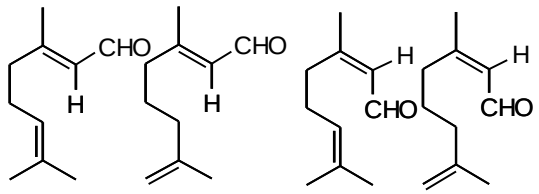
I. Acyclic monoterpenoid aldehydes

Citral

Source

- Lemon grass, *Cympobogon citratus* (and other *Cympobogon* spp. as *C. nardus*, Gramineae/ Poaceae) (70-80%).
- *Ocimum pilosum* (35%).
- Citral occurs as a mixture of at least 2 or probably 4 possible isomers. **Citral a** (geranial) and **citral b** (neral) are geometrical isomers (cis/ trans). They may occur in either limonene or terpenolene forms (such as the corresponding alcohols).

Structure



Citral a or ***geranial*** ***Citral b*** or ***neral***

Isolation

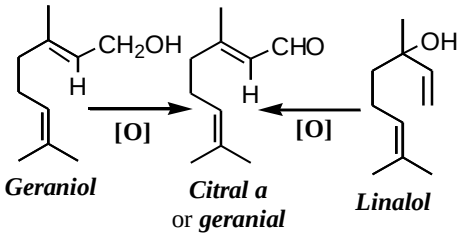
- The oil is treated with $NaHSO_3$. Citral bisulfite is precipitated and purified by washing with alcohol and ether.
- Citral is regenerated by treatment of the bisulfite product with NaOH or by distillation in vacuum.

Separation of citral a from citral b

Citral a could be obtained free from citral b during the regeneration from the bisulfite compound by taking advantage of the fact that **the crystalline sodium bisulfite compound of citral a is sparingly soluble while that of citral b is readily soluble.**

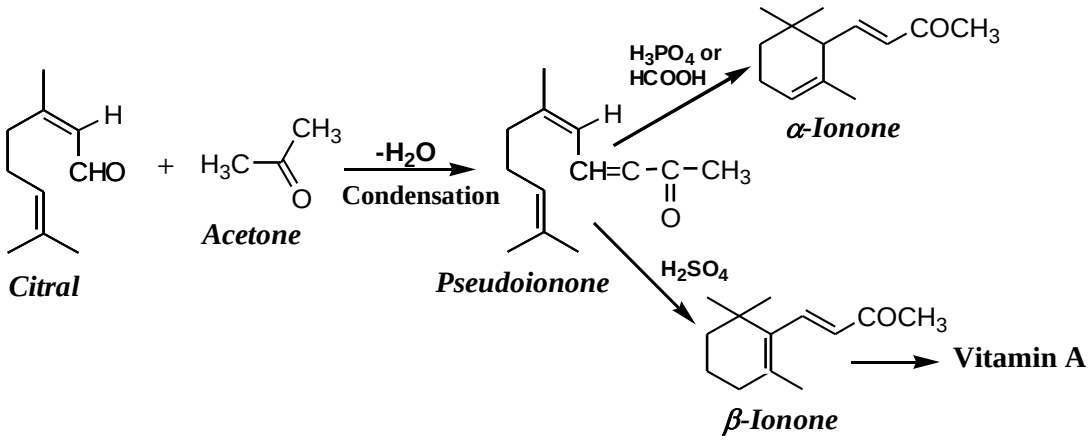
Synthesis

Citral could be obtained from geraniol or linalol by oxidation.



Synthesis of ionones from citral

- The property of **citral** to **condense with substances with an active -CH_2 group** is applied in the preparation of **ionones** that can be used as starting material for semisynthesis of **vitamin A**.
- Condensation of **citral** with **acetone** yields the aliphatic ketone **pseudoionone,** which is cyclized under the influence of concentrated **H_2SO_4 or H_3PO_4** to yield the violet-smelling ketones known as **ionones**. Treatment with H_2SO_4 yields mainly β-ionone, while that with either H_3PO_4 or HCOOH results in the formation of α- ionone.



Uses

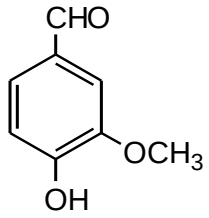
- In compounding synthetic lemon, lime and orange flavors.
- **Citral** possesses antioxidant activity and cytotoxic properties (against Hela cell line). In addition to antifungal activity against *Candida* species (*Candida albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis*)
- Synthesis of ionones, methyl ionones (irones) and vitamin A.
- **Ionones** have a **cedar wood-like odor** and in very dilute alcoholic solution resemble the **odor of violets**.
- **Irone** or 6-methyl ionone is the fragrant principle of **violets** and is generally a mixture of isomers. It is also isolated from iris rhizomes.

Citral is **unstable to alkalis** (due to high unsaturation and polymerization due to aldol condensation) and, therefore, **not used in perfuming white soaps and alkaline cosmetics** to prevent darkening of the final product.

B. Aromatic aldehydes

Vanillin

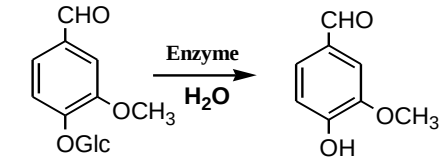
Source



Vanillin

- It occurs either free or combined as glycoside in several plants.
- The most important source is the **vanilla fruits** (pods), which contain about 1.5 – 3% vanillin **combined as glucovanillin glycoside**.
- It occurs in the **free form** as a constituent of benzoin, balsam Peru, clove oil etc…

Isolation from vanilla pods



Glucovanillin ***Vanillin***

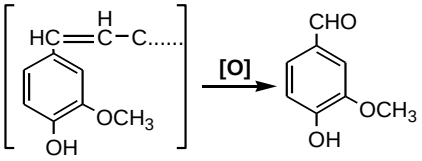
- During **curing or fermentation** of vanilla pods **before marketing**, hydrolysis of most of glucovanillin is achieved with liberation of free vanillin.
- The **fermented pods are extracted with ether**, the solvent evaporated and the crude vanillin recrystallised from alcohol.
- If present as glucoside (e.g **glucovanillin**), it is first subjected to **hydrolysis** followed by isolation of the aldehyde by **distillation**, formation of derivatives or by extraction with organic solvent etc…

Synthesis

Due to its wide range of applications, vanillin is prepared by different synthetic ways. The most commonly used methods are those using either **lignin** or **eugenol** as starting materials.

1. **Synthesis from lignin**

   Lignin is obtained as a by-product in the manufacture of paper pulp from woody plants. On oxidation it yields vanillin as final product.

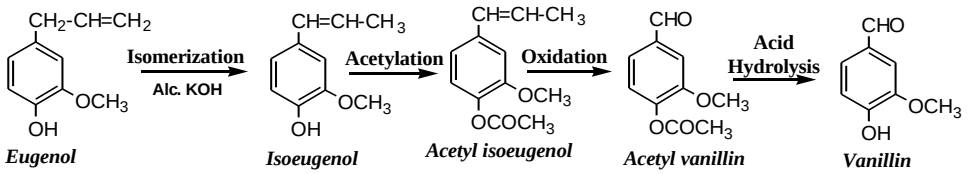


Lignin ***Vanillin***

   This is the cheapest method and is used for the commercial production of vanillin.

2. **Synthesis from eugenol:**

   This is carried through conversion of eugenol to isoeugenol, followed by acetylation, oxidation and hydrolysis. This procedure is applied for preparation of **high-grade** vanillin.



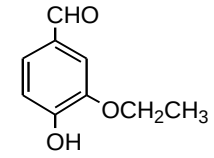
Eugenol ***Isoeugenol*** ***Acetyl isoeugenol*** ***Acetyl vanillin*** ***Vanillin***

Uses

Vanillin is commonly used as flavoring agent in food industries and in perfumery.

Ethyl vanillin (Bourbonal ,vanillal, Ethavan®, Ethovan®)

Properties



Ethyl vanillin

It is a solid with a **finer and more intense vanilla-odor than vanillin**.

Replacement of the methyl group by an ethyl group intensifies the fragrance.

Uses

Ethyl vanillin is used as a flavoring agent and in perfumery.

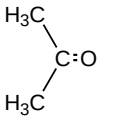
Vanillin is a potential inhibitor of acetylcholinesterase activity

Ketones

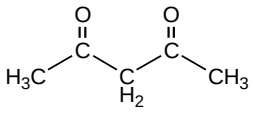
- **Ketones** detected in volatile oils belong **mainly** to the **alicyclic** group.
- Aliphatic and aromatic ketones are of rare occurrence.

A. Acyclic or aliphatic ketones

- These are generally produced during steam distillation by decomposition of more complex compounds.
- They are water soluble and present in the water of cohobation, i.e. usually not detected in the distilled oils.



Acetone



Diacetyl

- The most common are **acetone** and **diacetyl**.
- **Terpenoids** of the group are rare and can be represented by the monoterpenoid ketone, tagetone.

B. Alicyclic terpenoid ketones

These represent the most important group **of ketones detected in volatile oils. The most common belong to the groups of:**

I. **Monocyclic monoterpenoids** such as **menthone**, **carvone**, and **diosphenol**.
II. **Bicyclic monoterpenoids** such as **fenchone** and **camphor**.
III. **Ketones arising from terpene degradation** such as the ionones and irones.

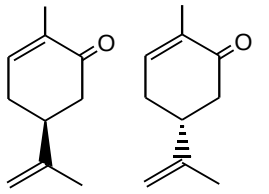
C. Aromatic ketones

Compounds belonging to this group are of **rare** occurrence in volatile oils.

I. Monocyclic monoterpenoid ketones

Carvone

Source



(+)-Carvone ***(-)-Carvone***

It occurs in nature in ***l, d*** and ***dl*** forms.

- ***l*-form**: in spearmint oil (70%) (*Mentha spicata*, Labiateae /Lamiaceae)
- ***d*-form**: in caraway (*Carum carvi)*, and dill fruits oils (Umbellifereae/ Apiaceae) (50 – 60%).
- ***dl*-form**: in ginger grass oil.

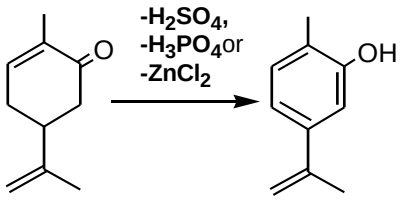
Isolation

It is obtained from the hydrodistilled caraway and dill oils after fractional distillation and collection of the fraction boiling between 220-235º C (containing mainly carvone) by one of the following methods:

1. Treatment with concentrated aqueous **sodium sulfite solution (Na_2SO_3)** with continuous neutralization of the liberated NaOH with dilute acid (to ensure completion of reaction).
2. Removal of non-reacting compounds by extraction with ether.
3. Treatment of the hydrosulfonic acid Na salt **with NaOH to regenerate carvone**, which is steam-distilled.

Properties

Isomerisation to the phenol, carvacrol, on treatment with acids or zinc chloride.



Carvone *Carvacrol*

Uses

- It is used as carminative and substitute for caraway oil.
- As a relaxant it helps relieve from stress, emotional exhaustion, and clear respiratory tracts by acting as an expectorant in the treatment of coughs, bronchitis, and bronchial asthma.

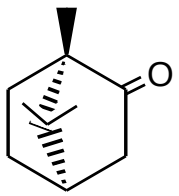
II. Bicyclic monoterpenoid ketones
Camphor

Source

It occurs in *d*-, *l*- and *dl* forms:

***d*-form** is found in all parts of the **camphor tree** , *Cinnamomum camphora* (Lauraceae), also found in oils of sassafras , rosemary , lavender and sage.

- ***l*-form** occurs in oils of certain species of **sage** (*Salvia officinalis*, Labiateae / lamiaceae) lavender and artemisia.
- ***dl*-form is** found in oil of sage etc…



(+)-*Camphor*

Isolation

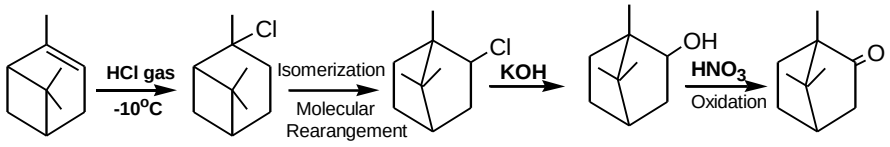
Natural camphor could be obtained by different methods:

1. **From the camphor tree**:
   - The chipped wood is **steam distilled** and the distillate is received in special chambers.
   - Camphor solidifies on the walls, on **cooling**, and is collected at the bottom of the chambers.
   - The crude camphor is **purified** by mixing with a mixture of soda lime, sand and charcoal, followed by **sublimation**.
   - The sublimed camphor is obtained as fine crystals and could be pressed into cubes or plates.
2. By **freezing** oils rich in camphor.
3. By **fractional distillation** of other oils with low camphor %, followed by **cooling**. Raw camphor may be purified by sublimation.

4. By **formation of insoluble complexes** with **strong acids** such as 80% sulfuric acid.

Synthesis

Camphor is semi-synthesized from **α-pinene**, which is the **main constituent of turpentine oil**.



α-Pinene *Pinene hydrochloride* *Bornyl chloride* *Borneol* *Camphor*

Differentiation between natural and synthetic camphor

The main differences between natural and synthetic camphor are as follows:

Property	Natural camphor	Synthetic camphor
Optical Rotation	Optically active (d or l)	Optically inactive (dl)
Test for Chlorides	Negative	Positive
Vanillin /HCl and careful heating	Yellow to green to blue color (due to other volatile contaminants)	Yellow

Separation of camphor from borneol

The components of this mixture may be separated by one of the following methods:

1. Addition of **phthalic** or **succinic anhydride** to form the acid ester that on treatment with **NaOH** yields borneol Na phthalate or succinate that are soluble in the aqueous alkaline layer. Camphor is separated by extraction with organic solvent e.g. ether or by distillation.
2. Addition of **stearic anhydride** to yield bornyl stearate (high b.p.) and camphor that is separated by **fractional distillation**.
3. Addition of **hydroxylamine** where upon camphor separates as its **oxime** that is dissolved in dilute H_2SO_4 and borneol removed by extraction with ether.

Uses

- **Excellent plasticizer for cellulose esters and ethers; used in manufacture of plastics especially celluloid.**
- **It is used in manufacture of lacquers, varnishes and explosives, as a moth repellent and preservative in pharmaceuticals and cosmetics.**
- **Camphor is an ingredient of camphorated parachlorophenol and paregoric.**
- **It is applied as a topical anti-infective, anti-pruritic and counter irritant.**

Camphor derivatives of pharmaceutical importance:

- **Bromo *d*-Camphor** is powerful **counter irritant**.
- ***d*-Camphor carboxylic acid** was used as its basic bismuth sodium salt as **antisyphylitic**.
- **Camphotamide**, prepared by condensation of camphor sulphonic acid with niketamide, is used as **analeptic**.

Esters in Volatile Oils

- Many volatile oils owe their aroma to the presence of esters.
- The most common esters are the acetates of terpineol, borneol and geraniol.

- **Aging of perfumes** is generally carried to allow esterification to take place and thus improving the fragrance.

- Some oils are formed mainly of esters e.g. oil of wintergreen that contains 99% methyl salicylate.

Isolation and identification

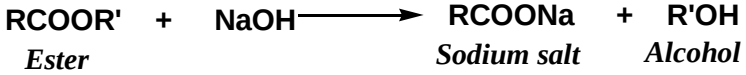
1. Few esters solidify at room temperature and could be separated by **cooling**.
2. **Crystalline derivatives**, which could be prepared directly from esters, are few (c.f. alcohols, aldehydes and ketones).

   However, **methyl anthranilate** can be isolated from essential oils readily by shaking with H_2SO_4, cooling for crystallization of the **sulfate** and recrystallization from alcohol, regeneration is done by treatment with NaOH.

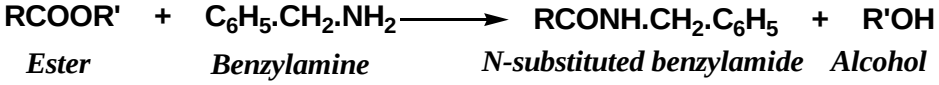
3. Isolation could be carried by **fractional distillation** with the following precautions:
   - Application of a good vacuum (to lower the temperature).
   - Removal of acids before distillation (to prevent hydrolysis).

   The process is, however, sometimes not efficient because esters have close b.p. (especially those of isomeric alcohols).

4. Certain esters are identified only **after saponification** through **identification of their hydrolytic products** (acids and alcohols) but this indirect method is time-consuming.



5. **Aminolysis** (i.e. hydrolysis using amines) is valuable as a method of identification of the acyl group (acid radical) e.g. refluxing the ester in N-benzylamine to give N-substituted benzylamides that have specific melting points.



Determination

General procedure

Estimation of the ester content is of great importance in the evaluation of many essential oils.

Principle:

- Saponification of the esters using a known excess of standard alcoholic alkali (KOH).
- Determination of the amount of alkali consumed for ester(s) hydrolysis after back titration of the excess non-reacting alkali with standardized acid.
- Calculation of the ester percentage.

Classification

Esters isolated from volatile oils could be classified according to the **acyl radical** into:

A. Esters of aliphatic acids.
B. Esters of aromatic acids.
C. Esters containing nitrogen.

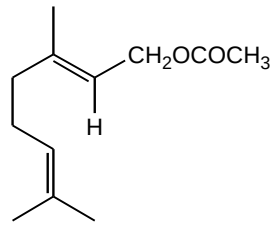
A. Esters of aliphatic acids

Examples of esters belonging to this group are geranyl acetate, linalyl acetate and benzyl acetate

Geranyl acetate

Source

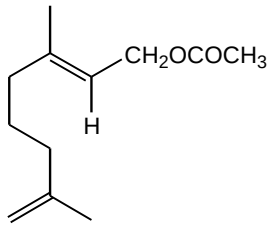
It is widely distributed, found in **oil of citronella** (*Cympobogon nardus*), **lemon grass** (*Cympobogon citratus*), geranium, petit grain, lavender, coriander etc…



Geranyl acetate
Terpinolene form

Isolation

It can be obtained by fractional distillation in vacuum of the volatile oil.



Geranyl acetate
Limonene form

Properties

- Colorless liquid with pleasant **rose-like** odor.

- B.p. 242 - 245 º C with decomposition at atmospheric pressure.

Identification

This is carried by saponification followed by identification of geraniol and acetic acid.

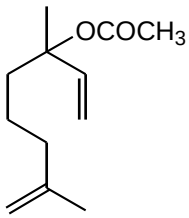
Uses

Widely used in perfume, cosmetic and soap industries to **imitate rose oil**.

Linalyl acetate

Source

It is the principal constituent of **lavender**, bergamot and other essential oils e.g. oil of petit grain, jasmine flower oil, sage oil etc…



Linalyl acetate

Properties

- Colorless oily liquid that possesses a pleasant **lavender-like** odor.
- It is readily hydrolyzed and decomposed on steam distillation.

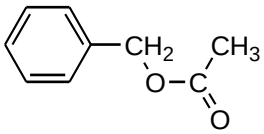
Isolation

It is obtained by fractional distillation under reduced pressure of the volatile oil.

Benzyl acetate

Source

It is a constituent of **Jasmine** oil.



Benzyl acetate

Uses

- It is used in perfumery and as solvent for cellulose acetate.
- If ingested, it causes GI irritation with vomiting and diarrhea. It is also irritating to skin, eyes and respiratory tract.

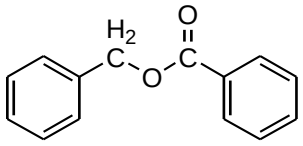
B. Esters of aromatic acids

Examples belonging to this group are: benzyl benzoate; methyl, benzyl and cinnamyl cinnamate; and Methyl salicylate

Benzyl benzoate

Source

It occurs in oil of **ylang ylang** (*Cananga* spp., Anonaceae), **balsam Tolu**, **balsam Peru** and oil of tuberose.



Benzyl benzoate

Isolation

It is obtained from oils containing high amounts by cooling and crystallization (as it solidifies at 20 ºC).

Properties

- Optically inactive, viscid liquid that congeals at moderate temperature to yield white crystals m.p. 20 ºC.
- Sparingly volatile with steam.

Identification

After saponification, the hydrolytic products are identified as benzoic acid and benzyl alcohol.

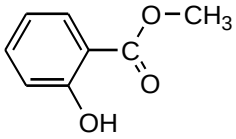
Uses

Although **it may cause eye and skin irritation**, benzyl benzoate has a wide variety of uses as:

- **Scabicide** and **pediculicide**.
- Solvent for cellulose acetate, nitrocellulose and artificial musk.
- Substitute for camphor in celluloid and plastic pyroxylin compounds.
- **Perfume fixative**.
- Flavoring agent in confectionery and chewing gum.

Methyl salicylate

Source



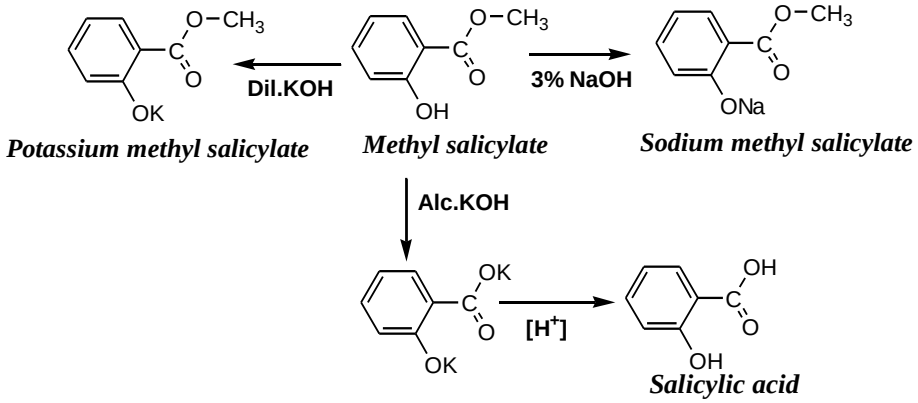
Methyl salicylate

- Oils of **wintergreen** (*Gaultheria procumbens*) and **sweet birch** (*Betula lenta*) contain up to 99% **methyl salicylate**.
- The ester is present in a **combined form**; it constitutes the **aglycone part** of the glycosides **gaultherin** and **monotropin** that must first be hydrolyzed by the aid of enzymes or acids to liberate the ester.
- Therefore, without previous digestion in warm water for several hours (i.e. enzymatic hydrolysis), the plant material would yield almost no oil on distillation.

Properties

- Colorless oily liquid, b.p. 224 ºC, with **powerful odor** and flavor very characteristic of wintergreen and a **pungent** warm **taste**.
- On treatment with cold saturated **aqueous solution of $FeCl_3$,** methyl salicylate develops a **red violet** color, which disappears after 15 min.
- **Action of alkalis:**

- In **dilute aqueous alkalis** e.g. KOH, it readily dissolves yielding K methyl salicylate (the phenolic OH group gives the phenate).
- In **more concentrated aqueous solutions**, e.g. 3% NaOH, it is sparingly soluble due to the formation of sodium methyl salicylate which precipitates (Na phenates are less soluble than the K salts).
- When heated with **excess alcoholic alkalis** (KOH or NaOH), **both ester** and **phenolic groups are involved** in the reaction (saponification and formation of phenate) and on acidification salicylic acid is obtained.



Identification

This can be carried through:

- Saponification of the ester and identification of salicylic acid and MeOH, salicylic acid (m.p. 158 ºC).
- Preparation of derivatives due to the presence of the free phenolic OH group with: acetic anhydride, benzoyl chloride, phenyl isocyanate, etc….

Uses

- Methyl salicylate has **local irritant**, **antiseptic** and **antirheumatic** properties.
- It is also used in flavoring food products, oral preparations, toothpastes and pharmaceuticals.

Toxicity:

- **Ingestion** of relatively small amounts of methyl salicylate **may cause severe poisoning and death** (average lethal dose 10 ml in children and 30 ml in adults).
- **Symptoms of toxicity** are nausea, vomiting, acidosis, pulmonary edema, pneumonia, convulsions and death.

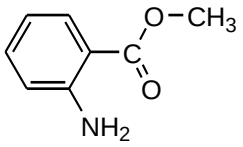
C. Esters containing nitrogen

These are **organo-nitrogen compounds** containing an ester group. The most common are derivatives of anthranilic acid or o-aminobenzoic acid.

Methyl anthranilate

Source

Oils of **neroli**, **jasmine**, tuberose, gardenia, **ylang ylang** and oils obtained from leaves of sweet orange, bergamot etc…



Methyl anthranilate

Isolation

It can be readily isolated by:

- Shaking the oil with **dilute H_2SO_4** (due to basic amino group).
- Cooling to crystallize the sulfate formed.
- Purification by recrystallization of the sulfate from alcohol.
- Regeneration of the ester by treatment with NaOH.

Properties

- It has a beautiful **violet fluorescence** that is apparent in any volatile oil containing it.

Identification

- By physical examination of the characteristic violet fluorescence.

Oxides and Peroxides in Volatile Oils

Oxides

These are **internal ethers**, the most widely distributed is the monoterpenoid **cineole**, and other examples are the sesquiterpenoids **bisabolol oxides**.

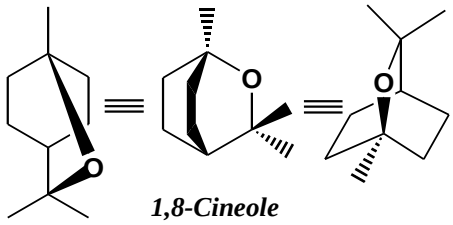
Cineole, Eucalyptol or **Cajuputol**

Source

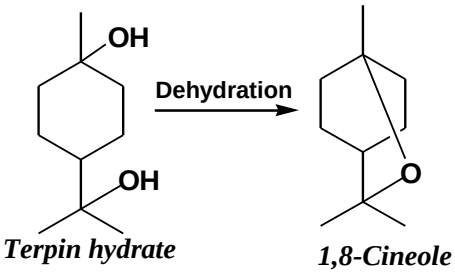
- The most common is **1,8 cineole**.
- It is widely spread in essential oils, next constituent after □-pinene in frequency.
- Major constituent of oils obtained from certain ***Eucalyptus*** species (30 - 70 %), oil of **Cajuput** (*Melaleuca leucadendron,* Myrtaceae) (40 %), and oil of Laurel leaf (50 %).
- Minor constituent in cardamom, lavender, spearmint and wormseed oils.

Structure and Synthesis

It can be obtained by dehydration of terpin hydrate.



1,8-Cineole

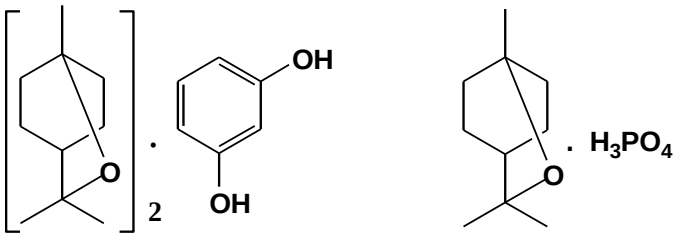


Terpin hydrate ***1,8-Cineole***

Isolation

It can be isolated by one of the following methods, **based on its amount in the oils**:

- Fractional distillation followed by **cooling** the fraction collected at 170-180 ºC, crystalline cineole is collected, m.p. 1 ºC.
- Formation of **additive products** with:
  1. **Halogen acids** HCl or HBr to yield the crystalline $C_{10}H_{18}O$. HCl or $C_{10}H_{18}O$. HBr.
  2. **Phenols** e.g. 50% resorcinol.
  3. **Syrupy phosphoric acid**, which is decomposed by hot water (regeneration). This reaction is also used for estimation in case of oils rich in the constituent.



Cineole resorcinol complex *Cineole Phosphoric acid complex*

Determination

Several methods are proposed including:

1. **The congealing point method** (Kleber's and Von Rechenberg's method)
   The congealing point of the cineole-containing oil is determined, and cineole content is read from specific tables. A disadvantage of the method is that the whole process should be carried at low temperatures (below zero degree).
2. **The o-cresol method** (Cocking's method)
   This depends on that the freezing point of a **mixture of cineole and o-cresol** is lowered due to the amount of other constituents in the assayed oil. The percentage w/w of cineole corresponding to the freezing point is then calculated from special tables.
3. **The phosphoric acid method** (Scammel's method , modified by Baker and Smith)
   - This depends on that cineole forms with syrupy phosphoric acid (sp.gr. 1.75), on the cold, a loose additive compound from which it is regenerated by the addition of warm water.
   - The volume of cineole liberated is measured in the graduated neck of a Cassia flask and its percentage in the oil calculated.
   - This method is used for large amounts of cineole (not less than 50 %).
4. Other **colorimetric** methods have been devised and are useful in case of low cineole-containing oils.

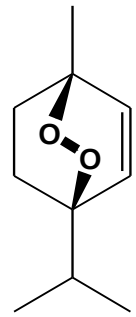
Uses

Cineole is widely used in many kinds of pharmaceutical, cosmetic and household preparations:

- It is used **locally** as **anaesthetic**, **antiseptic** and **anti-inflammatory**.
- **Internally**, cineole serves as a **stimulant**, and **expectorant** in cases of chronic bronchitis.
- It is used in room sprays, skin lotions and other types of cosmetics.
- It has a cockroach repellent activity.

Peroxides

Ascaridole



Ascaridole

Ascaridole is the only naturally occurring monoterpenoid peroxide.

Source

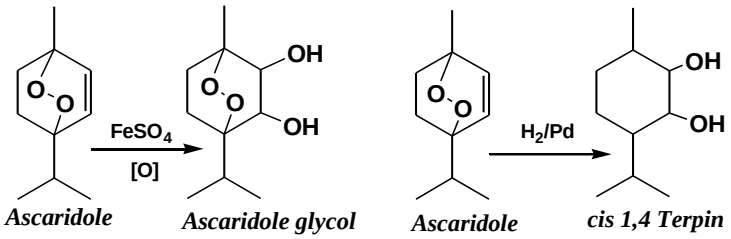
Ascaridole is the main constituent of **oil of chenopodium** (65 - 70 %) [C*henopodium ambrosioides* var. *anthelminticum,* Chenopodiaceae].

Isolation

It is obtained by repeated fractional distillation of the oil in vacuum and collecting the fraction boiling at 95 - 98 ºC (avoid raising the temperature to prevent explosion).

Properties

- Viscid yellow oily liquid, with **disagreeable odor** and flavor.
- Insoluble in water but **soluble in dilute acetic acid**.
- It can not be distilled at atmospheric pressure.
- It **explodes** with violence if :
  - heated to 130 -135º C (decomposition).
  - treated with H_2SO_4 , HCl , HNO_3 or H_3PO_4 .
- It liberates iodine from **KI in acetic acid** solution (**used for determination**).



Ascaridole *Ascaridole glycol* *Ascaridole* *cis 1,4 Terpin*

Uses

Ascaridole is used as **anthelmintic** in ascariasis.

Organo-nitrogen and organo-sulphur compounds in volatile oils

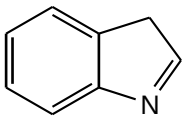
Plant materials containing high amounts of albuminous matter (e.g. seeds) yield on distillation volatile nitrogenous or sulfurous compounds, which can be detected in volatile oils, others may be original constituents responsible for the odor.

I. Organo-nitrogen compounds

Indole (Benzyl pyrrole)

Source

Indole is detected in flower oils such as oils of jasmine, neroli, lemon flower, etc …



Indole

Properties

- It has a powerful, persistent, disagreeable odor especially if not purified (synthetic product).
- If **pure** and **strongly diluted** it has a **flowery**, **heavy odor** which is not more disagreeable.
- It is volatile in steam, soluble in hot water and organic solvents.

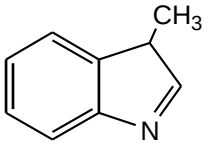
Uses

Indole is used, **in traces**, in perfumes of heavy oriental types, and as **fixative**.

Skatole (3-methyl indole)

Source

It is obtained from the volatile oil of the wood of *Celtis reticulosa*, Ulmaceae.



Skatole

- It is present in human excrements (as a degradation product of albuminous matter)
- It could be produced from strychnine alkaloid by heating with soda lime (semi-synthesis).

Uses

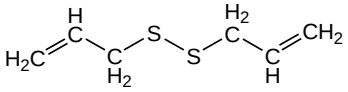
It is used in minute amounts as a **modifier** and **fixative** of floral perfume composition.

II. Organo-sulphur compounds

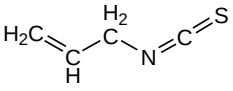
These are detected in certain volatile oils as degradation products. Decomposition is mainly due to hydrolysis of glycosides during steam distillation of the oils.

Examples are:

- Hydrogen sulphide, **H_2S**, in unrectified oils of anise and caraway.
- Carbon disulphide, **CS_2**, in volatile oil of mustard.
- Methyl sulfide or dimethyl sulfide, **$(CH_3)_2S$**, in unrectified oil of peppermint.
- Allyl disulfide and diallyldisulfide **$(C_3H_5)_2S_2$**, in oils of onions and garlic.
- Allyl isothiocyanate (or mustard oil).



Diallyl disulfide

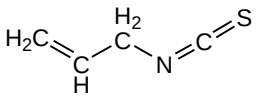


Allyl isothiocyanate

Allyl isothiocyanate

Source

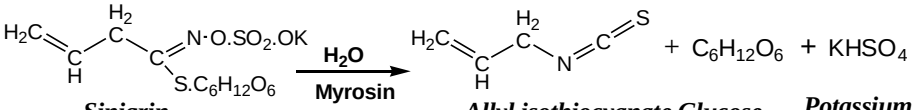
- It occurs in **glycosidal combination** as **sinigrin** in the seeds of black mustard (*Brassica nigra*, Cruciferae).
- Sinigrin (or K myronate), when hydrolysed with the enzyme myrosin, yields allyl isothiocyanate, D-glucose and potassium hydrogen sulfate.



Allyl isothiocyanate

Preparation

- The ground defatted black mustard seeds are macerated in water for about 5 hours at a temperature of 37 - 40 º C to induce enzymatic action, then steam distilled.

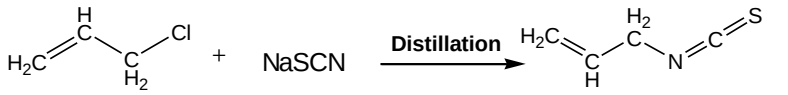


- The oily layer, heavier than water, is collected over anhydrous $CaCl_2$ and rectified by fractional distillation collecting the portion boiling between 145 - 152 ºC (nearly pure allyl isothiocyanate).

- During distillation an amount of the oil decomposes into CS_2, S and certain volatile amines which are generally present up to 0.3 - 0.5 %.

Synthesis

It could be obtained from **allyl chloride** by treatment with **Na thiocyanate** in alcoholic solution and distillation of the mixture.



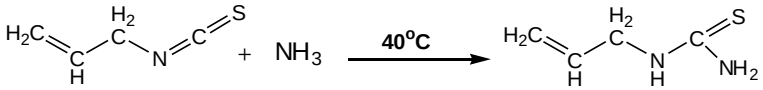
Allyl chloride *Sodium thiocyanate* *Allyl isothiocyanate*

Properties

- Allyl isothiocyanate is a colorless or pure yellow strongly refractive liquid, with **pungent irritating odor** and an acid taste.
- It is sparingly soluble in water but soluble in organic solvents.
- It decomposes on exposure to light and air and should be stored in dark well-filled bottles.

Identification

It can be identified by the **formation of allyl thiourea**, on warming at 40 ºC with aqueous NH_3 solution in the presence of alcohol.



Allyl isothiocyanate *Allyl thiourea*

Determination

- This is carried by treatment of the oil sample with an **excess of standard $AgNO_3$ in the presence of ammonia**.
- The precipitated Ag_2S is filtered and the unreacted $AgNO_3$ titrated against NH_4SCN using ferric alum as indicator (**Volhard's** method).

Uses

- Locally, it is used as rubefacient or vesicant in certain plasters.
- It is used for flavoring of food products especially mustards.
- Internally it causes severe **GIT irritation.**

RESINS AND RESIN COMBINATIONS

- **"Resins"** is a term applied to indicate a group of **solid** or **semi-solid substances** of complex chemical nature and variable composition.
- They are **brittle** secretions or **exudations** of plant tissues, produced either **normally** or due to **pathogenic** conditions.

"Artificial resins" are synthetic products having the general physical characters of natural resins. They could be obtained through formaldehyde condensation.

"Balsams" are resinous **mixtures** that **contain cinnamic acid**, **benzoic acid**, or both, or **esters** of these acids

Physical Properties

1. **Condition**: Resins are hard, amorphous (rarely crystallizable), transparent or translucent brittle substances.
2. **Specific gravity**: They are generally **heavier than water**.
3. **Effect of heat**:
   - Heating at low temperature results in melting to form a sticky adhesive fluid without decomposition.
   - Heating in a closed vessel results in decomposition generally to hydrocarbons.
   - Heating in open-air results in formation of a smoky flame due to the large number of carbon atoms.
4. **Solubility**:
   - They are insoluble in water and petroleum ether.
   - Almost completely soluble in alcohol, chloroform and ether to give solutions, which on evaporation results in the formation of a varnish-like film.
   - Soluble in acetone, CS_2, fixed and volatile oils.
   - Soluble in chloral hydrate (clarification of sections in microscopical mounts).
5. **Color**: Resins vary in color. Most resins darken on storage due to slow oxidation.

Resin Combinations

1. Resins occur frequently in **homogeneous mixtures** with either **volatile oils** or **gums** or **both** to form:
   - **Oleoresins.**
   - **Gum-resins.**
   - **Oleo-gum-resins**.

   This classification is rather artificial because trace of any of these constituents may be present in any of the combinations.

Separation of the components of an oleo-gum resin:

- **Gums** could be easily separated by extraction with water and precipitated by alcohol.
- **Volatile oils** are separated by steam distillation.
- **Resins** are soluble in alcohol and precipitated by water.

2. When present in **glycosidal combination** with sugars, they form **glycoresins.**

Preparation

According to the method of preparation, two types of resins are distinguished the **natural** and the **prepared resins.**

1. **Natural resins** are those collected from the plants in which they are produced through natural or artificial punctures e.g. mastic and balsam of Peru.
2. **Prepared resins** (or resin combinations), these are obtained by one of the following methods:
   - Extracting the **plant containing a resin** with **alcohol** then the solvent is either evaporated or the solution poured in water to precipitate the resin e.g. **Podophyllum**, or
   - Extracting the **plant containing oleoresin** with **ether** or **acetone** followed by distillation to remove the oil, e.g. preparation of **Colophony**.
   - Extracting the **plant containing gum resin** with **alcohol** in which the gum is insoluble.

A. Official resins and resin-combinations

Examples of official resin combinations are:

Resins: Colophony, guaiacum, scammomy, jalap and podophyllum.

Oleo resins: Filix- mas extract and copaiba.

Oleo-gum-resins: Asafoetida, galbanum and myrrh.

Balsams: benzoin, balsam of Peru, balsam of Tolu, balsam of Peru and storax.

Selected Examples

A. Resins

Colophony or Rosin

Source

Colophony is the *solid resin* ***obtained from*** Pinus *species* ***especially*** **Pinus palustris *[Fam. Pinaceae].***

Composition

1. **Resin acids:** 80-90% of isomeric **diterpene acids**, mainly **abietic** acid present as its anhydride from which it is obtained by treatment with alcohol. It also contains **sapinic** and **pimaric** acids.
2. **Resene:** represented by a small proportion of hydrocarbons.

Test for identification

Copper acetate test (for abietic acid):

- Extract the powder with petroleum ether and filter.
- Shake the filtrate with twice its volume of Cu acetate.
- The petroleum ether layer turns green due to the formation of the copper salt of abietic acid.

Uses

In pharmaceutical industries, colophony is used as ingredient in cerates plasters and ointments. Commercially, it is used in the manufacture of varnishes, paint dryers, printing inks, soap, sealing wax, floor coverings etc.…

Cannabis, Indian hemp, or Marihuana

Source

- It consists of the **dried flowering tops of the pistillate plants** (female plants) of *Cannabis sativa* (Fam. Moraceae). The resin is known in Persia and Arabia as **hashish.**
- Through selective cultivation, **two genetic types of Cannabis** are produced:
  1. The **drug type**, which is **rich in the psychoactive constituent** , and
  2. The **hemp type**, which is **mainly cultivated for its elongated phloem fibers**.
- The **resin is secreted into trichomes found on the bracts** (small leaves) and **bracteoles** that enclose the **ovary of the flowering tops of the female plant**.
- For medicinal purposes either the **resin** (**hashish**) is used or the **flowering tops** of the female plants **(marihuana**).
- The male plant produces an equivalent amount of active constituents distributed through out the plant not concentrated into a resin.

- The **amount of resin decreases when the plants are cultivated in temperate regions**. Thus, **Indian cannabis** contains **20%** or more resin, **hemp** cultivated in **temperate regions** produces **6%** or less.

Constituents

- These are **meroterpenoid** compounds collectively known as **cannabinoids**.
- The main active constituent of the resin is **(-)-Δ^9-trans-tetrahydrocannabinol** (Δ^9-THC).
- Other constituents isolated from the resin include **cannabinol**, **cannabidiol**, **cannabichromene**, cannabigerol, and Δ^8-trans- **tetrahydrocannabinol (Δ^8-THC).**

Test for identification

Modified Beam's test:

1. Extract the drug with **methanol**, evaporate the extract to dryness and extract the residue with **petroleum ether**.
2. Filter and **wash** the filtrate with **dilute Na_2CO_3** followed by **dilute H_2SO_4** and **water**.
3. Decolorize with **charcoal** and evaporate to dryness.
4. To the residue, add few drops of **N/10 alcoholic KOH** a **purple color** is produced.

Pharmacological action

- **Tetrahydrocannabinols (THC)** possess **euphoric** activity.
- **Cannabinol** is **weakly active**.
- **Cannabichromene** and **cannabidiolic acid** are **sedative**.

Uses

- Medicinally, **Indian hemp** has been used as a **sedative** and **hypnotic**.
- **Δ^9-Tetrahydrocannabinol** or **Dronabinol** has an **antiemetic** effect and is used orally in the treatment of the **nausea and vomiting associated** with **cytotoxic drugs** used in cancer chemotherapy. It acts probably by **blocking the opiate receptors in the brain** and **indirectly inhibiting the emetic center.**
- It also **stimulates the appetite** and therefore used in treatment of anorexia associated with **weight loss** in patients suffering from **AIDS**.

Toxicity

On **ingestion** or **inhalation** by smoking, Indian hemp may cause euphoria, delirium, hallucination, weakness, hyperplesia and drowsiness.

Podophyllum resin [Podophyllin]

Source

It is a mixture of resins obtained from the **dried roots** and **rhizomes** of ***Podophyllum peltatum*** (American podophyllum) and ***Podophyllum hexandrum*** (Indian podophyllum). Fam. Berberidaceae.

Constituents

- Chief constituents belong to the group of **lignans** (C18 compounds related to flavonoids, being dimers of 2 C6-C3 units). They are present **free and in glycosidal combination**.
- The main components of the resins are: **podophyllotoxin**, **α-peltatin** and **β-peltatin**.
- Their concentrations in the resins of the two species are different:
  - The **Indian resin**: **podophyllotoxin (40%) with little or no peltatins**;
  - The **American resin**: **20% of podophyllotoxin, 10% β-peltatin and 5% α-peltatin.**

Tests for identification

1. The alcoholic solution of the **Indian resin** gives a **stiff gel** when treated with **alkali** due to its **high content of podophyllotoxin**.

Podophyllotoxin contains a **lactone** ring, when treated with alkali will form the **alkali salt of podophyllic acid** which is **gelatinous**.

2. Treatment of the alcoholic solution of the resins with few drops of **Copper acetate** gives: a **bright green color** with the **American resin** and a **brown color or precipitate** with the **Indian** resin.

Pharmacological action

- The **lignans** have **antitumour** activities.
- The **peltatins** are responsible for most of the **purgative** effect of the drugs.
- The **resin** has also **antimitotic** activity.

Uses

- **Externally**: the tincture is applied as paint for **treatment of soft venereal warts**.
- **Internally**: the resin is used as **drastic but slow purgative**.

BITTERS

Definition and General Characters

- The term **Bitters** or **Bitter principles** is usually used to indicate a group of natural products that have an **intensely bitter taste** and were traditionally **used** in liquid medicaments **to stimulate appetite**.
- Many of these products and drugs containing them are still included in **tonic** formulations and are usually administered before meals.
- They are sometimes prescribed as **cholagogue** and in case of indigestion.
- They are mainly of vegetable, rarely of animal origin.
- They are abundant in certain plant families especially Compositae, Labiatae, Gentianaceae and Umbellifereae.
- They are generally formed of **carbon**, **hydrogen** and **oxygen**; **nitrogen is rarely present**.
- They constitute a **heterogeneous group of compounds** that belong to **different chemical classes**.
- Many of them possess a **terpenoid structure** being derivatives of mono-, sesqui-, di- and triterpenes and most contain a **lactone ring**.
- The bitter taste is often altered due to the presence of other constituents, therefore different types could be distinguished:
    1. **Pure bitters** (*Amara pura*) as gentian roots.
    2. **Mucilaginous bitters** (*Amara muciliginosa)* as *Lichen isladicus*.
    3. **Aromatic bitters** (*Amara aromatica*) as *Absinthium* herb.
    4. **Astringent bitters** (*Amara adstringentia*) as *Condurago* roots.
- Extracts of the following drugs have been used as bitter stomachic: gentian, quassia, calumba, cinchona (or quinine), nux vomica (or strychnine), hops, centaury, condurago, quebracho and *Taraxacum*. Many of these drugs are now mainly used for other pharmacological activities.

Pharmacological action

- The bitter constituents **stimulate the gustatory nerves** in the mouth and give rise to an **increase in the psychic secretion of gastric juice**.
- Yet, they apparently improve the gastric function through a **direct action on the stomach** and /or **duodenum.**
- They should be taken half an hour before meal.
- The plant extracts are used as tonic to stimulate appetite, in anemia and convalescence.

Determination of bitterness value

- Bitters belong to different chemical classes, therefore a general way for their detection and quantitative determination is difficult.
- A procedure to **determine the bitterness value based** on their **physiological effect** consists in **comparing** the **threshold concentration of the tested extract with** that of **quinine hydrochloride**. This should be carried by the same person in a short space of time.
- The bitterness value of the tested sample (extract or substance) is deduced from the following equation:

Bitterness value = 2000 X **C** / **A** X**B**

A = quantity of the material in mg / ml

B = volume of the tested in ml / 10 ml = dilution of threshold of bitter concentration

C = quantity of quinine HCl in mg / ml.

- **Examples**: The bitterness value of Quassia wood is 40-50000, that of gentian roots is 10-30000 and that of Marrubium herb is 3000.

Classification

I. Terpenoid bitters

This group includes isoprenoid bitters of different structures such as:

1. **Monoterpenoids** (C10) e.g. **iridoids** (aucubin), **secoiridoids** (gentopicrin); mostly in glycosidic forms.
2. **Sesquiterpenoids** (C15) containing a **lactone ring** and subclassified as germacranolides, eudesmanolides and guaianolides etc… such as santonin, cnicin and absinthin; as well as highly **oxygenated sesquiterpenoids** such as picrotoxinin and picrotin.
3. **Diterpenoids** (C20) having labdane, kaurane and pimarane structures e.g. marrubiin.
4. **Triterpenoids** (C30) e.g. cucurbitacins and quassinoids and **Secotriterpenoids** that are modified tetracyclic triterpenoids containing less than 30 C atoms e.g. limonoids.

II. Non-Terpenoid bitters

1. **Phenolic** bitters e.g. humulone and lupulone.
2. **Chromone** bitters e.g. khellin and visnagin.
3. **Coumarin** bitters e.g. xanthotoxin, imperatorin and bergapten.
4. **Coumarone** bitters e.g. rotenone.
5. **Anhydride** bitters e.g. cantharidin.

Terpenoid Bitters

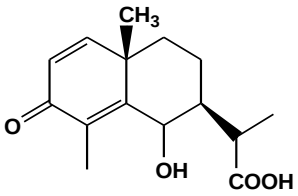
Santonin

Occurrence

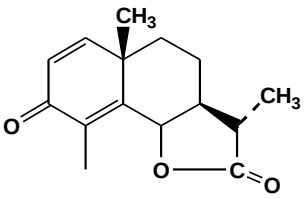
Santonin is the chief anthelmintic constituent of the dried unexpanded flower heads of *Artemisia cinna* (not less than 2 %) and other santonin-containing species of *Artemisia* (santonica or wormseed), Family Asteraceae (Compositae).

Structure

Santonin is the crystalline lactone of santonic acid**; it has** 15 carbon atoms **and is biogenetically related to** sesquiterpenes**.**



Santonic acid



α-Santonin

Properties

- Santonin occurs as colorless, odorless, shiny, tabular crystals or as white crystalline powder, m.p. 170 – 173 ºC.
- It is at **first tasteless**, but after sometimes becomes **slightly bitter**.
- It is slightly soluble in boiling water, castor oil, ether, soluble in alcohol and chloroform but insoluble in cold water and petroleum ether.
- On **exposure to light**, **santonin is transformed into the isomeric photosantonin** from which it can be obtained by crystallization from alcohol.
- Santonin is a **lactone** that yields **salts with alkali**. These, when treated with mineral acids give santonic acid, which separates as the lactone santonin (cyclization due to dehydration).

Tests for identification

1. **Santonin** gives a **violet color** with **alcoholic alkali** (KOH or NaOH) that gradually turns to **reddish-yellow**.
2. **Santonin** when dissolved in few drops of **alcohol containing furfural**, followed by addition of 2ml of **concentrated sulfuric acid**, then heated in a porcelain dish over water bath, a **purple color** is developed, which changes to **bluish-violet**, **dull blue** then nearly **black**.
3. **Santonin** (0.01 gm), when heated with **2 ml of H_2SO_4**, then diluted with an equal volume of water, no color is observed. On addition of 2 drops of dilute **$FeCl_3$** solution to the hot mixture a **blood red to reddish violet** color is obtained.

Uses

- **Santonin** is used as an **anthelmintic** in the treatment of **roundworm infestation** (e.g. *Ascaris*) in doses of 60 to 200 mg daily for 3 days. It has a **mild effect on threadworms and no effect on taenia.**

- It is generally used in combination with kainic (Digenic) acid to reduce its toxicity. A mixture of the two drugs was found appreciably more effective in the treatment of ascariasis than if each was used separately.

Toxicity

- Santonin affects vision causing **"xanthopsia"** (white objects look green, blue or yellow).
- It may also cause headache, vertigo, nausea, vomiting, apathy, profuse of sweating and diarrhea.
- **Large doses** may give rise to **epileptic convulsions** followed by coma, hearing disorders and heamaturia. Death may occur from respiratory failure.

Non–Terpenoid Bitters

Phenolic bitters

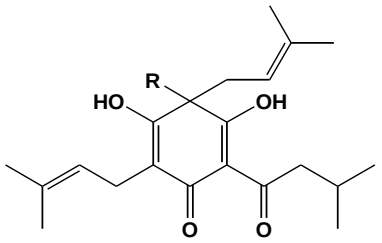
Humulone (α–Lupulinic acid) and Lupulone (β- Lupulinic acid)

Occurrence

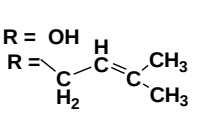
- Humulone and lupulone are the chief active constituents obtained from the dried strobiles of *Humulus lupulus* (hops, humulus or lupulus) family Moraceae.
- The bitterness of hops is mainly due to acidic bitter principles humulone, lupulone and about 10 % of resins.

Structure

These are **crystalline phloroglucinol derivatives**, the α-acids e.g. humulone and the β-acids e.g. lupulone



Humulone
Lupulone



Test for identification

An alcoholic solution of humulone gives a **reddish-violet color** with alcoholic **ferric chloride** (phenolic).

Uses

- Humulone and lupulone contribute to the bitterness of hops.
- They are used as **aromatic bitters** and exert a **mild sedative** action.
- Hops are used in the **manufacture of beer** for taste improvement (flavoring agent).
- Humulone was found to exert a **bacteriostatic** activity.

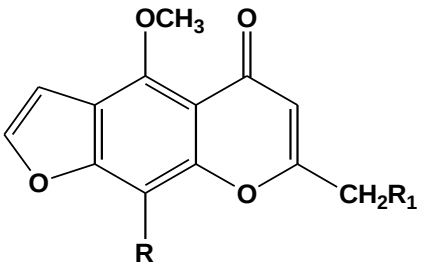
Chromone bitters

Khellin and Visnagin

Occurrence

- Khellin, Kellin, Visammin, Kelamin® or Kellicor® is the main constituent of the fruits of *Ammi visnaga* family Umbelliferae*.*
- The fruit contains about 1 % of khellin beside two other crystalline compounds, visnagin (about 0.1 %) and khellol glycoside (about 0.3 %).
- Together with the chromone compounds, coumarins such as samidin, dihydrosamidin and visnadin are also present in the fruits of *Ammi visnaga*.

Structure



	R	R_1
Khellin	**OCH_3**	**H**
Visnagin	**H**	**H**
Khellol glucoside	**H**	**O-Glucose**

Tests for identification

Khellin:

1. Mix few crystals of **khellin** with **solid KOH or NaOH** a **rose red color** is produced (not given with K_2CO_3 or Na_2CO_3 or bicarbonate).
2. Mix few crystals of **khellin** in a porcelain dish with a drop of **H_2SO_4** or of **phosphoric acid**, a **reddish-orange** color is produced, that turn to **bright yellow** on addition of 2-3 drops of **H_2O**.
3. Add about 1-ml **nitric acid** (1:1) to few mg of **khellin** in a porcelain dish, then add 5 ml **NaOH** solution, **violet** color is produced.
4. To few crystals of khellin, add an equal quantity of **ninhydrin** followed by 2 – 3 drops of concentrated **H_2SO_4**, an **emerald green color** is produced on stirring.

Visnagin:

Triturate few crystals of **visnagin** with solid **KOH** or **NaOH**, a faint rose red color is produced (paler than that produced with khellin)

Properties

Khellin:

- Khellin occurs as bitter needle crystals, m.p. 153 ºC.
- It is freely soluble in $CHCl_3$ and alcohol, less soluble in ether, **sparingly soluble** in **petroleum ether** and **cold H_2O**, but more soluble in hot water and hot CH_3OH.

Visnagin:

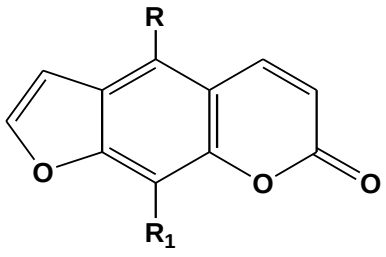
- Visnagin occurs as colorless thread-like needles.
- It is freely soluble in $CHCl_3$, sparingly soluble in alcohol, **insoluble in cold water** and **petroleum ether**.

Uses

- Khellin is available in tablets, injections and suppositories.
- It exerts a potent **selective coronary vasodilator** and **bronchodilator** activity.
- It is used in treatment of **coronary insufficiency**, **angina pectoris** and in **bronchial asthma**.
- Beside these actions, it plays a great role in **relieving kidney spasm**.

Coumarin bitters

Xanthotoxin, Imperatorin and Bergapten



These are **furanocoumarin** derivatives of the **psolaren** type.

	R	R_1
Psolaren	**H**	**H**
Xanthotoxin	**H**	**OCH_3**
Imperatorin	**H**	**OCH_2- $CH=C(CH_3)_2$**
Bergapten	**OCH_3**	**H**

Occurrence

- **Xanthotoxin (**Ammoidin or Methoxsalen**)** is obtained from the fruits of *Ammi majus* (Umbelliferae), the pericarp of the fruits of *Fragara xanthoxyloids* (Umbelliferae) and *Ruta graveolens* herb (Rutaceae).

- **Imperatorin** (Ammidin) occurs in the fruits of *Ammi majus* (Umbelliferae), in the rhizomes of *Imperatoria* spp.

- **Bergapten** is isolated from the leaves of *Ficus carica* (Moraceae), the herb of *Ruta graveolens*, the oil of *Citrus bergamia* (Rutaceae) and the fruits of *Ammi majus, Petroselinum sativa* and some other plants belonging to family Umbelliferae .

Test for Identification (see following table)

Test	Xanthotoxin	Imperatorin
1. Triturated few crystals in a porcelain dish with **concentrated H_2SO_4.**	Orange yellow color that turns to light green.	Deep orange color that turns to brown.
2. Test with **Marquis reagent**.	Orange color that turns to grass green.	Orange color that turns to brown.
3. Reduction of **ammoniacal silver nitrate** and **Fehling's solution**.	No reduction	Reduction
4. Test with **Wagner's reagent**.	Precipitate	No precipitate
5. Boil with **dilute HNO_3**.	Yellow color turns to crimson on treatment with alkali.	Yellow color turns to purple on treatment with alkali.

Uses

- **Xanthotoxin** is used in the treatment of **leucodermia** by increasing the level melanin pigments in the skin on exposure to ultraviolet light; where the *stratum corneum* is thickened and an inflammatory reaction initiated.
- It is also used to increase tolerance to sunlight and in the treatment of idiopathic **vitiligo**, mycosis fungoides, and **psoriasis**.
- It has no effect on leucodermia due to infections or trauma.
- It is sometimes used orally an initial high dose is given then the lowest effective maintenance dose in prescribed.
- It is contraindicated in case of patients suffering from liver diseases, lupus, erythematosis or other diseases associated with light sensitivity.

Toxicity signs

Nausea, vomiting, headache, edema, dizziness, nervousness, insomnia, pruritis, mental excitation and mental depression, undesirable skin reaction, carcinogenicity, cytogenetic effects on eye, liver and nails.

Chapter III

Structure Elucidation of Natural Products

Natural Products and their bio-discovery

Role of natural products in modern medicine

Herbal products represent an ever-increasing percentage of the total drug market; about 50% of the significant drugs used in medicine today are natural in origin. This significant role in modern medicine is due to that:

1. They provide a number of **useful drugs that cannot be commercially produced by synthesis** such as the opium, ergot and solanaceous alkaloids, the cardiotonic digitalis glycosides and most of the antibiotics.
2. They supply **basic compounds**, which through slight modification **could be rendered more effective or less toxic** such as variations in the morphine molecule.
3. They are used as **models for production of synthetic analogues** with same physiological activities, example procaine and similar local anesthetics.
4. They may be used as **starting materials for the production of potent drugs**, through chemical or biological transformations, although exhibiting themselves little or no activity. Examples are the production of the antitumour taxol alkaloid from baccatin III and that of hydrocortisone and related steroids from stigmasterol.

Although, the future of drug development may lie in the application of molecular biology and biotechnology, yet the plant kingdom will remain a tried and true source, which has to be conserved.

Different forms of plant products

Plant products are supplied in different forms depending on:

- The purpose of use,
- The nature of the active ingredients, and
- Economic factors.

The less complicated the processing of the plant material the cheaper will be the finished product. The different forms of plant products are:

1. **Fresh plant materials**: These are mainly used in folk medicine or in flavor and perfume industries.
2. **Dried plant materials**: Examples of this group are flavoring agents, spices and drugs, such as cathartics, where the dosage is not critical.
3. **Acellular products**: These are materials, which are derived directly from the plant by some physical processes, such as gum exudates, resins, balsams and fixed and volatile oils.
4. **Galenical preparations**: These are plant extracts or tinctures that may be directly used or further processed.
5. **Processed extracts**: These are extracts that have been standardized so that the concentration of the active principle is known.
6. **Pure compounds**: These are the most desirable for pharmaceutical formulations to facilitate proper standardization of biological activity, as well as, quality control.

Plant constituents

Primary and secondary plant metabolites

Plant constituents comprise a wide variety of organic substances that are formed and accumulated by plants. They include:

- **The primary metabolites** such as carbohydrates, proteins, fats, and nucleic acids which are **essential for life** and are commonly **present in all organisms.**

- **The secondary metabolites**, which may be, produced as a **defense against predators**, as volatile or colored **attractants**, as well as, **detoxifying agents**. They include most of the **pharmacologically active** plant products. They are **found in specific organisms or group of organisms**.
- However, **some** group of natural products **could be assigned to both division e.g. fatty acids and sugars**. Most of these compounds are described as primary metabolites, whilst some representatives are of rare occurrence and characteristic to certain plant species and are thus closer to secondary metabolites.

Factors influencing the production of plant secondary metabolites

The production of plant secondary metabolites is influenced by **three** major **factors**:

- **Heredity or genetic composition** that induces both qualitative and quantitative changes.
- **Ontogeny or stage of development** , and
- **Environmental changes** that result mainly in quantitative variations.

Classification of plant constituents

Plant constituents can be classified in different ways. They are mostly of complex chemical nature. They include hydrocarbons, oxygenated, organo-sulphur and organo-nitrogen compounds.

They may be:

- **Single chemicals** such as glycosides, terpenoids, steroids, phenylpropanoids and alkaloids, or
- **Mixtures** such as gums, fixed oils, fats, waxes, volatile oils, resins and resin combinations.

A **classification** of plant constituents **based on biosynthetic origin**, **solubility properties** and the **presence of key functional groups** is considered the most convenient. In this way the following classification was proposed:

1. **Phenolic compounds:**
   - These include a wide range of plant substances, which are recognized by their **hydrophilic** nature and their common origin from the aromatic **precursor shikimic acid**.
   - They possess **at least one aromatic ring** with one or more oxygenation sites.
   - Examples of plant phenolics are the **flavonoids and their glycosides**, the **phenyl propanoids, anthocyanins, xanthones, tannins and quinones.**
2. **Terpenoids:**
   - These are characterized by being **lipophilic** in nature.
   - They are biosynthetically **derived from isopentenyl pyrophosphate**.
   - They are **subclassified according to the number of their isopentene** (or isoprene, C = 5) **building units** into: mono- (C = 10), sesqui- (C = 15), di-(C = 20), tri- (C = 30), tetra (C = 40), and poly (C = ∞) terpenoids.
   - Mono- and sesqui-terpenoids are the main **constituents of volatile oils,** and **carotenoids** are example of tetraterpenoids.
   - **Steroids** are derived from isoprene **through formation of squalene**.
3. **Organic acids, lipids and related compounds:**
   - They have common **acetate precursor**.

- Examples of **simple organic acids** accumulating in plants are citric, malic, fumaric, oxalic, tartaric, malonic, shikimic, quinic and ascorbic acids.
- **Fatty acids** occur mainly in esterified forms with glycerol or higher aliphatic alcohols in the form of **fixed oils**, **fats or waxes**.
- Other compounds derived from acetate are **alkanes**, **polyacetylenes** and certain organo-sulphur compounds.

4. **Nitrogen containing compounds:**
   - These are distinguished from other classes by being usually **basic**.
   - They give a **positive response to either ninhydrin or Dragendorff's reagents.**
   - Examples are amino acids, peptides, enzymes, amines, **alkaloids**, **cyanogenic glycosides**, and chlorophylls.
5. **Water-soluble carbohydrates and their derivatives**:

   This group includes the **mono- and oligosaccharides** and the **water-soluble glycosides**.
6. **Macromolecules:**

   These can be separated from other plant constituents based on their **high molecular weight** such as the **nucleic acids**, **proteins and polysaccharides.**

Extraction, isolation, characterization and quantitative evaluation of plant constituents

The **phytochemical investigation** of a plant material involves the **extraction** of the plant material; **separation**, **isolation**, **characterization** and **quantitative evaluation** of the constituents of interest, and determination of their biosynthetic pathways.

Strategies for the **fractionation** of the plant extracts **based on biological activities** rather than on a particular class of compounds have been developed to meet the increasing interest in the potentialities of medicinal vegetable drugs. The chemical examination follows after the isolation of the active fraction.

Extraction of the plant material

- The term “extraction” is used, pharmaceutically, to indicate:

"The process used for **separation of the medicinally active portions** of plant or animal tissues **from the inactive or inert components** by using **selective solvents** in standard **extraction procedures".**

- The products, thus obtained, are relatively impure liquids, semisolids or powders intended for oral or external use.

Preparation of the plant sample for extraction

Before extraction the plant material should be:

1. Carefully selected to avoid the use of adulterated, contaminated or infected samples.
2. Carefully authenticated by a taxonomist.
3. Directly immersed in boiling alcohol within few minutes of its collection to stop enzymatic activity in case of fresh samples. These could be safely stored in dry plastic bags for few days.
4. Dried, if needed, before extraction under controlled conditions, as quickly as possible and in a good air draft.

Selection of the mode of extraction

Selection of the mode of extraction depends mainly on:

1. The texture and water content of the plant material
2. The type of substance to be isolated

Selection of the solvent

- Selection of the solvent suitable for extraction depends on the composition of the plant material. Based on their physiological activity, two major types of constituents are distinguished:
  1. The "**active constituents**" which are responsible for the therapeutic effect. Rarely, the plant contains **only one active constituent**. Most frequently, **a series of structurally related compounds**, often with similar pharmacological activity are found.
  2. The "**inert constituents**" that include **inert structural matter** (cellulose, lignin, suberin, and cutin) and **substances with no definite pharmacological** activities such as starches, albumin and coloring matters.

- The solvent of choice is that which will either:
  1. Selectively dissolves the active material, or
  2. Removes the inactive or inert material to leave higher concentrations of active compounds in the plant samples.

Factors influencing solubilization

- The most important factor influencing the solubility of material is **the polarity of the solvent and solute molecules**.

In general, **"the solubility of a solid in a liquid is a measure of the affinity of molecules of the solvent for molecules of the solute over their affinity to each other".** For efficient solubilization, the solvent molecules must have a high attraction for the solute molecules.

- In non-polar solvents, the **only force** present between molecules is that of **"dispersion"**.

- When solvent molecules have a permanent dipole, **"dipole-dipole forces"**, they are more strongly attached to each other and resist the introduction of a non-polar molecule (delay or hinder solubilization).

- **"Hydrogen bonds"** formed between molecules of certain solvents and electron-rich atoms of solute and may help solubilization.

- As a general **empirical rule**:
  1. **Non-polar solvents** such as light petroleum and hexane will dissolve non-polar compounds such as fats and waxes.
  2. **Polar solvents** such as, ethanol, methanol and water dissolve polar compounds such as alkaloidal salts and sugars.
  3. The use of **solvent mixtures** may increase the solubility of certain compounds.

- In **order of increasing polarity**, the following are the most common solvents used in plant extraction: cyclohexane, carbon tetrachloride, benzene, ether, chloroform, acetone, ethyl acetate, ethanol, methanol, water, acids and bases.

Principal methods for extraction

1. **Maceration:**

   The plant material is **immersed** in the suitable solvent, in a stoppered container, and allowed to stand for a period of at least **three days**, at **room temperature** or **in a warm place**, with frequent **agitation**. After filtration, the filtrate is adjusted to volume if needed.

2. **Percolation:**

   The ground plant material is subjected to **maceration** followed by a **continuous flow** of **fresh solvent** and **a continuous drainage of the extract** in a specially designed glass or metal equipment known as **percolator**.

3. **Digestion:**

   This is a form of **maceration** in which **gentle heat** is used during the whole process of extraction. The moderately elevated temperature is generally applied to increase the efficiency of the solvent without alteration of the active principles.

4. **Infusion:**

   This is a dilute solution of the readily soluble constituents of crude drugs prepared by **maceration for a short period of time with either cold or boiling water**.

5. **Decoction:**

   This process is carried for extracting thermostable water-soluble constituents by boiling with water for 15 min, straining and adjusting to volume if needed with cold water.

6. **Continuous hot extraction:**

   - This classical procedure could be described as a **repeated infusion with hot solvent**. On laboratory scale, it is carried in a **Soxhlet extractor (Figure 3).** The solvent in the flask is heated to boiling point and the vapor condensed in the reflux condenser. The hot liquid drips onto the sample, which is contained in a porous thimble. When the liquid in the extraction chamber reaches the top of the siphoning tube it flows back into the heated flask, taking with it the dissolved material.
   - The plant is repeatedly extracted and the soluble components are concentrated into the flask. For **thermolabile constituents a low-boiling point solvent is used**.
   - The process may be repeated after drying the plant powder with successive solvents of increasing polarity.

7. **Supercritical fluid extraction**:

   - The method is based on the fact that: **"above a certain temperature, and pressure, single substances do not condense or evaporate but exist as a fluid; under these conditions the gas and liquid both possess the same density, there is no sharp**

distinction between the characteristics of the two phases. This is the critical state". For water, t_c =374^{o}C and p_c =220 atmosphere and for CO_2, t_c =31^{o}C and p_c =74 atmosphere. Where, t_c is the critical temperature and p_c the critical pressure.

- Conditions above the critical temperature and pressure for a particular substance are usually used and these **supercritical fluids showed properties intermediate between those of the liquid and gaseous phases.** These properties are utilized to **maximize the extraction of plant constituents**.
- The technique involves the use of either **liquefied and supercritical fluid carbon dioxide** or **liquefied nitrous oxide** for the extraction of various plant constituents including pyrethrins and certain alkaloids.
- Several gases can be used, **the most advantageous is carbon dioxide** since:
  a. It is a readily available inexpensive natural product.
  b. It is chemically inert.
  c. It is non-inflammable.
  d. It is non-toxic.
  e. It is easy to eliminate.
  f. It has a low critical temperature (31 ^{o}C at P=74 bar.)
- Despite of the expenses relative to other extraction methods, **supercritical fluid extraction using carbon dioxide is currently spreading due to the following advantages:**
  a. The extracts obtained are of very close composition to natural products.
  b. The temperature and pressure could be changed, by fine-tuning, to adjust selectivity and viscosity (simultaneous extraction and fractionation).
  c. The absence of hydrolysis or rearrangements in the product components.

8. **Extraction by distillation**

- **Steam distillation** is much used to isolate volatile oils, hydrocyanic acid and other volatile constituents from plant materials. The presence of water allow the process to be carried at a temperature below 100^{o}C (Dalton's law of vapor pressure) to avoid decomposition or polymerization of thermolabile constituents.

- **Thermo-micro-distillation**: involves steam distillation on a semi-micro scale for the **direct transfer of volatile materials from a powdered drug to a thin-layer plate**. The procedure is performed in special ovens known as **thermo-micro-analysis and**

separation ovens (**TAS ovens**), **Figure (3),** which permits the rapid steam distillation of very small quantities of a drug sample and the retention of the distillate for analysis. The method is used for volatile constituents such as essential oils and certain alkaloids and is considered as an **analytical separation procedure rather than a preparative extraction method**.

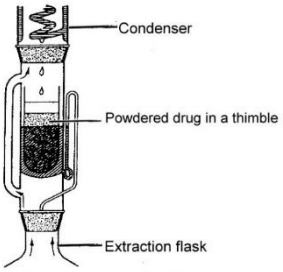


Fig.3A. Soxhlet continuous extraction apparatus

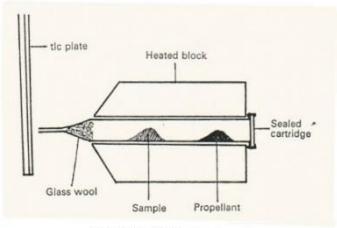


Fig.3B. TAS oven

Fractionation of crude extracts

- Fractionation is desirable to separate the main classes of constituents present as complex mixtures in the crude extracts to facilitate further isolation of their individual components.
- Before fractionation crude extracts are generally clarified by filtration through celite and concentrated under reduced pressure in a rotary evaporator at temperatures not exceeding 30-40 ^{o}C.

Several procedures are adopted such as:

1. **Solvent / solvent precipitation** :
   - The concentrated **extract** is **mixed with a less polar miscible solvent** causing the selective precipitation of the less-soluble plant constituents. Examples are:
     - The precipitation of crude saponin mixtures such as that of *Phytolacca decandra* by addition of acetone to its methanolic extract.
     - The precipitation of gum from the aqueous extract of *Olibanum* by addition of alcohol.
   - The **extract** may also be **treated with a more polar solvent** in which the required constituent is insoluble such as the precipitation of resins from alcoholic extracts by addition of water or acidulated water.

2. **Liquid / liquid extraction:**

- This technique involves the **partition of the solute molecules between two immiscible solvents.**
- The amount of solute in each phase depends upon the relative solubility in each solvent, which in turn is related to their polarity. It is measured by the **partition coefficient**, which, for any system, is constant, provided that neither phase becomes saturated with the solute molecules.

$$\text{Partition coefficient: } K = \frac{\text{Mole fraction of solute in phase 1}}{\text{Mole fraction of solute in phase 2}}$$

The success of the method depends on both the selectivity of the solvents for the required compounds and the technique of extraction whether carried out by **single or multiple contacts** or by more improved and refined discontinuous and continuous **counter-current techniques.**

i. **Single contact:**

A given volume of the crude extract in the selected solvent (stationary phase) is shaken once with a certain volume of an immiscible solvent (mobile phase) in a separating funnel and the two layers separated.

ii. **Multiple contact:**

Several successive extractions are carried out using separate portions of the mobile phase in a multiple contact manner with the same volume of the stationary phase containing the required solutes. This laboratory procedure, using a series of separating funnels results in better extraction of the solutes until almost complete exhaustion.

iii. **Counter-current Extraction (CCE):**

- This technique can be used to separate compounds with small differences in their partition coefficient.
- It is carried in a special apparatus called "**Craig's counter-current apparatus**" which relies on the same principle as ordinary liquid / liquid extraction except that it is a **multiple partition process**, each extraction being separate and step-wise in nature.

- There may be a hundred of separating chambers (special tubes), each formed of two compartments **(Figure 4)**.
- Equilibration and transfer are carried out automatically. **The heavier, stationary phase is placed in the apparatus and the lighter, migratory (mobile) phase is moved along the apparatus after successive equilibration in the apparatus units**.

The **greater the partition coefficient** of a solute in the migratory phase, the **faster it will migrate through the apparatus.**

- The distribution of a single compound with K = 1 along the vessels of Craig's counter-current apparatus are illustrated in **Figure (4)**

iv. **Droplet-counter-current technique (DCC):**

- This is an extension of CCE in which fractionation is achieved by passing droplets of the mobile phase through an immiscible liquid stationary phase, thus the solutes are distributed between the two phases.
- The liquid stationary phase is held in glass tubes (or columns) of narrow diameter, from 200-600 in number, and connected with Teflon tubes.

- The **glass tubes are filled with the liquid stationary phase** and the **mobile phase containing the sample is introduced into the first column**: from the **bottom**, if a **lighter mobile phase** is used (**ascending technique**) or at the **top**, if a **heavier mobile phase** is used (**descending technique**).

- The process continues by the flow of more mobile phase and fractions containing the solutes are collected at the end of the apparatus. A schematic representation of DCC is represented in **Figure (5).**

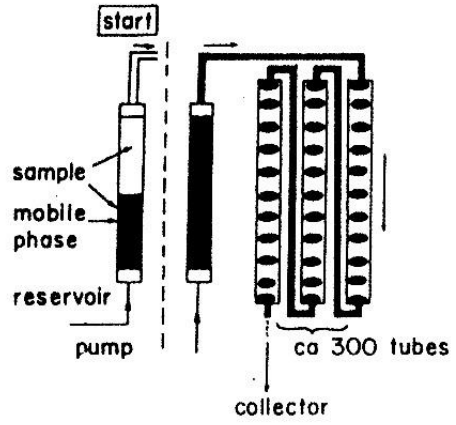
The **advantage of this technique over CCE** is that it can be easily applied to the separation of **saponins** as no shaking is applied during the process so that foaming is prevented. The method is useful in separation of highly polar compounds, such as alkaloids, tannins, iridoids, anthraquinones etc….

v. **Liquid / liquid acid-base extraction technique:**

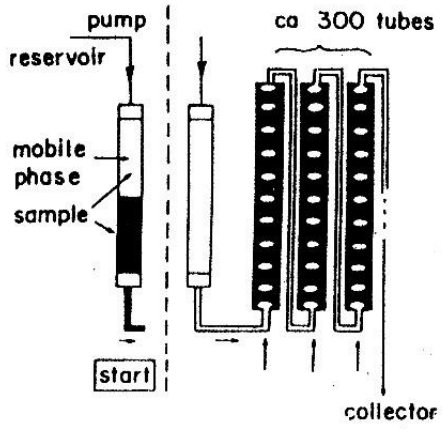
- This method is applied when the required solute is acidic or basic and achieves a high degree of separation by alteration of the pH of the aqueous phase in a liquid / liquid partition system.

- As a general rule, salts being ionic are soluble in polar and insoluble in non-polar solvents. For examples alkaloidal bases are soluble in chloroform and on acidification their salts will be extractable with water.

- In the same way, carboxylic acids and phenols are soluble in chloroform and on alkalinization their salts will be extracted by water.



A- Descending mode



B- Ascending mode

Fig.5. Schematic illustration of DCC apparatus

vi. **Preparation of acellular products:**

Acellular products are plant isolates resulting from specific methods of preparation directly from plant material. Chemically, they are complex mixtures. They could be obtained by:

- **Exudation** from certain trees after the bark has been damaged or removed, followed by **collection of the exudate** such as balsams and resins.
- **Cold expression** of the fresh plant material such as fixed oils.
- **Steam distillation, solvent extraction** or in certain cases **scarification** followed by **expression** as for volatile oils.

Separation and Isolation of Individual Plant Constituents

The most difficult step in the phytochemical investigation of a plant material is the isolation and purification of individual constituents after fractionation of the original extracts.

- The **chemical properties** of functional groups and moieties contained in compounds such as aldehydes, phenols and alkaloids can be used as basis for their separation from other materials through derivatization.

- However, such chemical methods may well not fractionate components within the same class. Therefore, in this area various **physical techniques** are rather used. These are sublimation, fractional distillation, fractional liberation, fractional crystallization and the various old and new techniques of chromatography.

1. **Sublimation:** This may be directly performed on the crude drug such as : the isolation of **caffeine from tea** and that of balsamic acids (benzoic and cinnamic acids) from balsams. It can also be used for purification of materials present in crude extracts. Modern techniques allow now a strict control of temperature and pressure.
2. **Fractional distillation:** This is a traditional method for separation of components of volatile mixtures such as **volatile oils**.
3. **Fractional liberation:** This could be achieved through the use of **pH gradient**, an example is the separation of a **mixture of alkaloidal salts** in aqueous solution. Thus, on addition of aliquots of alkali, the weakest bases will first be liberated in the free state followed by base liberation in ascending order of basicity. If the mixture is shaken after each addition with organic solvent, then a fractionated series of bases will be obtained.
4. **Fractional crystallization:** This method is based on that the components of a mixture have different solubility in a particular solvent. Frequently, derivatives of the components are used rather than the original compounds, such as the picrates of alkaloids and the osazones of sugars.
5. **Chromatography:** The separation and purification of plant constituents is mainly carried out using one or a combination of chromatographic techniques, such as preparative paper (PC), thin layer (TLC), gas (GC) and High pressure liquid chromqtography (HPLC). In addition more specific techniques are also used such as, ion exchange, gel filtration and affinity chromatography.

Identification of Plant Constituents

Once a plant constituent has been isolated and purified, it is necessary to determine the class of compounds to which it belongs and then to find out which particular substance it is within the class.

1. **Check for homogeneity and purity:** This is carried by repeated chromatography in several TLC and/or PC systems: the compound should travel as a single spot.
2. **Determination of the class of the compound:** This is usually clear from its response to colour tests, its solubility and R_f properties and its UV spectral data.
3. **Identification within the class:** This depends on measuring other properties and then comparing with published data. These properties include:
   - Melting points for solids and boiling points for liquids.
   - Optical rotation for optically active compounds.
   - R_f and RR_t values under standard chromatographic conditions.
   - Spectral characteristics including ultra violet (UV), infra red (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) measurements.
4. **Confirmation of identity:** This is performed by direct comparison with an authentic sample if available or by comparison with published data. For new compounds, it is preferable to confirm the identity by degradation and laboratory synthesis.

Quantitative Determination of Plant Constituents

- Crude plant **extracts** should be **standardized before use** in therapy.
- **Determination of the total amount of the active components** can be carried by several methods that depend mainly on the nature of the constituents to be determined. Procedures are liable to errors due to the interference of other constituents. The most commonly used methods are:

1. **Gravimetric methods:** A known weight of plant material is used and the amount of the isolated product determined and the yield calculated on dry weight. The losses during extraction and purification can be calculated by adding a known amount of a pure substance to the crude extract, repeating purification and determining the amount recovered (% recovery).
2. **Titrimetric methods:** Examples are the acid-base determinations of alkaloids in aqueous or non-aqueous media, and redox determination of sugars and peroxides.

3. **Spectrophotometric methods:** These are mostly colorimetric methods, based on measuring the absorbance of a solution of the purified extract in the presence of a specific color developing agent in a suitable spectrophotometer (or colorimeter). The intensity of the color produced should be proportional to the concentration of the determined constituents (following Beer's law), under the experimental conditions. The percentage may be calculated by referring to a standard curve.
4. **Biological methods:** These are special methods for standardization of extracts that contain toxic materials and failed to be accurately evaluated with other methods e.g. cardiac glycosides.

5. **Chromatographic methods:** Ideally, the determination of individual components within the class should be determined; this is readily achieved by chromatographic techniques such as HPLC and GLC.

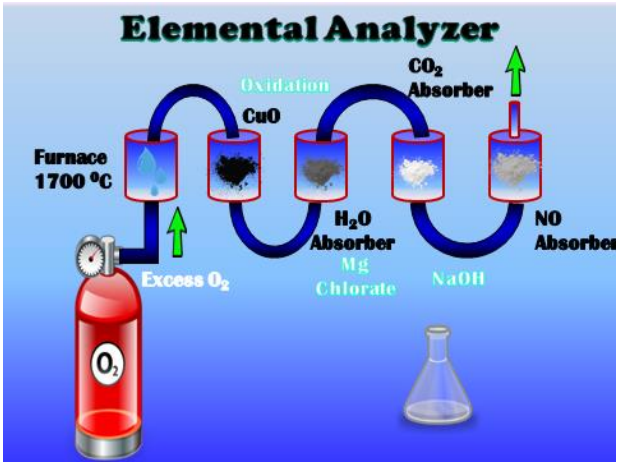
Structural analysis of natural products

In order to predict the structure of a natural compound several techniques can be applied and the sum of their results can reveal the structure of the compound.

1- Elemental analysis

The components of an organic natural compound and the percentage of each atomic species in the molecule is given by several methods.

- Carbon is determined by combustion as CO_2.
- Hydrogen is determined by combustion as H_2O.
- Nitrogen is converted into NaCN.
- Halogens are converted to inorganic salts.
- Sulphur is determined as oxides of sulphur.
- Phosphorous is determined as metallic P.



At the end of these procedures, a sheet of information is reached, indicating the percentage of each component.

<u>Calculations:</u>

An organic compound gave the following percentages in elemental analysis: C = 48.76 % and H = 8.07%, deduce the molecular formula.

1- Oxygen percentage = 100 - (C% + H%)

O% = 100 - 56.83 = 43.17.

2- Each atomic percentage is divided by its atomic weight.

$\frac{C}{12}$: $\frac{H}{1}$: $\frac{O}{16}$: $\frac{N}{14}$

$\frac{48.76}{12}$: $\frac{8.07}{1}$: $\frac{43.17}{16}$

= 4.06: 8.07: 2.69

3- The resulting figures are then divided by the least figure in the element group.

$\frac{4.06}{2.69}$: $\frac{8.07}{2.69}$: $\frac{2.69}{2.69}$

= 1.51: 3: 1

4- This ratio is translated into integral numbers by multiplying by any number.

1.5 : 3 : 1 multiplied by 2

= 3.0 : 6 : 2

Giving the molecular formula $C_3 H_6 O_2$

2- Determination of rings and double bond equivalent (DBE):

Using the molecular Formula obtained from the elemental analysis the number of rings and or/and double bonds in the molecule can be calculated. In order to form a double bond, two protons should be eliminated:

$$CH_3\text{–}CH_3 \xrightarrow{-2H} CH_2 = CH_2$$

Thus each double bond or ring in the molecule is equivalent to two protons. Numbers of double bond or rings (i.e. double bond equivalent) can be calculated using the following equation: if the molecular formula is $C_a : H_b : O_c : N_d$

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

where:

a is number of tetravalent atoms (C, Si)

b is number of monovalent atoms (H, Cl, Br)

d is number of trivalent atoms (N,P)

the number of divalent atoms (O,S) does not play a role here.

Examples:

1- n-hexane C_6H_{14}

DBE= ((12+2)-14)/2 = 0, so there is no double bonds or rings

2- Cyclohexane C_6H_{12}

DBE= ((12+2)-12)/2 = 1, so there is one double bond or ring (here we know it's a ring)

3- Benzene C_6H_6

R= ((12+2)-6)/2 = 4 and this is for four double bonds and/or rings (in this case 3 double bonds and one ring)

4- Using this molecular formula: $C_3\ H_6\ O_2$

DBE= ((6+2)-6)/2= 1 this means one double bon or one ring

5- Using this molecular formula: C_5NH_5

DBE= ((10+2)-(5-1))/2 = 8/2 = 4 meaning 4 double bonds or rings or both

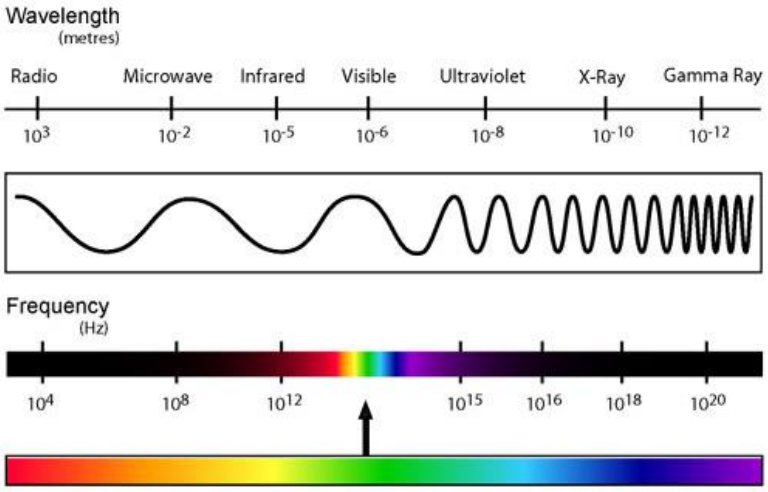
Note the following:

- One saturated ring is equivalent to one DBE
- One aromatic ring is equivalent to four DBE: one ring and three double bonds
- A triple bond is equivalent to two DBE

3- Spectroscopy

Electromagnetic radiation:

Electromagnetic radiation is the transmission of energy in the form of waves having both an electric and a magnetic component. The forms of electromagnetic radiation are radio waves, infrared radiation, light waves, ultraviolet light, X rays and gamma rays, all of which constitute the electromagnetic spectrum . All of these forms are essentially the same physical phenomenon, differing principally in the wavelength (λ) and frequency (ν) of the radiation.



The electromagnetic radiation spectrum

All electromagnetic waves travel through empty space with the same velocity c, the velocity of light which equals to 299,792 km/sec. Electromagnetic radiation is characterised by its frequency ν or wavelength λ and these two quantities are related:

$$\nu \lambda = c$$

$$\text{So: } \nu = c / \lambda$$

The relation between the energy and the velocity of light is given by the following equation:

$$E = h \nu = h c / \lambda$$

Where h is Planck's constant

Thus: $E \alpha \nu$

Thus: $E \alpha c / \lambda$

So: $E \alpha 1 / \lambda$

So as the frequency of an electromagnetic wave increase its energy increases and when the wavelength increase the energy decreases.

Definition of Spectroscopy:

The word 'spectroscopy' is used as a collective term for all the analytical techniques based on the interaction of light and matter. Spectrophotometry is one of the branches of spectroscopy where we measure the absorption of light by molecules that are in a gas or vapour state or dissolved molecules/ions.

Spectroscopy is the use of light, sound or particle emission to study a substance and investigate its structure and properties. There are many spectroscopic techniques, however the main four techniques with most applications are:

1- Ultraviolet spectroscopy (UV)

2- Infrared spectroscopy (IR)

3- Nuclear Magnetic resonance (NMR)

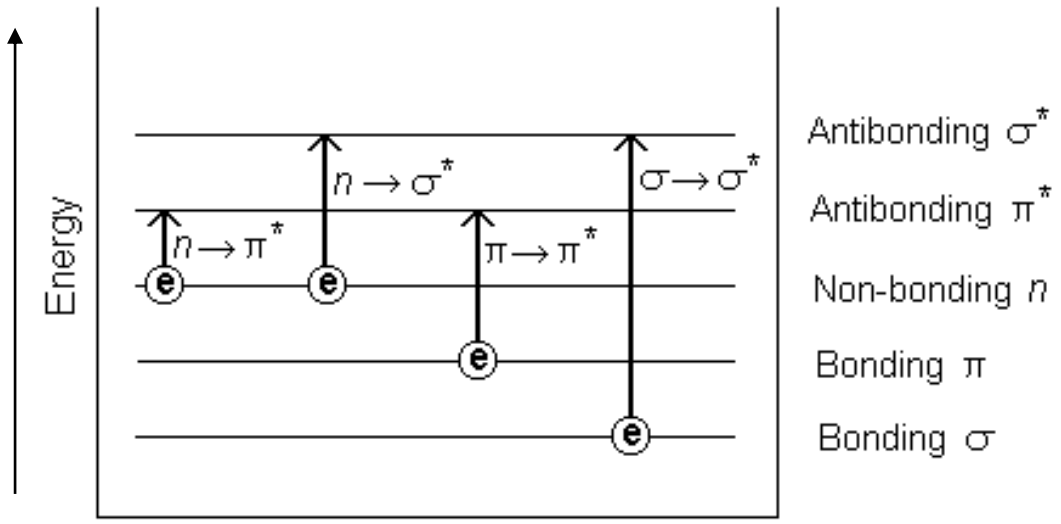
4- Mass spectroscopy (MS).

A) Ultraviolet Spectroscopy

Theory and principles

Electronic excitation:

The absorption of light energy by organic compounds in the visible and ultraviolet region of the spectrum involves electron transition from the ground state molecular orbitals σ, π, and n to the higher-energy antibonding orbitals σ* and π* . As the n electrons do not form bonds, there are no antibonding orbitals associated with them.



The electron transitions between different molecular orbitals

The electronic transitions that are involved in the ultraviolet and visible regions are of the following types: π → π* and n → π* because the energy required by

other transitions is very high and cannot be supplied by the UV or visible light energy.

Consequently, compounds in which all valence shell electrons are involved in single-bond formation, such as saturated hydrocarbons, do not show absorption in the ordinary ultraviolet region.

Transitions to antibonding π* orbitals are associated only with unsaturated centres in the molecule; these are of lower energy requirement and occur at longer wavelengths, within the region of the ordinary ultraviolet spectrophotometer. π → π* are associated with double bonds and conjugation and n → π* are associated with atoms with lone pair of electron such as oxygen and nitrogen.

A classical example of such transitions is saturated aldehydes and ketones which exhibit an absorption of low intensity around 285 nm, which is attributed to an n → π* transition, and an absorption of high intensity around 180 nm, which is attributed to π → π* transition.

Instruments

In order to obtain useful information from the ultraviolet or visible spectrum of a compound the wavelength of maximum absorption (λ_{max}) and the intensity of absorption (ε) must be measured accurately.

The compound should be dissolved in some suitable solvent that does not itself absorb light in the region under investigation. The most commonly used solvent for ultraviolet spectral determinations is 95% ethyl alcohol. Water and hexane are also commonly used.

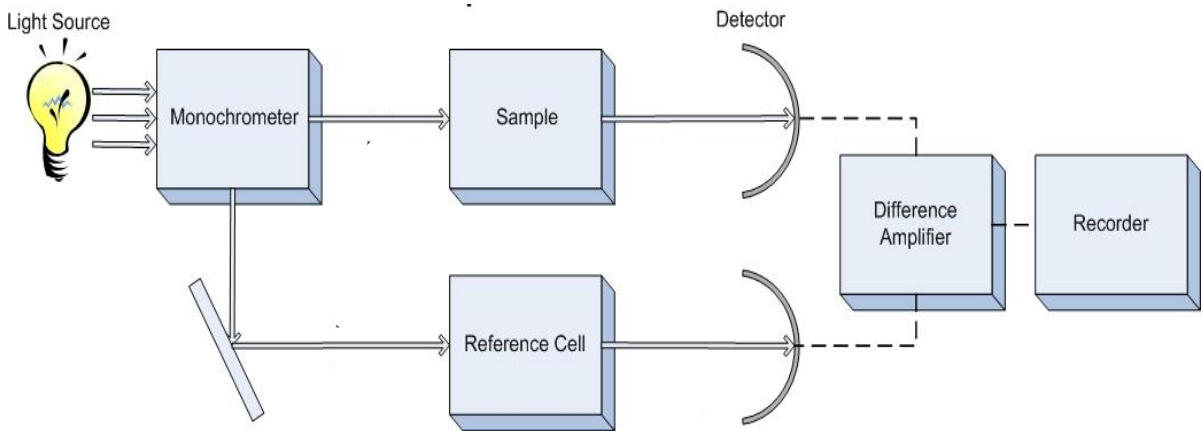
The solution must be placed in some suitable container that is transparent to UV/visible light, so quartz cells must be used in case of UV measurements, however normal glass or plastic cuvettes can be used in case of visible light measurements. The most commonly used cells have 1.0 cm path length.

UV/Visible spectra can be recorded using a machine called UV/Visible spectrophotometer which can measure both wavelength and intensity of transmitted light.

UV/Visible spectrophotometer

Light source: The most suitable source of light in the ultraviolet region (180- 400 nm) is the hydrogen-discharge lamp. A tungsten-filament lamp is usually used for the visible region (400-800 nm) of the spectrum.

Most spectrophotometers are double beam instruments. The primary source of light is split into two beams, one of which passes through a cell containing the sample solution and the other which passes through a cell containing the reference solvent. The spectrophotometer electronically subtracts the absorption of the solvent in the reference beam from the absorption of the solution in the sample beam. Thus, effects owing to absorption of light by the solvent are minimized.



Double beam UV/Visible spectrphotometer

Definitions

1- **Chrormophore**: A covalently unsaturated group responsible for electronic absorption (for example, C=C, C=O, and NO_2).

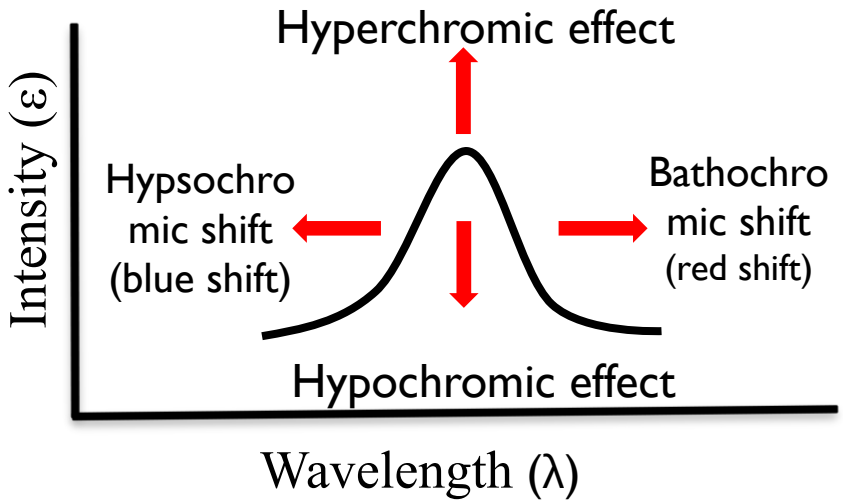
2- **Auxochrome**: A saturated group with non-bonding electrons which, when attached to a chromophore, alters both the wavelength and the intensity of the absorption (e.g., OH, CH_3, Cl).

3- **Bathochromic shift (a red shift)**: The shift of absorption to a longer wavelength due to substitution or solvent effect.

4- **Hypsochromic Shift (a blue shift)**: The shift of absorption to a shorter wavelength due to substitution or solvent effect.

5- **Hyperchromic effect**: An increase in absorption intensity.

6- **Hypochromic effect**: A decrease in absorption intensity.

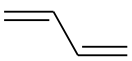


Calculation of maximum absorbance (λ_{max})

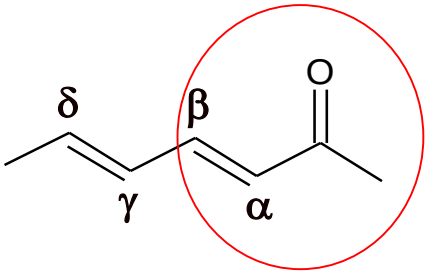
A) Woodward-Fieser Rules:

Woodward's and Fieser's rules are a set of rules that can be used to predict the position of absorption of dienes and enones

a- In dienes

	Homoannular (cisoid) 	Heteroannular (transoid) 
Chromophore (Parent or base)	253 nm	214
Increments for:		
Exocyclic double bond	5	5
Double bond extending conjugation	30	30
Alkyl substituent or ring residue	5	5
Auxochromes:		
-OH	5	5
-O-acetyl	0	0
-O-alkyl	6	6
Halogen (-Cl, -Br)	5	5
$-NH_2$	30	30
$-NR_2$	60	60

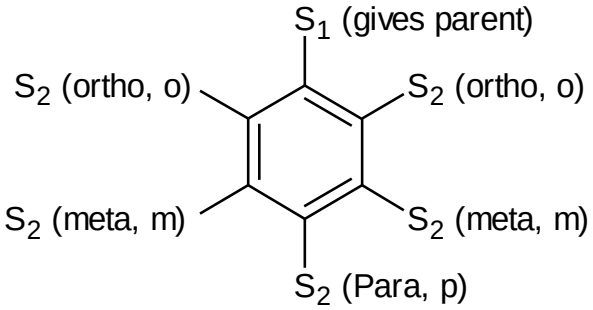
b- In enones and dienones



	α	β	γ	Δ
Chromophore (Parent or base): Aldehyde	210 nm			
Ketone	215 nm			
Acid and ester	195 nm			
Exocyclic double bond	5			
Double bond extending conjugation (over the enone system)	30			
Alkyl substituent or ring residue	10	12	18	18
Auxochromes:				
-OH	35	30	-	50
-O-alkyl	35	30	17	30
-O-acetyl	6	6	6	6
Halogen: -Cl	15	12	-	-
-Br	25	30	-	-
$-NR_2$	-	95	-	-

B) Scott's Rules for Aromatic Systems:

Benzene absorbs strongly at 184 (ε 47, 000), at 202 nm (ε 7,000) and around 250 nm and this last band is the one which will be used for calculations.



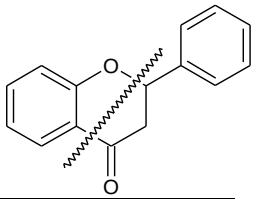
	o	m	P
Chromophore (Parent or base):			
Ethers (Ar-O-R)	246 nm		
Aldehyde or Ketone (Ar-CHO, Ar-CO-R)	250 nm		
Acid (Ar-COOH)	230 nm		
Ester (Ar-COO-R)	230nm		
Exocyclic double bond	Not applied		
Double bond extending conjugation (over the enone system)	Not applied		
Alkyl substituent or ring residue	3	3	10
Auxochromes:			
-OH	7	7	25
-O-alkyl	7	7	25
Halogen:			
-Cl	0	0	10
-Br	2	2	15
$-NH_2$	13	13	58
$-NR_2$	20	20	85

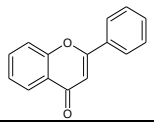
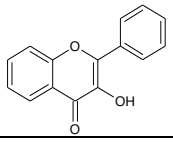
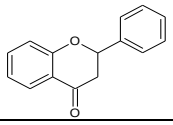
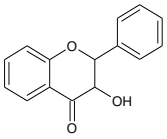
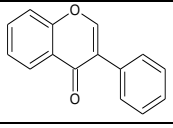
The rules listed above apply only to diene, enone or benzoyl containing compounds. If a compound does not contain one of these three chromophores, you will not be able to predict or calculate a diagnostic λ_{max} value using these tables. Also, if a compound contains one of the listed chromophores, but it has an auxochrome that is not listed, then, the best that you will be able to do is to predict a minimum value for the diagnostic λ_{max}. In these cases, the diagnostic λmax will be greater than the predicted λ_{max} using the tables. In the event that you have a compound that fits the rules, the calculated λ_{max} should be within (+/-) 3 nm of the λ_{max} observed in the UV-Vis spectrum.

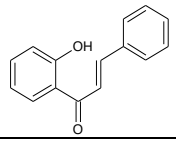
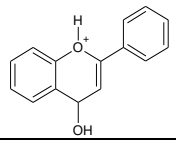
General UV Spectroscopy of flavonoids:

Generally, flavonoids produce two bands in UV spectrum: band I due to the cinnamoyl part (around 350 nm) and band II due to the benzoyl (around 250 nm). Table below shows the UV maximum absorbance for different flavonoids classes.

Band II Band I



Name	Band II (nm)	Band I (nm)	Structure
Flavone	250-280	300-350	
Flavonol	250-265	350-380	
Flavanone	270-295	300-330	
Flavanol	290	320	
Isoflavone	245-270	300-340	

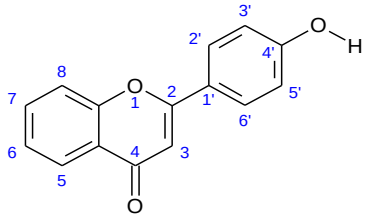
Chalcone	220-270	340-390	
Anthocyanine	270-280	460-550	

Effect of shift reagents:

Shift reagents are a group of reagents that react with free OH groups on the skeleton of a flavonoids leading to bathochromic shift in band I or band II of the flavonoids methanol spectrum.

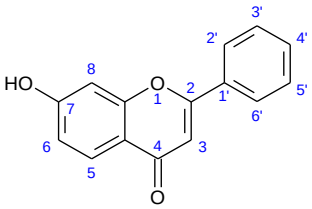
1- Effect of Sodium methoxide ($NaOCH_3$):

$NaOCH_3$ is a strong alkaline that will react with free OH on 4' in ring B, abstracting the proton and resulting in a bathochromic shift in band I by 40-80 nm. If 4' OH does not exist or it is occupied (by a glycoside for example) the shift will not occur.



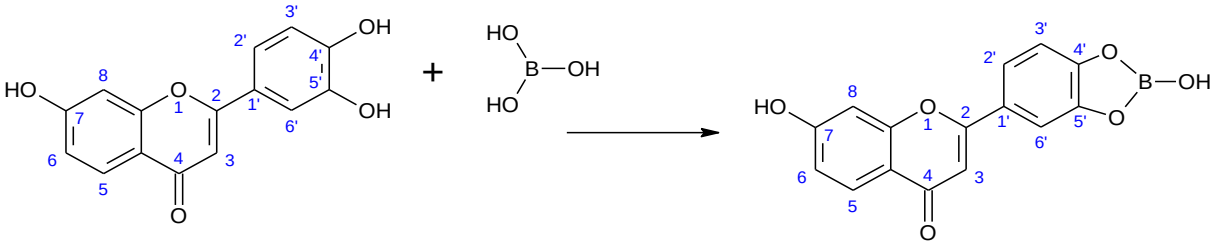
2- Effect of sodium acetate ($NaOCOCH_3$):

When the fused sodium acetate is added to the methanol spectrum it usually induces bathochromic shift of about 10-20 nm in Band II due to the presence of free OH group at C7.



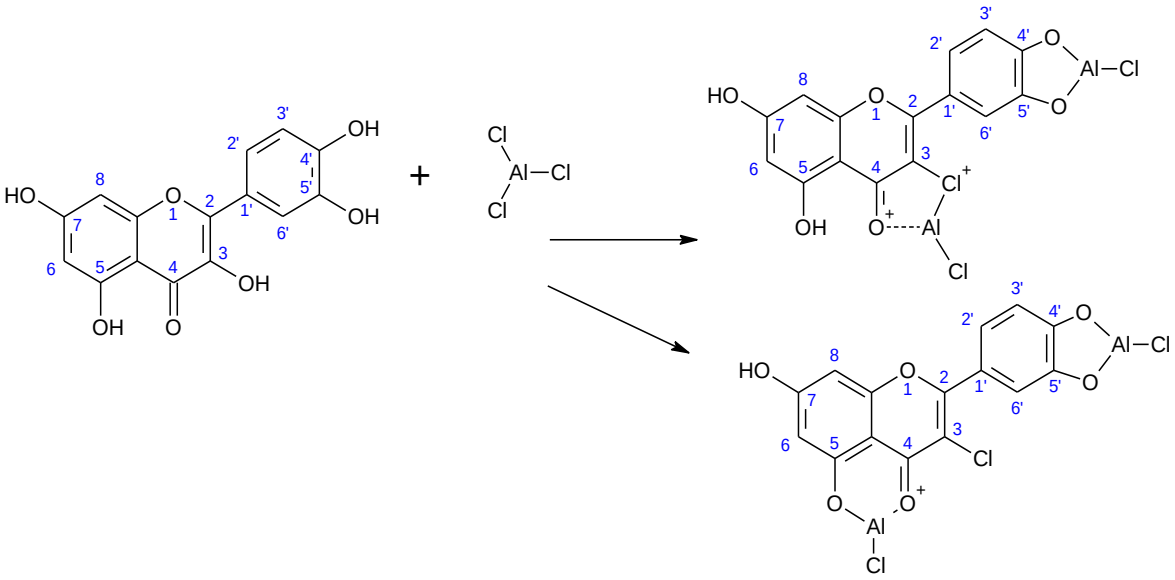
3- Effect of Boric acid (H_3BO_3):

When boric acid is added to the sodium acetate spectrum a bathochromic shift (5-20 nm) is observed when free ortho-dihydroxy groups are found on the skeleton of Flavonoid.



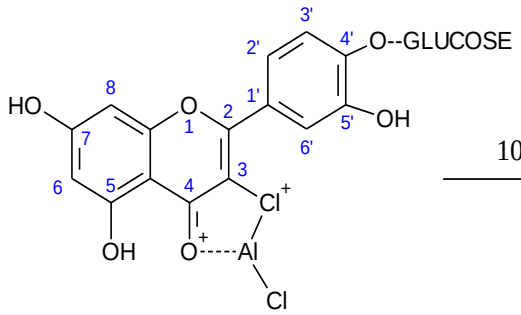
4- Effect of Aluminium chloride ($AlCl_3$):

A1C1$_3$ forms complex with any orthodihydroxy system and with C5 or C3 with the carbonyl of the pyrone system resulting in a bathochromic shift of about 40-65 nm is observed in Band I.



A1C1$_3$ complex has different stability in hydrochloric acid (HCl): The addition of 10% HC1 on the $AlCl_3$ spectrum will destroy the orthodihydroxy complex and will not affect the C3 or C5 complexes. S the addition of HCl will result in the following:

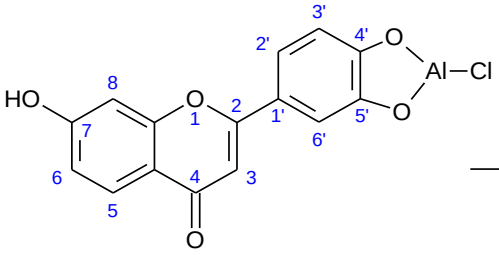
a- No change from the A1C1$_3$ spectrum, this means that C5 and C3 had free OH and no free orthodihydroxy system exist.



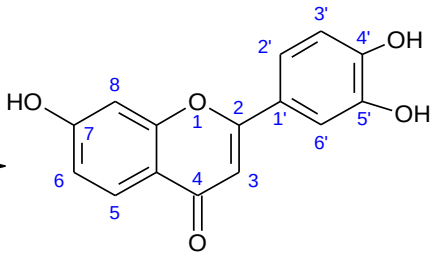
10 % HCl

No Change from the $AlCL_3$ -methanol spectrum

b- Blue shift back to the methanol spectrum, means only orthohydroxylation were exist and no C3 or C5 free OH.

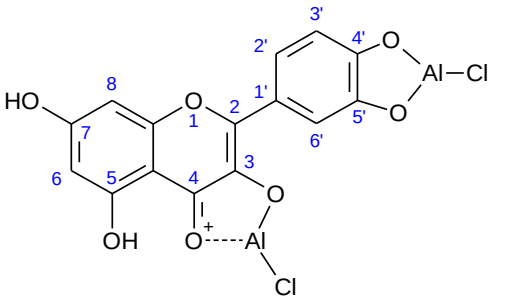


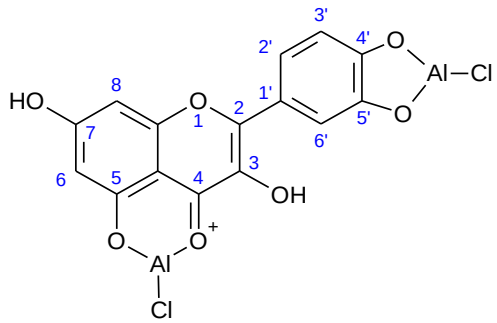
10 % HCl



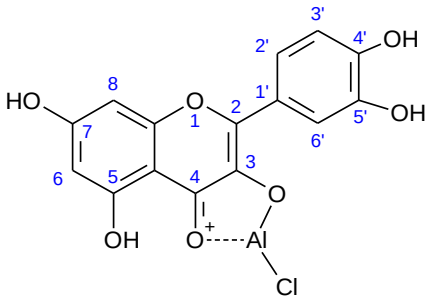
Blue shift to the original methanol spectrum

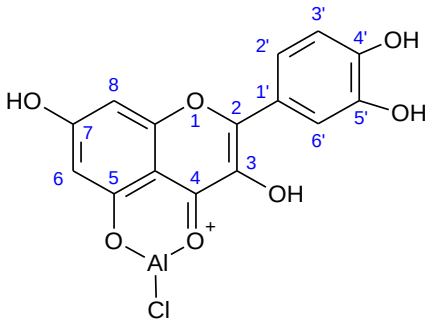
iii. Blue shift back but not completely to methanol spectrum, means orhthohydroxylation exist, and either C5 and/or C3 had free OH.





10 % HCl





Blue shift not to the original methanol spectrum

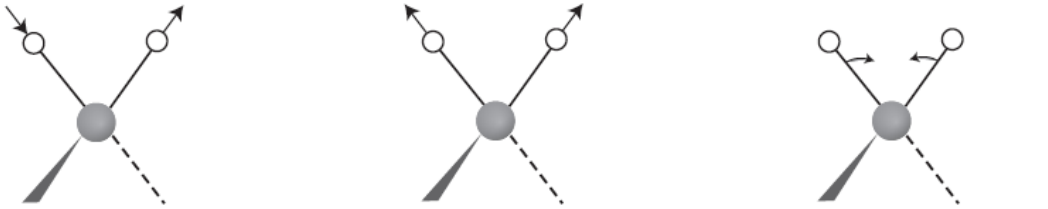
B) Infrared (IR) Spectroscopy

IR spectroscopy is one of the most widespread spectroscopic techniques structure elucidations. It involves the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample.

Theory and principles

Molecular vibration

At temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other. A molecule is not a rigid assemblage of atoms, but is said to resemble a system of balls of varying masses, corresponding to the atoms of a molecule, and springs of varying strengths, corresponding to the chemical bonds of a molecule. IR radiation causes vibrations in the molecules.

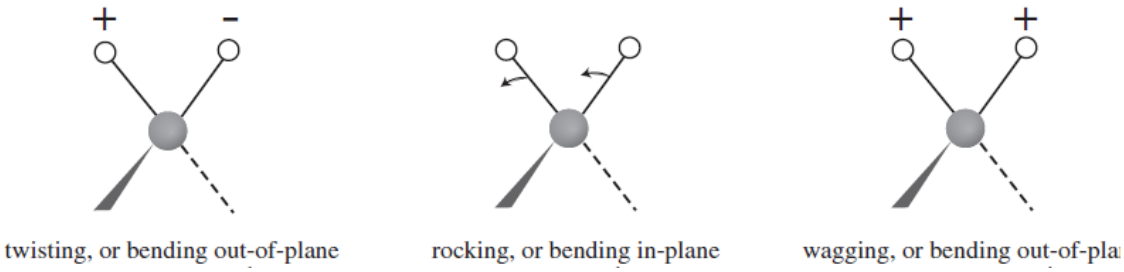


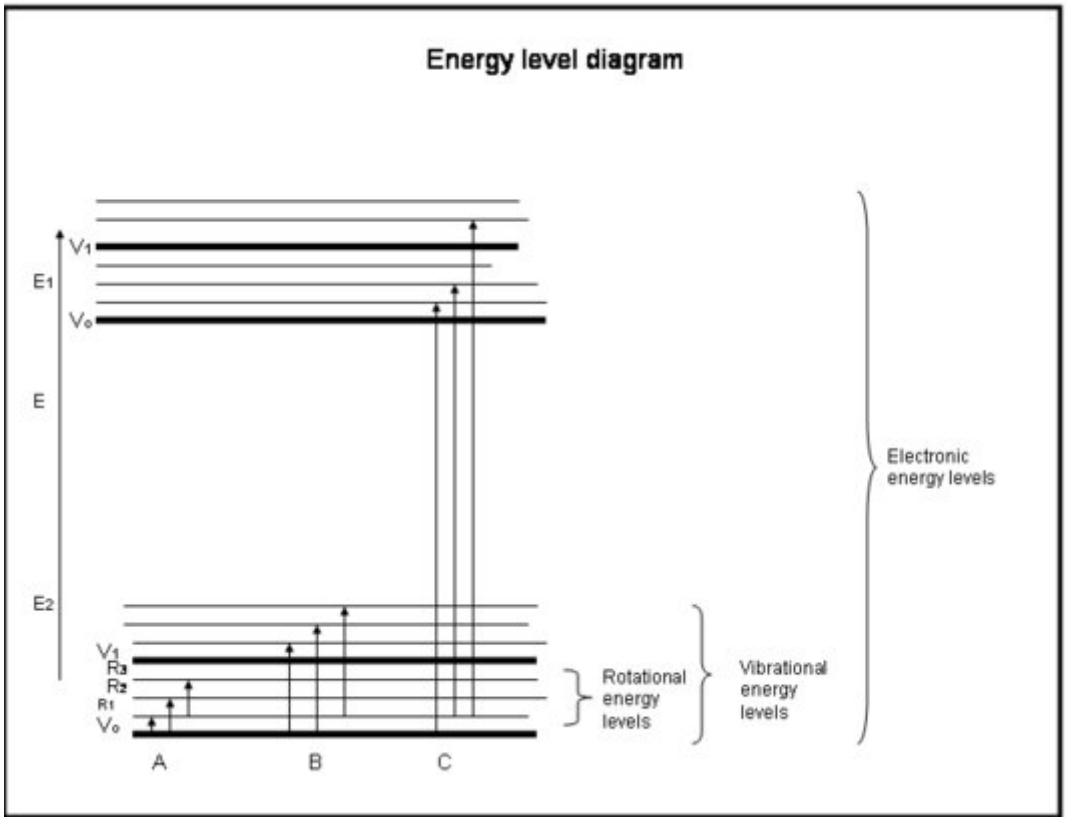
asymmetrical stretching symmetrical stretching scissoring, or bending in-plane

The major types of molecular vibrations are stretching and bending. In stretching vibrations, the distance between two atoms increases or decreases, but the atoms remain in the same bond axis.

In bending vibration, the position of the atom changes relative to the original bond axis.

When infrared light of that same frequency is incident on the molecule, energy is absorbed and the amplitude of vibration is increased. When the molecule reverts from the excited state to the original ground state, the absorbed energy is released as IR radiation. Bending vibrations generally require less energy and occur at longer wavelength than stretching vibrations.





IR Spectrum

The ordinary infrared region extends from 0.8 to 200 μm. Because of the difficulty in reading the absorption in the form of microns, the wave number (ν' in cm^{-1}) was considered. The wave number is directly proportional to the absorbed energy and the wavelength is inversely proportional to the absorbed energy.

$$\nu'_{(\text{in cm-1})} = (1/\lambda_{(\text{in } \mu\text{m})}) \times 10^4$$

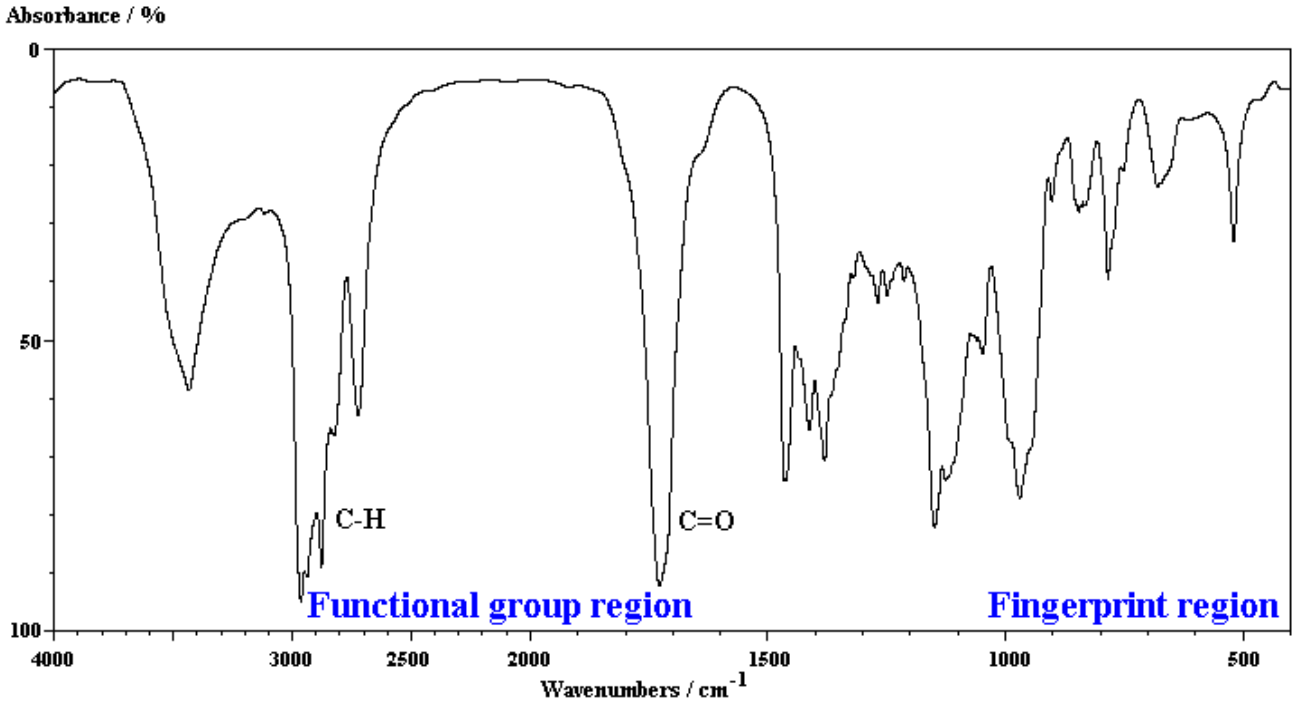
Therefore, IR region extends from 0.8 to 200 μm (12500 to 50 cm^{-1}).

- The region from 0.8 to 2.5 μm, (12500 to 4000 cm^{-1}) is called the **near infrared**.
- The region from 2.5 to 15 μm (4000 to 667 cm^{-1}) is called the **middle infrared** and it is the most informative region.
- The region from 15 to 200 μm (667 to 50 cm^{-1}) is called the **far infrared**.

Stretching vibrations occur at much higher wave number (4000 - 1600 cm^{-1}). The region to the right-hand side of the IR spectrum (from about 1500 to 500 cm^{-1}) usually contains a very complicated series of absorptions.

These are mainly due to all manner of bending vibrations within the molecule. This is called the **fingerprint** region. It is much more difficult to pick out individual bonds in this region than it is in the "cleaner" region at higher wave numbers.

The importance of the fingerprint region is that each different compound produces a different pattern of troughs in this part of the spectrum.



IR Spectrum diagram

Instruments and sample preparation

Sample is prepared for IR investigation by one of these methods:

1- Solid samples can be milled with potassium bromide (KBr) to form a very fine powder. This powder is then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR. Alternatively, solid samples can be dissolved in a solvent such as $CC1_4$, $CHC1_3$ or nujol (highly purified paraffin) and the solution is placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

2- For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride (salt). Salt is transparent to infrared light. The drop forms a thin film between the plates.

3- For gas samples: the sample is allowed to enter a special gas cell.

An IR spectrometer consists of three basic components: radiation source, monochromator (or an interferometer in Fourier Transform-type Spectrometers), and detector. The common radiation source for the IR spectrometer is an inert solid heated electrically to 1000 to 1800 °C. Three popular types of sources are Nernst glower (constructed of rare-earth oxides), Globar (constructed of silicon carbide), and Nichrome coil. They all produce continuous radiations, but with different radiation energy profiles. The monochromator is a device used to disperse a broad spectrum of radiation and provide a continuous calibrated series of electromagnetic energy bands of determinable wavelength or frequency range. Prisms or gratings are the dispersive components used in conjunction with variable-slit mechanisms,

mirrors, and filters. Most detectors used in dispersive IR spectrometers can be categorized into two classes: thermal detectors and photon detectors. Thermal detectors measure the heating effect produced by infrared radiation. Photon detectors rely on the interaction of IR radiation and a semiconductor material. Nonconducting electrons are excited to a conducting state. Thus, a small current or voltage can be generated. Thermal detectors provide a linear response over a

wide range of frequencies but exhibit slower response times and lower sensitivities than photon detectors.

Infrared spectrophotometer

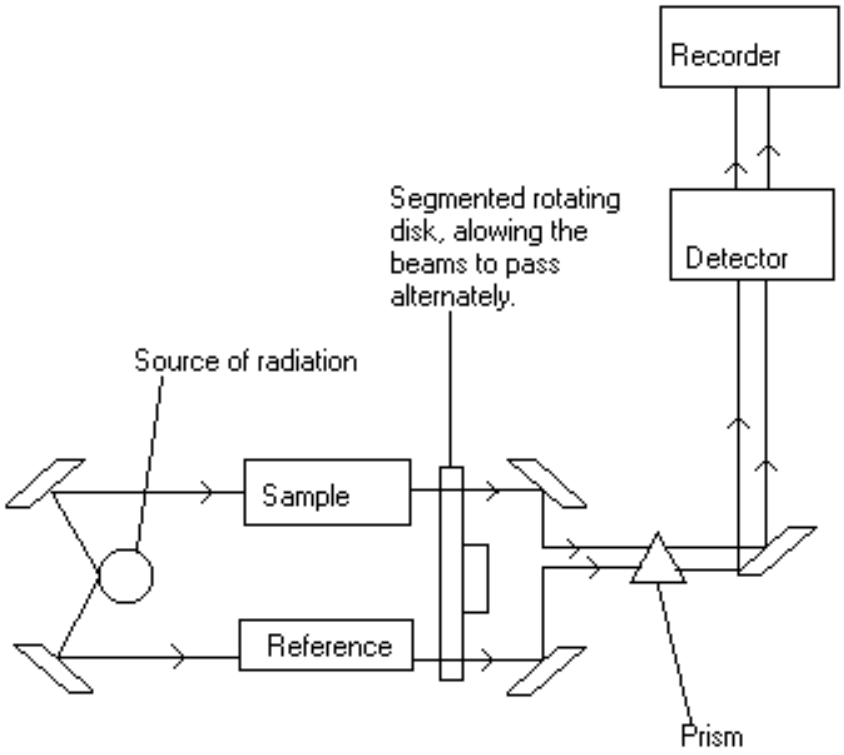
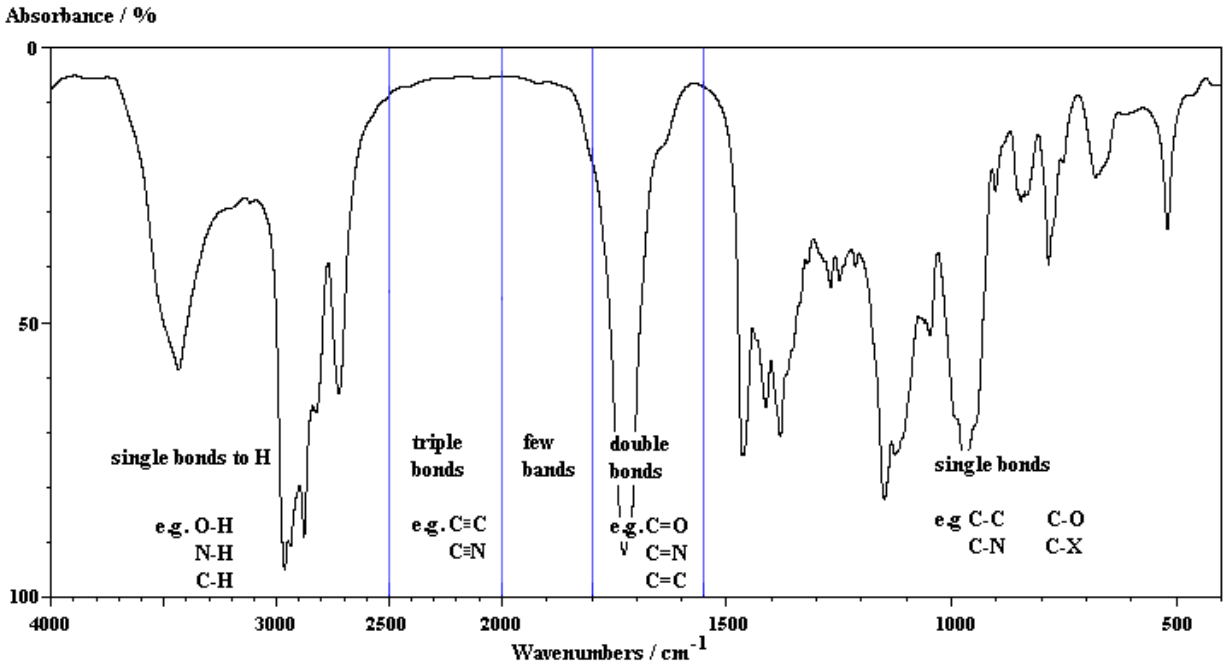


Diagram for IR spectrophotometer

Absorptions of common functional groups



A) Stretching peaks

1- OH stretching:

Non-bonded OH: 3600 - 3200 cm^{-1} (sharp peak because there is no H-bonding)

Bonded OH: (Polyhydoxy or glycosides) 3500-3200 cm^{-1} (broad due to H-bonding)

Carboxylic acids OH: 3300-3200 cm^{-1}

2- NH (NH_2):

NH_2 gives two peaks at 3500 – 3400 cm^{-1}

NHR gives one peaks

NR_2 gives no peak

3- CH stretching

Aliphatic CH_2 and CH_3: 2960 - 2850 cm^{-1}

Aromatic and Alkene: 3000-3030 cm^{-1}

Acetylenic: 3300 cm^{-1}

4- C=C stretching: 1600 – 1680 cm^{-1}

5- C=O stretching: 1630-1850 cm^{-1}

Amide CO-NH_2 : 1630-1700

Ketone: 1705 - 1725 cm^{-1}

Acid: 1700-1725 cm^{-1}

Aldehyde: 1720 - 1740 cm^{-1}

Ester: 1735- 1750 cm^{-1}

Anhydride: 1800- 1850 cm^{-1}

6-Alkyne, Acetylenic C=C: 2200-2100 cm^{-1}

7 - Cyanide (nitrile) C-N: 2260-2240 cm^{-1}

B) Binding peaks:

1- C-O:

Pirmary alcohols: 1000-1050 cm^{-1}.

Secondary alcohols: 1050-1100 cm^{-1}

Tertiary alcohols: 1100-1150 cm^{-1}

Phenols: 1200 cm^{-1}

Ester: 1200- 1250 cm^{-1}

2- C-C: 1300 to 1350 cm^{-1}

3- Halogen bending vibration:

C-F: 960 cm^{-1}

C-Cl: 859 cm^{-1}

C-Br: 667 cm^{-1}

C-I: 667 cm^{-1}

Functional Class	Stretching Vibrations			Bending Vibrations		
	Range (cm^{-1})	**Int.**	**Assignment**	**Range (cm^{-1})**	**Int.**	**Assignment**
Alkanes	2850-3000	Str	CH_3, CH_2 & CH 2 or 3 bands	1350-1470 1370-1390 720-725	med med wk	CH_2 & CH_3 deformation CH_3 deformation CH_2 rocking
Alkenes	3020-3100 1630-1680 1900-2000	med var str	=C-H & $=CH_2$ (usually sharp) C=C (symmetry reduces intensity) C=C asymmetric stretch	880-995 780-850 675-730	str med med	=C-H & $=CH_2$ (out-of-plane bending) cis-RCH=CHR
Alkynes	3300 2100-2250	str var	C-H (usually sharp) C≡C (symmetry reduces intensity)	600-700	str	C-H deformation
Arenes	3030 1600 & 1500	var med-wk	C-H (may be several bands) C=C (in ring) (2 bands) (3 if conjugated)	690-900	str-med	C-H bending & ring puckering
Alcohols & Phenols	3580-3650 3200-3550 970-1250	var str str	O-H (free), usually sharp O-H (H bonded), usually broad C-O	1330-1430 650-770	med var-wk	O-H bending (in-plane) O-H bend (out-of-plane)
Amines	3400-3500 (dil. soln.) 3300-3400 (dil. soln.) 1000-1250	wk wk med	N-H (1°-amines), 2 bands N-H (2°-amines) C-N	1550-1650 660-900	med-str var	NH_2 scissoring (1°-amines) NH_2 & N-H wagging (shifts on

						H-bonding)
Aldehydes & Ketones	2690-2840(2 bands) 1720-1740 1710-1720 1690 1675 1745 1780	med str str str str str str	C-H (aldehyde C-H) C=O (saturated aldehyde) C=O (saturated ketone) aryl ketone α, β-unsaturation cyclopentanone cyclobutanone	1350-1360 1400-1450 1100	str str med	α-CH_3 bending α-CH_2 bending C-C-C bending
Carboxylic Acids & **Derivatives**	2500-3300 (acids) overlap C-H 1705-1720 (acids) 1210-1320 (acids) 1785-1815 (acyl halides) 1750 & 1820 (anhydrides) 1040-1100 1735-1750 (esters) 1000-1300 1630-1695 (amides)	str str med-str str str str str str str	O-H (very broad) C=O (H-bonded) O-C (sometimes 2-peaks) C=O C=O (2-bands) O-C C=O O-C (2-bands) C=O (amide I band)	1395-1440 1590-1650 1500-1560	med med med	C-O-H bending N-H (1°-amide) II band N-H (2°-amide) II band
Nitriles **Isocyanates, Isothiocyanates, Diimides, Azides & Ketenes**	2240-2260 2100-2270	med med	C≡N (sharp) -N=C=O, -N=C=S -N=C=N-, $-N_3$, C=C=O	1350-1470 1370-1390 720-725	med med wk	

Other Functional Groups

Functional Class	Characteristic Absorptions
Sulfur Functions	
S-H thiols	2550-2600 cm^{-1} (wk & shp)
S-OR esters	700-900 (str)
S-S disulphide	500-540 (wk)
C=S thiocarbonyl	1050-1200 (str)
S=O sulfoxide sulfone sulfonic acid sulfonyl chloride sulphate	1030-1060 (str) 1325± 25 (as) & 1140± 20 (s) (both str) 1345 (str) 1365± 5 (as) & 1180± 10 (s) (both str) 1350-1450 (str)
Phosphorous Functions	
P-H phosphine	2280-2440 cm^{-1} (med & shp) 950-1250 (wk) P-H bending
(O=)PO-H phosphonic acid	2550-2700 (med)
P-OR esters	900-1050 (str)
P=O phosphine oxide phosphonate phosphate phosphoramide	1100-1200 (str) 1230-1260 (str) 1100-1200 (str) 1200-1275 (str)
Silicon Functions	
Si-H silane	2100-2360 cm^{-1} (str)
Si-OR	1000-1110 (str & brd)
Si-CH_3	1250± 10 (str & shp)
Oxidized Nitrogen Functions	
=NOH oxime O-H (stretch) C=N N-O	 3550-3600 cm^{-1} (str) 1665± 15 945± 15
N-O amine oxide aliphatic aromatic	 960± 20 1250± 50
N=O nitroso Nitro	1550± 50 (str) 1530± 20 (as) & 1350± 30 (s)

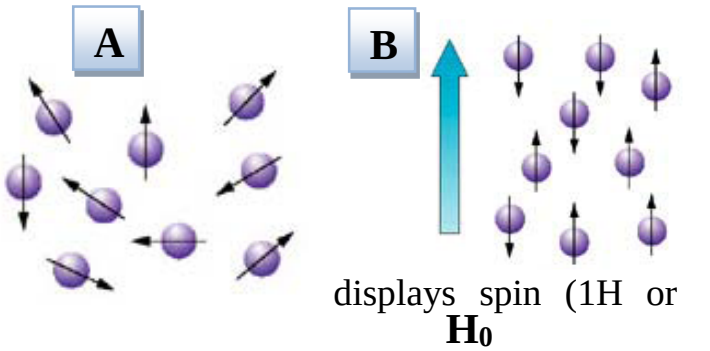
Standard abbreviations (str = strong, wk = weak, brd = broad & shp = sharp) are used to describe the absorption bands.

Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful tools available for determining the structure of organic compounds. NMR was first developed in 1946 by research groups at Stanford in the USA. The radar technology developed during the Second World War made many of the electronic aspects of the NMR spectrometer possible. Over the next 50 years NMR developed into the premier organic spectroscopy available to chemists to determine the detailed chemical structure of the chemicals they were synthesizing. Another well-known product of NMR technology has been the Magnetic Resonance Imager (MRI), which is utilized extensively in the medical radiology field to obtain image slices of soft tissues in the human body.

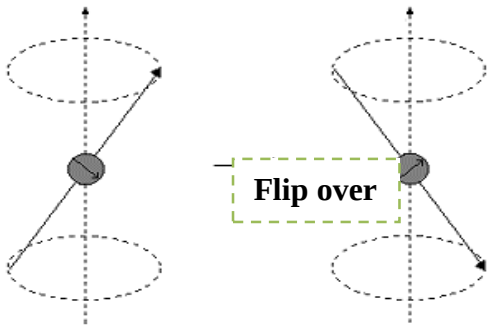
Theory of NMR

NMR relies on the ability of atomic nuclei to behave like a small magnet and align themselves with an external magnetic field. When irradiated with a specific radio wave the nuclei in a molecule can change from being aligned with the magnetic field to being opposed to it. The energy frequency at which this occurs can be measured and is displayed as an NMR spectrum. Not all nuclei display spin. In order to display spin a nucleus must have an odd number of protons or neutrons. If the number of neutrons and the number of protons are both even, the nucleus has no spin such as ^{12}C and ^{16}O. If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2). If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3). Hydrogen has one proton, so displays spin (1H or proton NMR).



The nuclei can align themselves with an external magnetic field with a strength H_0: A: the nuclei with no field and no alignment; B: the nuclei are aligned with an external magnetic field.

Deuterium has one proton and one neutron and also displays spin. Other atoms that display spin are isotopes of common elements such as ^{13}C, ^{19}F, ^{15}N and ^{29}Si.



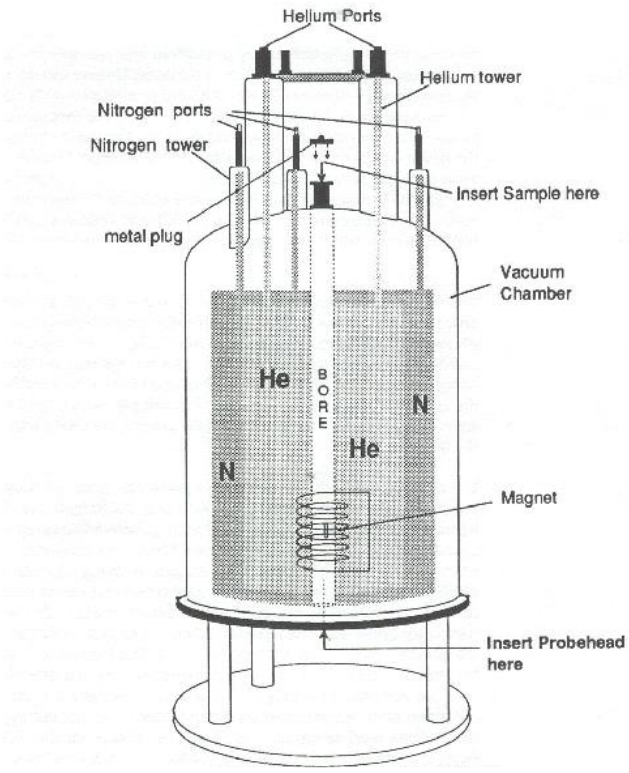
Processional movement and flipping over of a nucleus

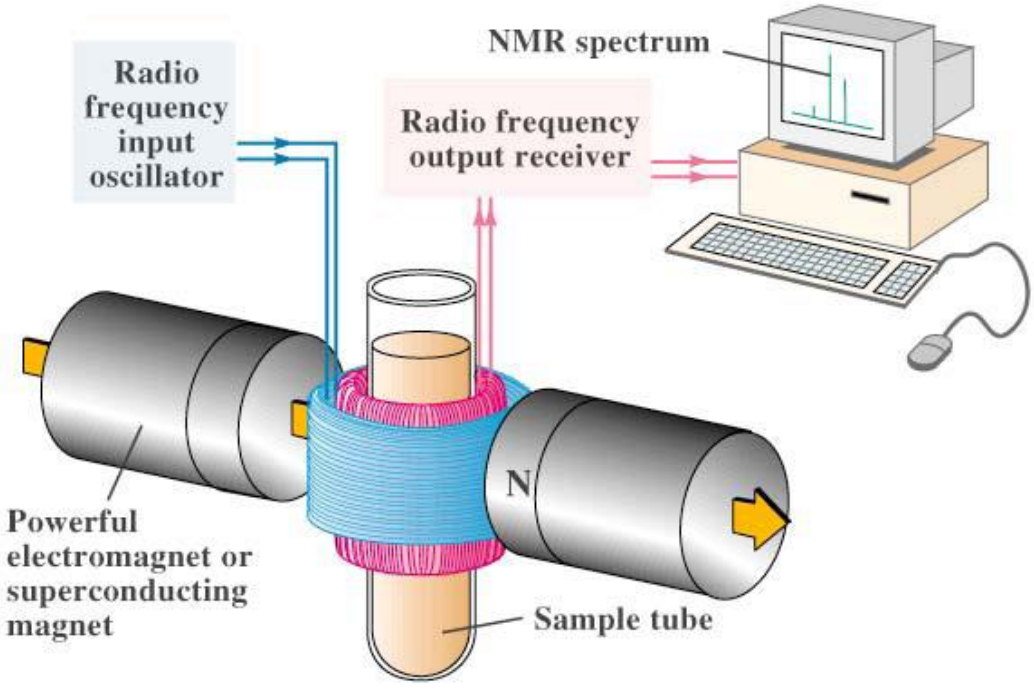
The nuclei are now spinning, their rotational axis draws out a magnetic circle perpendicular to the applied field and this motion is called precession. The energetically preferred orientation of the nuclei has the magnetic moment aligned parallel with the applied field (spin m=+1/2) and is often given the notation α, whereas the higher energy anti-parallel orientation (spin m=-1/2) is referred to as β. If the sample was irradiated with radio waves (in the MHz frequency range) the atom will absorb the energy and be promoted to the less favourable higher energy state (from α to β). This energy absorption is called resonance because the frequency of the applied radiation and the precession of the nuclei coincide or resonate.

Instrument:

There are two general types of NMR instrument; continuous wave and Fourier transform. Early experiments were conducted with continuous wave (CW) instruments, and in 1970 the first Fourier transform (FT) instruments became available. This type now dominates the market, and currently there is no commercial CW instruments being manufactured at the present time.

To get the nuclei in a molecule to all align in the same direction, a very strong magnetic field is generated using a superconducting electromagnet, which requires very low temperatures to function. The coils of the magnet are surrounded by liquid helium (-269°C), which is prevented from boiling off too quickly by a surrounding layer of liquid nitrogen (-77°C). These coolants are all contained in double-layer steel with a vacuum between the layers, to provide insulation just like a thermos. There is a narrow hole through the middle of the magnet, and the sample tube and radio frequency coils are located there.





NMR spectrometer

Sample Preparation:

NMR spectra are usually measured using a solution of the compound of interest. For ^{1}H NMR, the solvent must be modified so that the solvent ^{1}H signal does not overwhelm the solute signals. Many solvents are available in deuterated form, such that all the ^{1}H atoms are replaced by 2H (deuterium). The deuterium nuclei resonate at 30.7 MHz in a 200 MHz magnet, so they are effectively invisible to ^{1}H NMR spectroscopy. Commonly used solvents include deuterated water (D_2O), chloroform ($CDCl_3$), acetone (CD_3COCD_3), DMSO (CD_3SOCD_3), methanol (CD_3OD) and benzene (C_6D_6). These solvents are 99% or more deuterium at each site, but the residual ^{1}H still shows up as a peak in the proton spectrum (Table 1). The ^{2}H signal from the solvent is used by the spectrometer as a "lock" signal to prevent the magnetic field from changing during the experiment. Tetramethylsilane ($(CH_3)_4Si$, "TMS") is often added to the solvent to provide a reference peak at zero ppm for ^{1}H and ^{13}C. Samples should be prepared as homogeneous solutions with 5-10 mg (^{1}H spectra) or 30-50 mg (^{13}C spectra) of solute in a total volume of about 0.7 mL (4.5 cm deep in a 5-mm NMR tube). Larger volumes are wasteful of deuterated solvent, and smaller volumes make it very difficult to obtain a homogenous magnetic field

Deteriorated NMR solvents and their chemical shift

solvent	Chemical shift (δ ppm)	Splitting
$CDCl_3$	7.26	singlet
CD_3COCD_3	2.04	quintet
CD_3SOCD_3	2.49	quintet
D_2O	4.6	singlet
CD_3OD	3.31	quintet
C_6D_6	7.15	Multiplet- broad

NMR Spectrum:

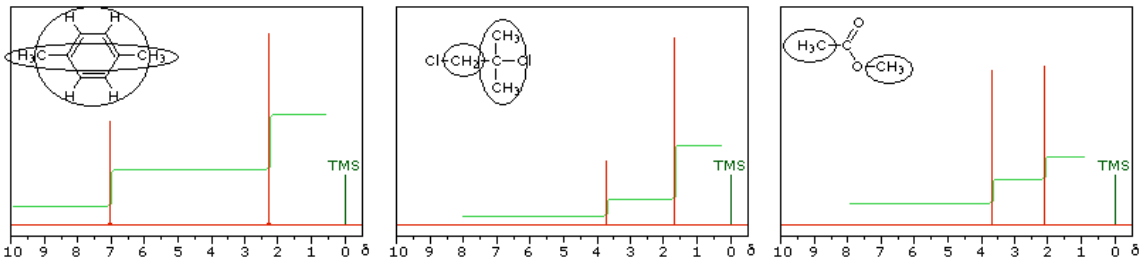
An NMR spectrum appears as a series of vertical peaks/signals distributed along the x-axis of the spectrum. Each of these signals corresponds to an atom (or atoms) within the molecule being observed. The position of each signal in the spectrum gives information about the local structural environment of the atom(s) producing the signal. The interpretation of NMR spectrum depends on the investigation of four different parameters:

1- Number of signals
2- Position of signals
3- Intensity of signals
4- Splitting of signals

Proton nuclear magnetic resonance (^{1}HNMR)

A) Number of signals (Equivalent and non-equivalent protons)

Protons within a compound experience different magnetic environments, which give a separate signal in the NMR spectrum. Protons that reside in the same magnetic environment are termed chemically equivalent protons. As a general rule protons in CH_3 and CH_2 groups are usually equivalent. Symmetrical compounds, such as benzene, are also equivalent; however, since many compounds are not symmetrical, it is important to know how to identify nonequivalent protons. Protons that are different in any way (even in their stereochemistry) are not equivalent and will absorb at different frequencies (give a separate signal on the NMR spectra). All of these compounds below have 2 signals in their NMR spectrum



4 H's are on a plane symmetry with each other. CH_3 are also on a plane of symmetry so 2 signals.	The CH_2 H's are attached to a C that's attached to a Cl. The CH_3 are attached to the same C H's and have the same neighbours.	One CH_3 is attached to an O while the other CH_3 is attached to a C.

B) Position of Signals (Chemical Shift)

The difference in absorption position of a particular proton, from the absorption position of a reference proton is called the chemical shift of that particular proton. Chemical shift is measured in units called δ (Delta) or τ (tau) and expressed in part per million (ppm) and given by the equation:

$$\delta\ (\text{ppm}) = \frac{\text{Chemical shift (Hz)}}{\text{Applied Frequancy}}$$

Reference compound: Tetramethylsilane (TMS)

In order to standardize the NMR spectra, the chemical shifts are positioned in relation to a reference proton set at 0.00 ppm. Tetramethylsilane, $(CH_3)_4Si$, is the standard for ^{1}H and ^{13}CNMR. TMS is practical as a reference compound because of its inert quality that prevents it from reacting with the sample and its highly volatile nature (27°C) that makes it easy to evaporate out of samples. It is soluble in most organic solvents and gives a very sharp single peak. Few compounds have a lower frequency reading than TMS and it has 12 equivalent protons that read strongly on the NMR spectra.

Shielding and deshielding:

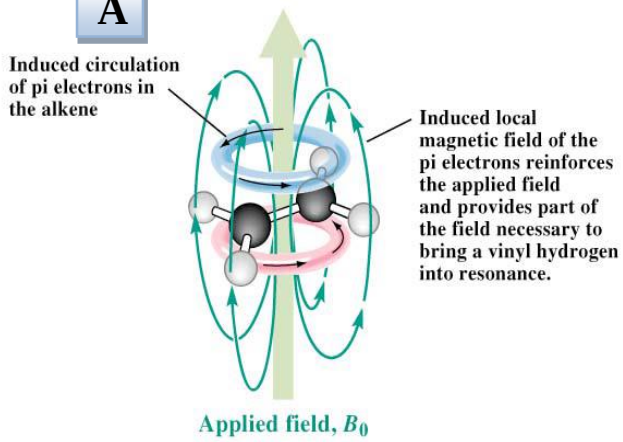
Under an applied magnetic field, circulating electrons in the electron cloud produce a small opposing magnetic field, ultimately decreasing the effective magnetic field felt by the proton, shifting the signal to the right (or upfield). This effect, in which the electron cloud "**shields**" the proton from the applied magnetic field, is called local **shielding**. Protons that are attached to more electronegative atoms experience higher chemical shifts. Electronegative atoms also remove electrons from the electron cloud, which decreases their density and results in less shielding; hence electronegative atoms are said to **deshield** the proton and cause it to have a higher chemical shift, moving it to the left (or downfield). The magnitude of the deshielding effect, however, rapidly decreases as the distance between the proton and electronegative atom increases. For example, values of the methyl chemical shift as it move away from bromine:

$C\underline{\mathbf{H}}_3Br$	$C\underline{\mathbf{H}}_3CH_2Br$	$C\underline{\mathbf{H}}_3CH_2CH_2Br$	$C\underline{\mathbf{H}}_3CH_2CH_2CH_2Br$
2.69 ppm	1.66 ppm	1.06 ppm	0.93 ppm

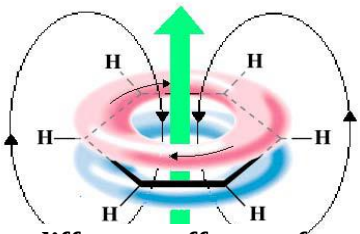
The electron cloud above and below the plane of benzene ring circulates in reaction to the external field so as to generate an opposing field at the centre of the ring and a supporting field at the edge of the ring. This is called the **anisotropic** effect and it results in downfield shift of aromatic protons' signal to be after 7 ppm. The same effect is found in double bonds however in triple bond, the anisotropic effect results in shielding of the protons and an up-field shift of their signals.

Magnetic field induced in the pi bond of a carbon-carbon double bond

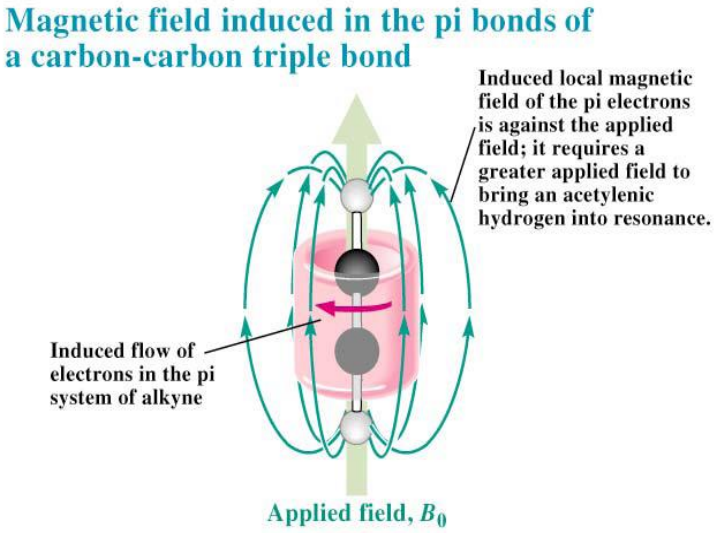
A



C



The different effects of anisotropy in aromatic systems (A), double bonds (B) and triple bonds (C)



Relative peak areas (integration)

The area under the signals (integration) corresponds to the number of protons responsible for that signal. Therefore, the relative intensities of the signal are proportional to the relative number of proton equivalents. It is important to remember that integration only provides ratios of protons, not the absolute number. For convenience in calculating the relative signal strengths, the smallest integration is set to 1 and the other values are converted accordingly.

Splitting (spin-spin coupling)

The interaction between nearby protons produce different spin flip energies as they can orient themselves in a pattern of parallel or antiparallel to the applied magnetic force. This phenomenon, where the spin of the nucleus of one proton is close enough to affect the spin of another, is called spin-spin coupling. As a result, NMR signals are not usually single (singlets), but a complex pattern of splits labelled as doublets (2 peaks), triplets (3 peaks), quartets (4 peaks), etc. The distance between

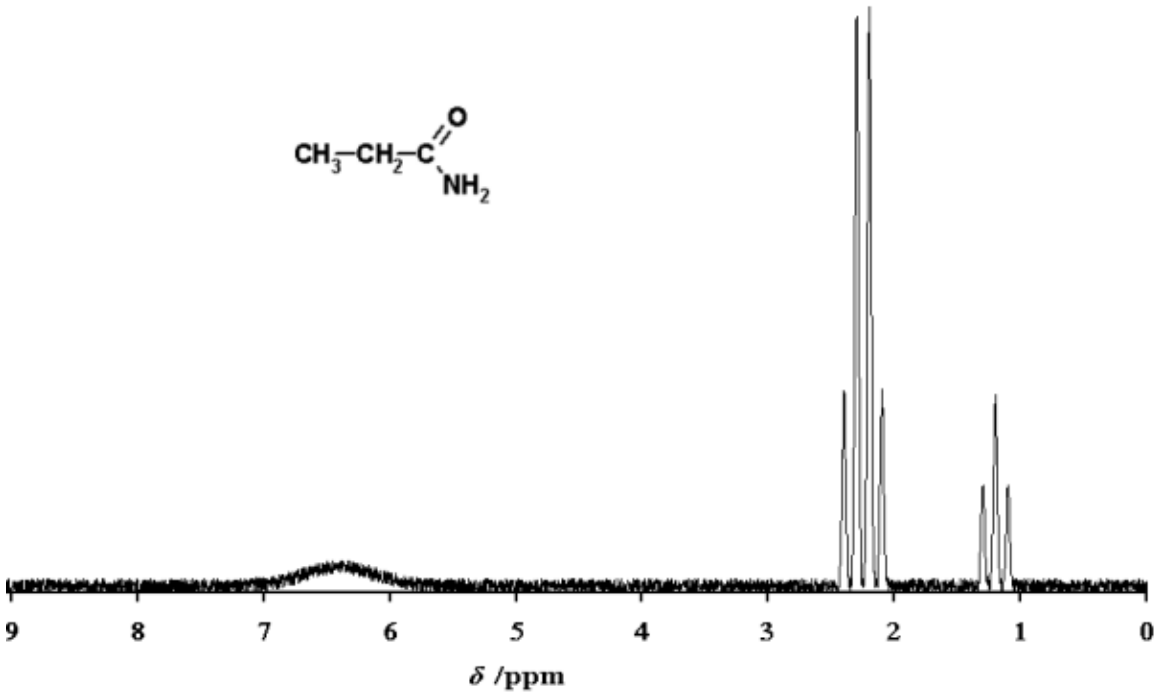
the split peaks is called the coupling constant (J). For a proton with n neighbours, its signal will be split into n+1 peak.

Rules for proton coupling:

1) Nuclei with the same chemical shift (i.e. equivalent protons) do not couple with each other. The protons must be nonequivalent in order to couple.

2) Geminal coupling (coupling through 2 bonds, i.e. on one carbon) can happen if the two protons are non equivalent. Vicinal protons (protons separated by 3 bonds, i.e. on two carbons) can couple with each other and this is the common type of coupling.

3) Hydrogens bonded to a Nitrogen or Oxygen usually do not couple with other protons and appear as singlets on the NMR spectra.



Spin- spin coupling

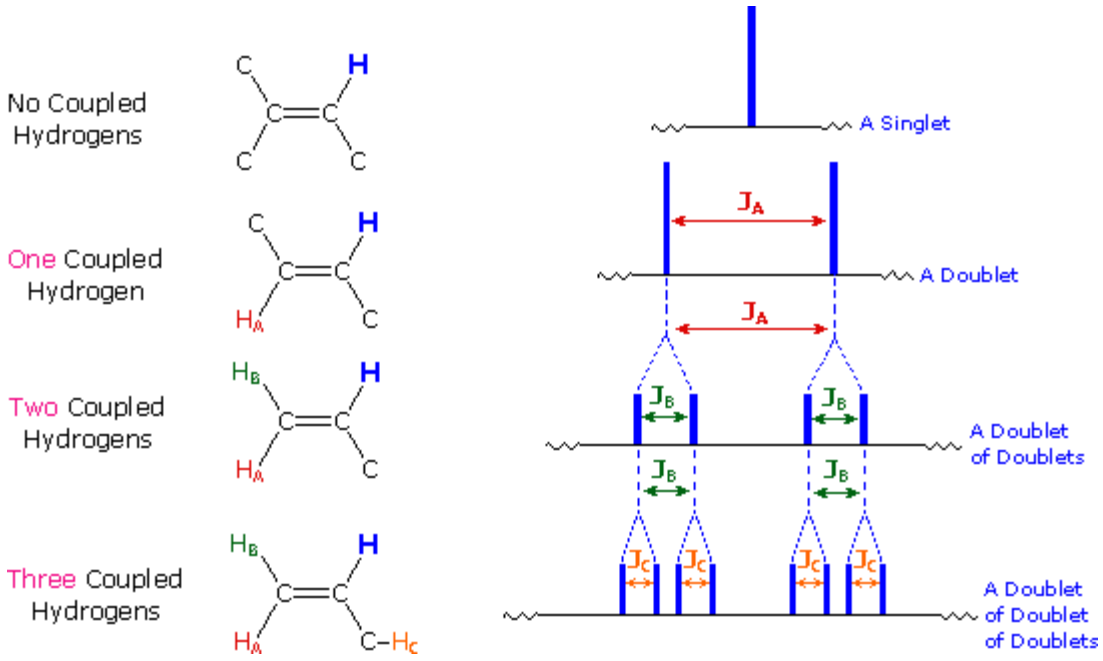
Coupling constant (*J*): This is the distance between two adjacent peaks of a split signal. The magnitude of J is a measure of how strongly the nuclear spins of the coupled protons influence each other. It is dependent upon the number and type of bonds that connect the coupled protons and their geometric relationship. Therefore,

for some molecules, the *trans* form could create a smaller coupling constant than the *cis* form. For nonequivalent hydrogens on the same carbon, the J is usually very small and unable to be observed. But, for non-equivalent hydrogens bonded to adjacent carbons, the J is usually large enough to be observed.

Long-range coupling: This occurs when the protons are separated by more than three bonds and one of the bonds is a double or triple bond. A small splitting is observed.

Non-first order splitting:

A given nucleus is spin-coupled to two or more sets of neighbouring nuclei by different J values, the n+1 rule does not predict the entire splitting pattern. Instead, the splitting due to one J set is added to that expected from the other J sets as shown in Figure 14.



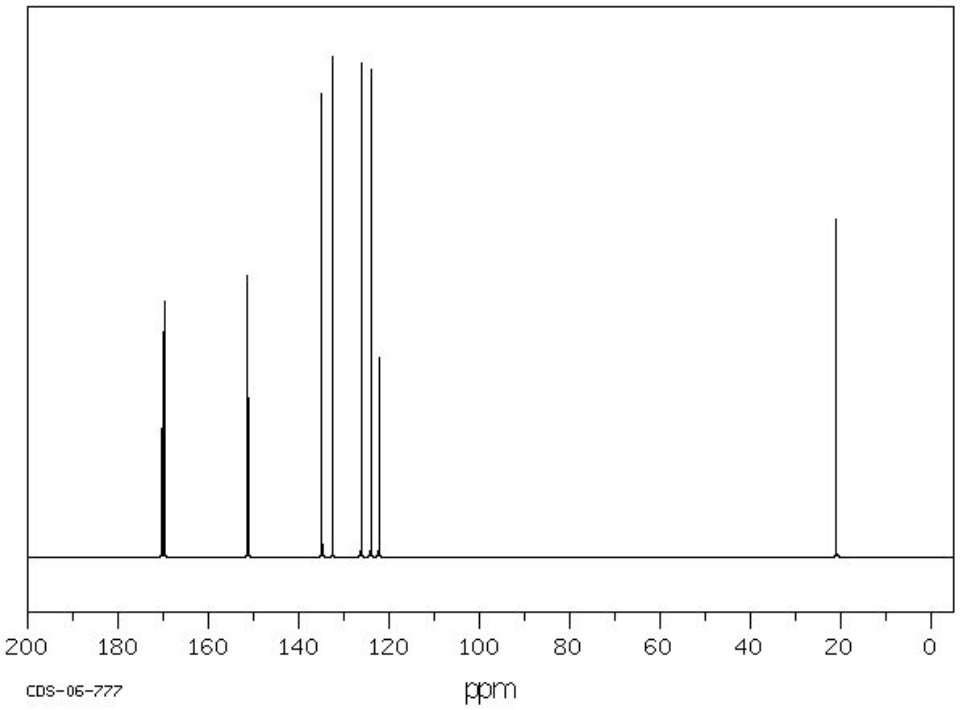
Non-first order splitting

Characteristic Proton Chemical Shifts

Type of Proton		Chemical shift (δ ppm)	Type of Proton		Chemical shift (δ
Primary	$RC\underline{H}_3$	0.9	Alcohols	$\underline{H}C$-OH	3.4 - 4
Secondary	$R_2C\underline{H}_2$	1.3	Ethers	$\underline{H}C$-OR	3.3 - 4
Tertiary	$R_3C\underline{H}$	1.5	Esters	RCOO-$C\underline{H}$	3.7 - 4.1
Vinylic	C=C-$\underline{H}$	4.6 - 6	Acids	$\underline{H}C$-COOR	2 – 2.2
Acetylenic	C≡C-$\underline{H}$	2 -3	Carbonyl	$\underline{H}C$-COR	2 - 2.7
Benzylic	Ar-C-$\underline{H}$	2.2 - 3	Aldehydic	$RC\underline{H}O$	9 -10
Allylic	$C=C-C\underline{H}_3$	1.7	Hydroxyli	R-$O\underline{H}$	1 - 5.5
Fluorides	$\underline{H}C$-F	4 - 4.5	Phenolic	Ar-$O\underline{H}$	4 - 12
Chlorides	$\underline{H}C$-C1	3 - 4	Enolic	C=C-$O\underline{H}$	15 - 17
Bromides	$\underline{H}C$-Br	2.5 - 4	Carboxyli	$RCOO\underline{H}$	10.5 - 12
Iodides	$\underline{H}C$-I	2 - 4	Amino	$RN\underline{H}_2$	1 - 5

^{13}C-NMR

The ^{13}C spectrum offers further characterisation of the molecule as it relates directly to the carbon skeleton. In carbon NMR, the carbon-13 nucleus is the one to observe as carbon-12 has no nuclear spin and is "NMR silent". Unfortunately, ^{13}C has a lower intrinsic sensitivity than the proton and has only 1.1 % natural abundance. The low abundance means that direct ^{13}C-^{13}C coupling is usually never seen in the spectrum and thus unable to assign carbon-carbon connectivities directly. Each carbon may be coupled to a number of protons in the molecule, typically over one, two or three bonds such that the resulting carbon resonances have complex structure which further reduces signal intensity by spreading the resonance. The spectrum is, therefore, usually recorded with broadband decoupling of all protons. This removes multiplicity in carbon resonances, so that the doublet, triplet and quartet patterns indicative of CH, CH_2 and CH_3 groups, respectively, are not seen and each carbon resonance appears a singlet (and this spectrum is called **^{13}C-^{1}H Decoupled NMR spectrum**). Typically one line is observed for each carbon atom in the molecule, as resonance overlap is rare. The broadband decoupling produces saturation of the proton resonances and this generates a nuclear Overhauser enhancement (NOE) of the carbon signal, further increasing signal intensity. The chemical shift of each resonance is again indicative of environment, and it is possible to identify certain functional groups for which there is no direct evidence in the proton spectrum e.g. carbonyls. Unlike proton spectra, the routine carbon spectrum is unsuitable for integration, for a number of reasons relating to relaxation times, the NOE and the way in which the data are digitised. However, this is not usually a limitation when attempting to identify a molecular structure as precise integral data would be of limited use. Despite this, it is often possible to distinguish non-protonated carbon resonances from their low intensity relative to protonated carbons. This effect is due principally to long carbon relaxation times which result from the lack of a directly-bonded proton.



^{13}C-^{1}H Decoupled NMR spectrum of aspirin

^{13}C suffers from lower sensitivity than the proton and this problem of sensitivity has been overcome in several ways. One way was the use of much larger samples (more than 50 mg) in order to increase the abundance of carbon isotope. Another method for enhancing the weak 13C signals was to run the spectrum over and over again and store the individual spectra in a computer which plots out the accumulated spectrum. This technique is very time-consuming.

Chemical Shifts

For almost all organic molecules, complete ^{13}C spectra appear between low-field carbonyl groups and high-field methyl groups in a range of just over 200 ppm downfield TMS, the same standard used in ^{1}H NMR.

Common carbon Chemical Shifts

Type of Proton		Chemical shift (δ ppm)	Type of Proton		Chemical shift (δ ppm)
Primary	R$\underline{C}H_3$	8-15	Alcohols	$\underline{H}$C-OH	65-90

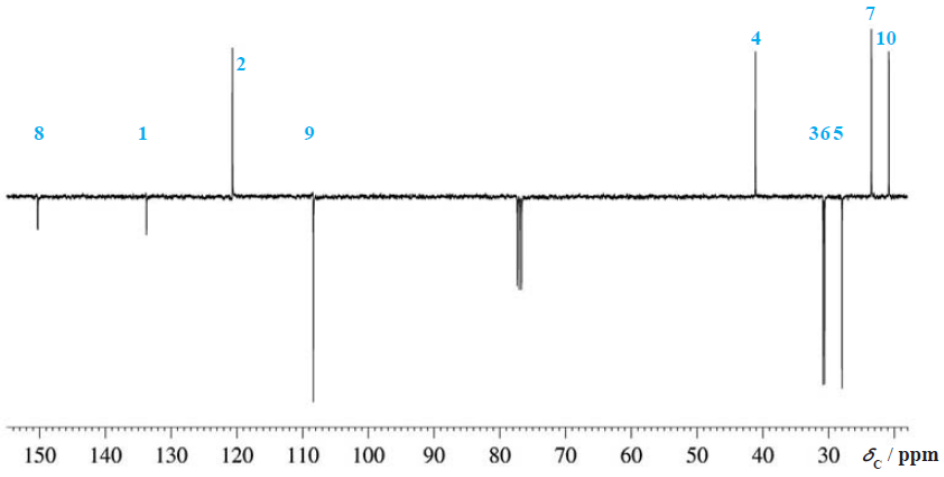
Secondary	$R_2C\underline{H}_2$	25-45	Ethers	$\underline{H}C$-OR	65-90
Tertiary	$R_3C\underline{H}$	25-45	Ketone	$\underline{H}C$-COR	190-200
Alkene	C=C-$\underline{H}$	110-140	Aldehydi	R$C\underline{H}$O	185-205
Acetylenic	C=C-$\underline{H}$	65-85	Carboxyl	RCOO$\underline{H}$	160-180
Aromatic	Ar	120-140	Halogens	$\underline{H}C$-F	30-50

Proton off-resonance decoupled spectrum

This mode allows only one-bond proton-carbon couplings so that each carbon will split by its own protons. In this spectrum the methyl carbon signal (CH_3) will be observed as a quartet, the methylene (CH_2) as a triplet, the methine (CH) as a doublet and the quaternary carbon (C) as a singlet. Although off-resonance proton-decoupled spectra contain much information, the technique has been supplanted by the DEPT experiment.

Attached proton test:

The attached proton test is a ^{13}C NMR experiment that is used to assign separating carbons unattached to protons and CH_2 signals from CH and CH_3 signals. The APT experiment yields methine (CH) and methyl (CH_3) signals positive (upper direction) and quaternary (C) and methylene (CH_2) signals negative (lower direction)

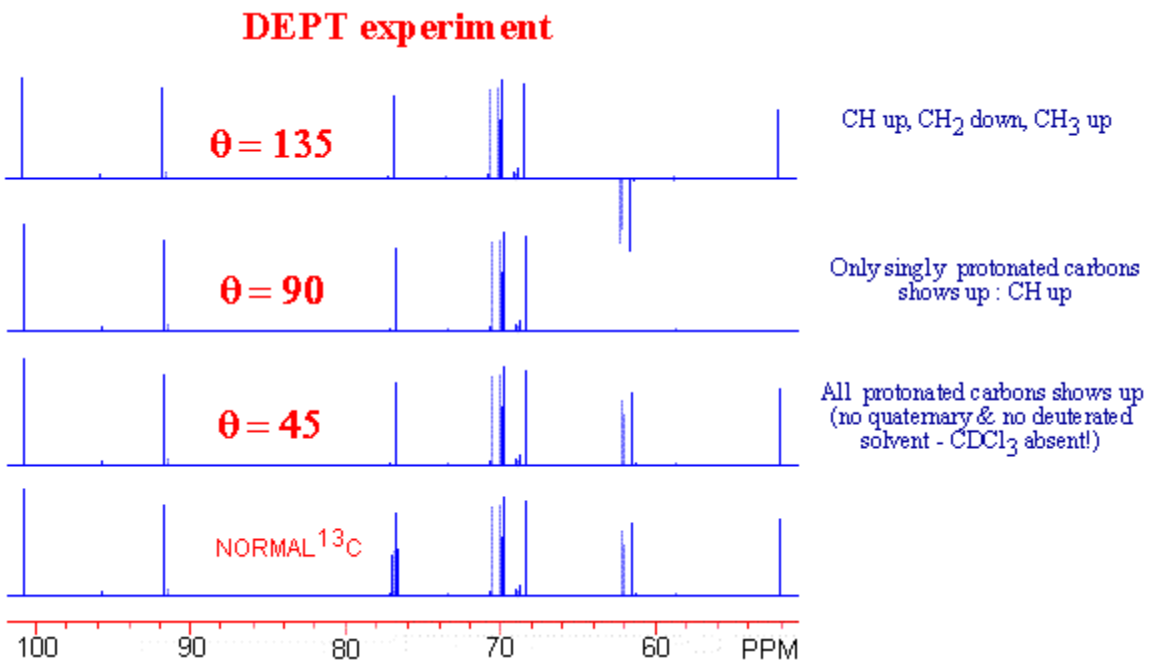


^{13}C DEPT

DEPT is "Distortionless Enhancement by Polarisation Transfer" and is used as a means of enhancing signal intensity and for editing spectra. As stated above, broadband proton decoupling removes multiplicity in carbon resonances, but the DEPT sequence allows one to establish the nature of the carbon atom whilst still acquiring broadband decoupled spectra, by making use of changes in signal intensities under differing experimental conditions. Three DEPT spectra are required for a full analysis and are termed DEPT-45, DEPT-90 and DEPT- 135 (the number indicates the flip angle of the editing proton pulse in the sequence), Figure 16. The signal intensities in these spectra are as follows:

	CH	CH_2	CH_3
DEPT-45	+ve	+ve	+ve
DEPT-90	+ve	zero	zero
DEPT-135	+ve	-ve	+ve

Non-proton-bearing carbons are not seen in DEPT spectra because the technique relies on polarisation transfer, that is, in this case, the transfer of proton magnetisation onto the directly bound carbon. Analysis of the three spectra reveals the carbon multiplicities directly, or they may be combined appropriately to yield sub-spectra containing CH or CH_2 or CH_3 resonances only. Often, it is not necessary to acquire all three experiments to assign multiplicities. Methyl resonances are often easily identified as they frequently resonate at lower frequency, so a DEPT-135 alone may be sufficient to distinguish between methine and methylene protons.



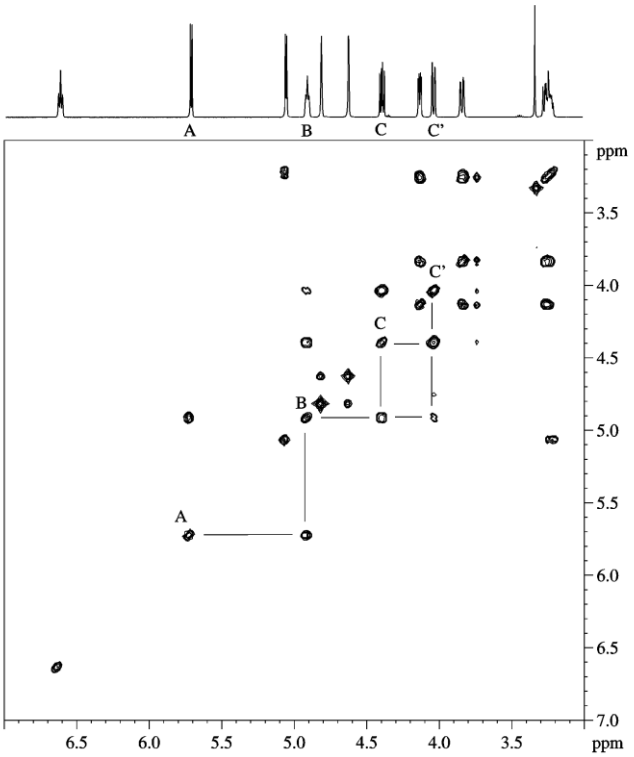
Different DEPT experiments

Two dimensional NMR:

Homo-nuclear correlation experiments:

^{1}H-^{1}H COSY

The COSY experiment (COrrelation SpectroscopY) provides a means of identifying mutually coupled protons and is the most widely used 2D experiment. The COSY experiment is a very efficient way of establishing connectivities when a large number of coupling networks need to be identified, as it maps all correlations with a single experiment. The experiment presents a two-dimensional contour map, each dimension representing proton chemical shifts and the contours representing signal intensity (just as contours are used to map mountains heights). The diagonal (running bottom left to top right) shows peaks that correspond with those in the usual 1D spectrum, and contain no new information. The peaks of interest are the off-diagonal or cross peaks. Each of these represents a coupling between the protons that are correlated by the cross peak.

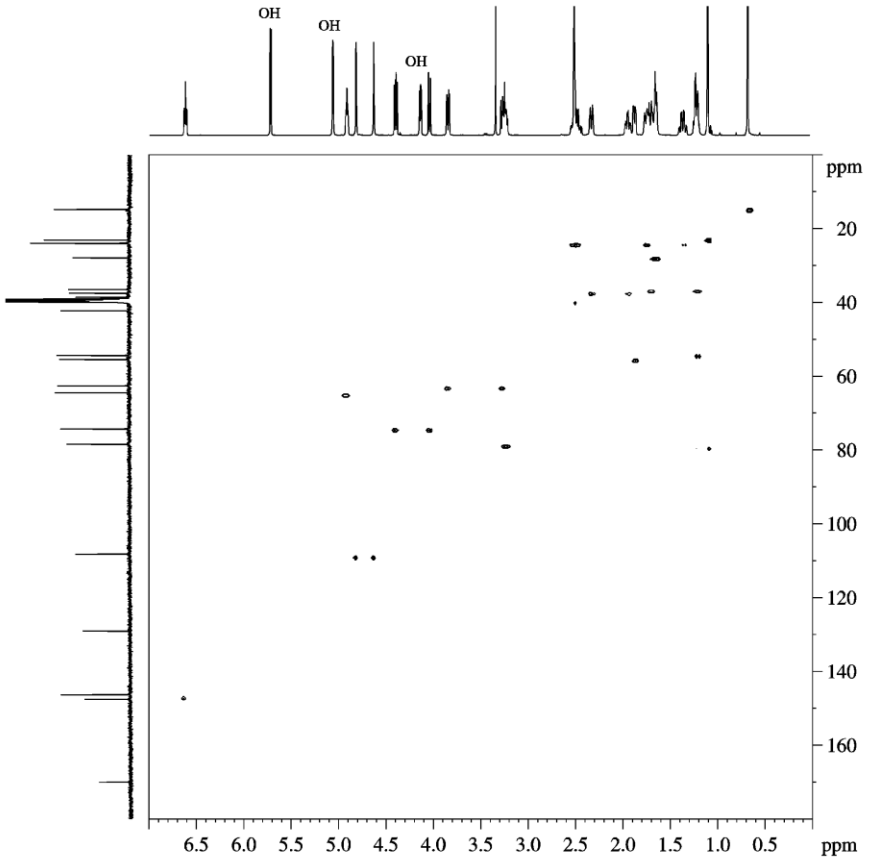


^{1}H-^{1}H COSY experiment

Heteronuclear correlation experiments

^{1}H-^{13}C HMQC

The HMQC (Heteronuclear Multiple-Quantum Correlation) experiment is a heteronuclear correlation technique that offers a means of identifying 1-bond H-C connectivities within a molecule. The results are displayed in a similar manner to those from COSY, with one dimension of the 2D map representing ^{13}C chemical shifts and the other representing ^{1}H chemical shifts, Cross peaks in the contour plot define to which carbon a particular proton (or group of protons) is attached, and it is therefore possible to map ^{13}C assignments from known ^{1}H assignments. The technique relies on magnetisation transfer from the proton to its directly attached carbon atom, and back onto the proton (for higher sensitivity) and so no responses are to be expected for non-protonated carbons or for protons bound to other heteroatoms.

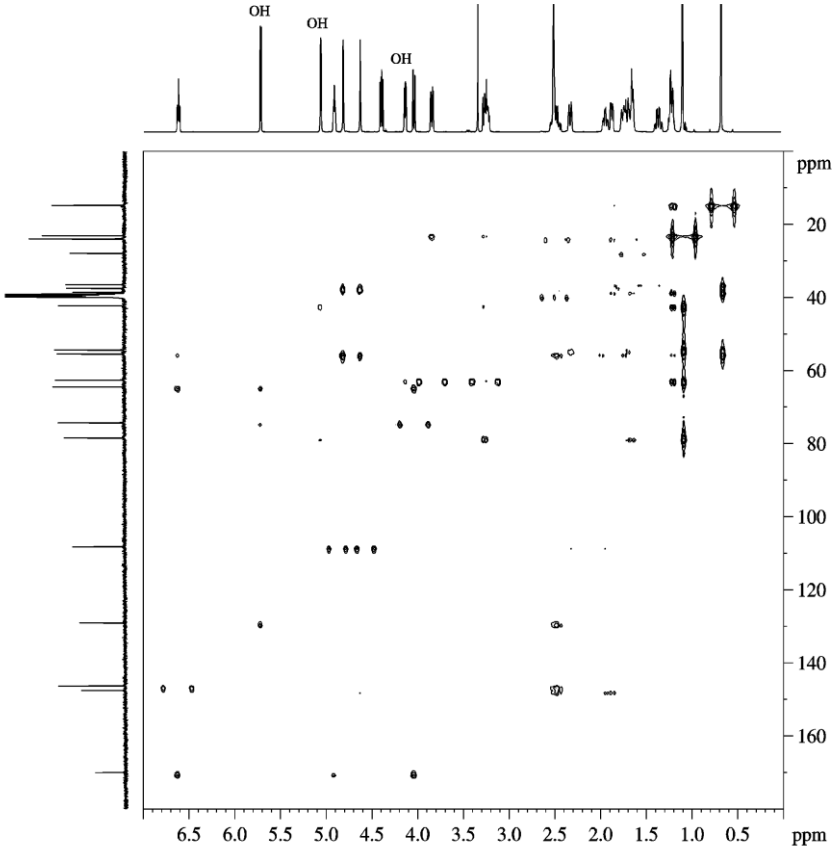


^{1}H-^{13}C HMQC

Usually, one peak is observed at the frequency of each protonated-carbon resonance, although occasionally two are seen and this is indicative of a diastereotopic CH_2 group. The HMQC experiment is also useful in the analysis of complex ^{1}H spectra as it provides dispersion of the proton spectrum along the ^{13}C dimension. Thus, a mass of overlapping multiplets in the proton spectrum can be spread apart by differences in ^{13}C chemical shifts, so allowing the ^{1}H chemical shift of each multiplet to be recognised.

^{1}H-^{13}C HMBC

This experiment (Heteronuclear Multiple-Bond Correlation) is closely related to HMQC and operates in essentially the same manner. In this case, however, the sequence timings are optimised for much smaller coupling constants and therefore seeks the correlations across more than one bond that arise from long-range couplings. These ^{1}H-^{13}C couplings typically occur with significant intensity over only 2 and 3 bonds, but may be apparent over 4 bonds in conjugated systems. Such experiments contain a mass of data on the molecular skeleton and can be extremely powerful tools in structure elucidation. The sequence also produces correlations across heteroatoms other than carbon. Those prove useful when attempting to link fragments identified, for example, from ^{1}H-^{1}H correlation spectra, and which show no ^{1}H-^{1}H couplings between the fragments themselves. Correlations can also be observed to quaternary centres and can be used, for example, in the assignment of carbonyl resonances. This experiment relies on correlations through small couplings; it is significantly less sensitive than the HMQC experiment.



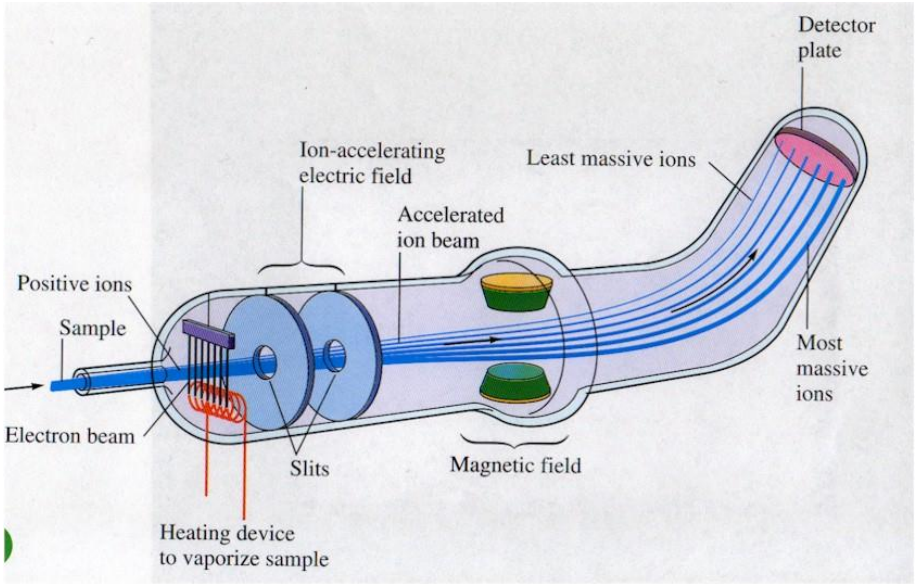
^{1}H-^{13}C HMBC experiment

Mass Spectrometry

Mass spectromerty is a technique for the identification of compounds based on ionization of gas phase molecule followed by analysis of the masses of the ions produced.

Instrumentation:

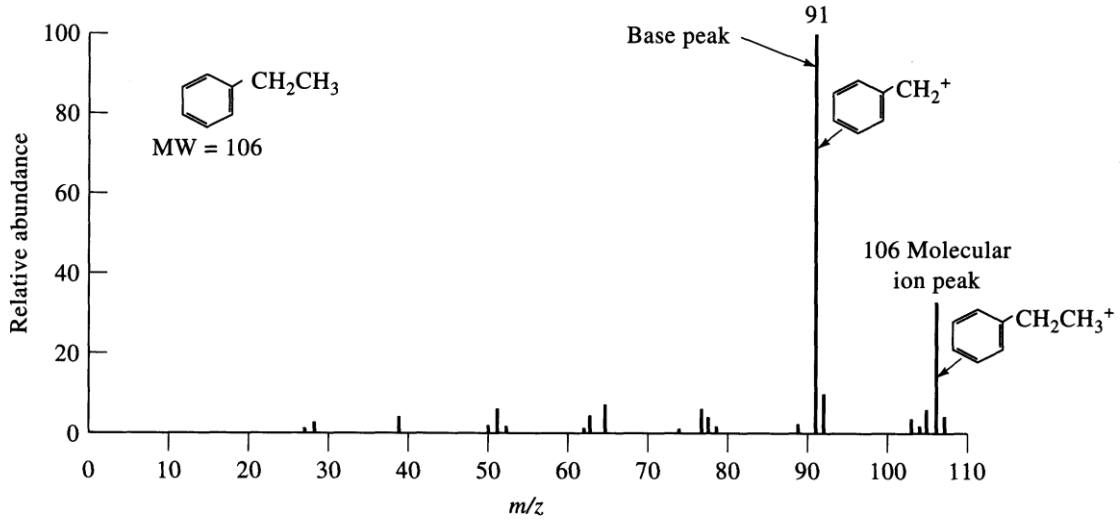
A composition of a mass spectrometer is shown in Figure 20. The sample is directed into a region called **ion source** where it gets ionized, usually using high energy electrons in the range of 20-100 eV. Many techniques are used to ionize molecules for mass spectrometry. These include electron impact (EI), photon ionization (PI), field ionization (FI), chemical ionization (CI), electrospray, Fast Atom Bombardment (FAB) and others. Understanding the ionization process is crucial to understanding the mechanisms by which mass spectral data are produced. The ions are sorted and separated according to their mass and charge in the **Mass Analyzer**. In order to avoid recombination, or neutralization of the ions, the pressure is kept low. **Detection** is normally performed with an electron multiplier which scans the spectrum of the ions to produce a record of intensity of the ions across it



Mass spectrometer composition

Mass Spectrum:

The mass spectrum is a graph of ion intensity versus mass-to-charge ratio (m/z). Any mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the base peak. Base peak is the most stable ion fragment in the spectrum. Molecular ion peak (M^+) corresponds to the molecular weight of singly charged molecule. For a pure compound, the molecular ion, if present, must be found at the highest m/z in the spectrum. Unfortunately, for a number of types of compounds, the molecular ion is not sufficiently stable to be found in appreciable abundance. The Molecular ion must be capable of yielding the important ions in the high mass region of the spectrum through the loss of logical neutral species.



A typical mass spectrum chart

Logical neutral species:

Only a certain number of low mass neutral fragments are commonly lost in decompositions of molecular ions. Mass losses of 4 to 14, 21to 25 and 33 to 38 are highly unlikely. The presence of an ion separated from the highest mass ion (i.e.

the molecular ion) by an anomalous mass will indicate that the latter ion is not the molecular ion.

The nitrogen rule:

If a compound contains an even number of nitrogen atoms (or no nitrogen atoms), its molecular ion will appear as an even mass number. If, however, a compound contains an odd number of nitrogen atoms, then its molecular ion will appear at an odd mass value.

Commonly Lost Fragments

m-15 ·CH_3

m-17 ·OH

m-26 ·CN

m-28 $H_2C{=}CH_2$

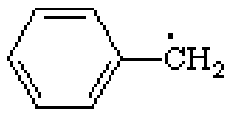
m-29 ·CH_2CH_3 ·CHO

m-31 ·OCH_3

m-35 ·Cl

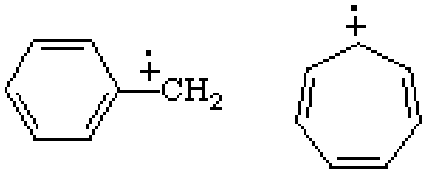
m-43 $CH_3\dot{C}{=}O$

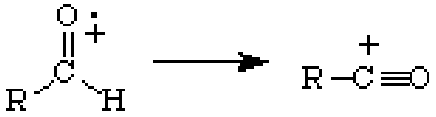
m-45 ·OCH_2CH_3

m-91 

Common Stable Ions

m/e = 43 

m/e = 91 

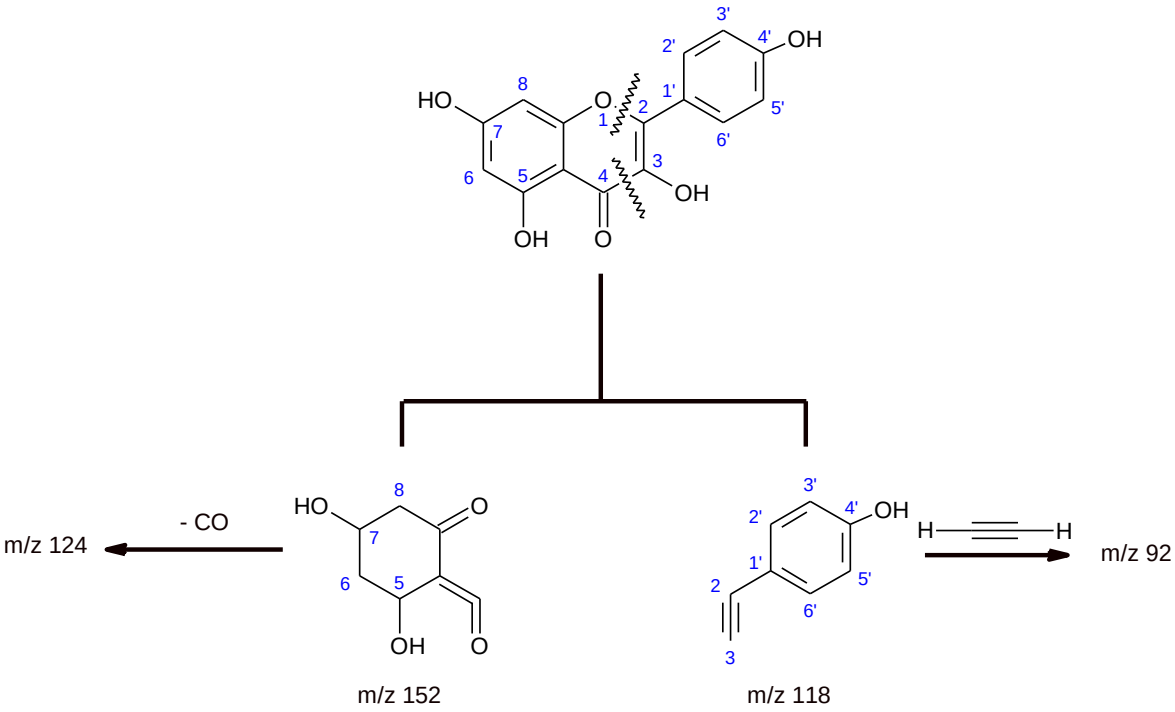
m/e = m-1 

Common fragment loss from different classes:

Ion Series	*m/z* and $[M–X]^+$ Ions	Remarks
carbenium ions	15, 29, 43, 57, 71, 85, 99, 113, 127, 141, ...	any alkyl group
acylium ions	29, 43, 57, 71, 85, 99, 113, 127, 141, 155, ...	aliphatic aldehydes, ketones, carboxylic acids and their derivatives
immonium ions	30, 44, 58, 72, 86, 100, 114, 128, 142, 156, ...	aliphatic amines
oxonium ions	31, 45, 59, 73, 87, 101, 115, 129, 143, 157, ...	aliphatic alcohols and ethers
sulfonium ions	47, 61, 75, 89, 103, 117, 131, 145, 159, ...	aliphatic thiols and thioethers
from benzyl	39, 51, 65, 77, 91	phenylalkanes
from benzoyl	51, 77, 105	aromatic aldehydes, ketones, carboxylic acids and derivatives
	$[M–16]^+$, $[M–30]^+$, $[M–46]^{+\cdot}$	nitroarenes
	45, 60, 73, $[M–17]^+$, $[M–45]^+$	carboxylic acids
	59, 74, 87, $[M–31]^+$, $[M–59]^+$	methyl carboxylates
	73, 88, 101, $[M–45]^+$, $[M–73]^+$	ethyl carboxylates
	44	McL of aldehydes
	58	McL of methyl ketones
	60	McL of carboxylic acids
	59	McL of carboxylic acid amides
	74	McL of methyl carboxylates
	88	McL of ethyl carboxylates
	$[M–20]^{+\cdot}$	fluorine compounds
	35, $[M–35]^+$, $[M–36]^{+\cdot}$	chlorine compounds (plus Cl isotopic pattern)
	79, $[M–79]^+$, $[M–80]^{+\cdot}$	bromine compounds (plus Br isotopic pattern)
	127, $[M–127]^+$, $[M–128]^{+\cdot}$	iodine compounds
	$[M–15]^+$	loss of methyl
	$[M–17]^{+\cdot}$	loss of ammonia from amines,
	$[M–17]^+$	loss of OH from (*tert.*) alcohols
	$[M–18]^{+\cdot}$	loss of water from alcohols
	$[M–27]^{+\cdot}$	loss of HCN from heterocycles or HNC from aromatic amines
	$[M–28]^{+\cdot}$	loss of CO, C_2H_4 or N_2
	$[M–44]^{+\cdot}$	loss of carbon dioxide
	$[M–91]^+$	loss of benzyl

General mass fragmentation of flavonoids

Flavone and flavonol should show the following pattern of fragmentation:



Phytochemistry II

PRACTICAL NOTES

For

SECOND YEAR PHARMACY STUDENTS

Department of Pharmacognosy
Faculty of Pharmacy
Menoufia University

Evaluation Sheet

For The practical Phytochemistry II

Name……..……..

Section ..... Number…….

Date	Subject	Signature
1		
2		
3		
4		
5		
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7		
8		
9		
10		
11		
12		
13		
14		

Activity (1)

After consulting reference books, published articles and internet seach, collect data bout biologically-important volatile components from animal origin.

Activity 2

Activity 3

Volatile oils

I- Determination of percentage of volatile oil in crude drugs

This process is carried out by subjecting a weighed amount of the crude drug to steam distillation in a specially designed apparatus provided with a trap of specified dimensions for collection of the oil.

The operating conditions depend on the nature and texture of the plant material as well as the concentration and nature of its volatile oil.

Clavenger's apparatus is usually used for this purpose.

Procedure

1. Introduce a known weight of drug in the distillation flask together with 3-6 times its weight of water or water and glycerin (to raise the boiling point in case of volatile oils rich in high boiling point constituents)
2. Allow to distill for a period of about 5-6 hours.
3. Leave the distillate to cool till a constant volume of oil is recorded in the graduated trap.
4. Calculate the percentage v/w of the oil (calculated on **dry weight** of the plant material) as follows:

$$\% \text{ v/w} = (v \times 100) / w$$

Where, **v** = volume of oil, and **w** = weight of dried plant material

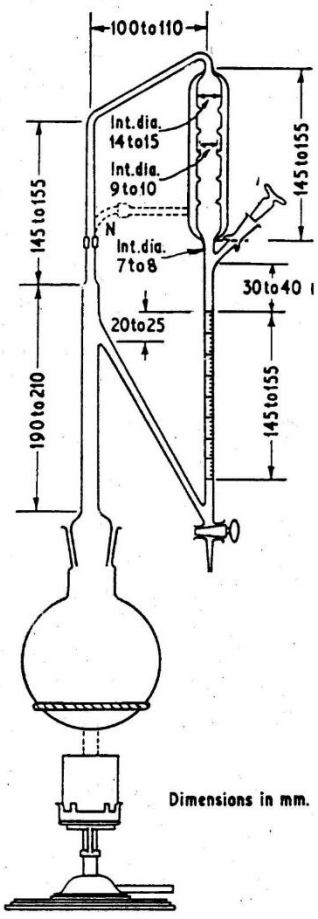
Analysis of volatile oils

Analysis of volatile oils is aiming at assuring their quality and purity and is based on the following criteria:

I. Physical examination.

II. Qualitative tests for the major oil constituents and some common adulterants.

III. Quantitative determination of the major oil constituents.



Clavenger's apparatus for determination of volatile oils

I- Physical examination of volatile oils

Sensory characters

1. Appearance.
2. Colour.
3. Odour.

B- Determination of physical constants

1. Solubility in different solvents especially in alcohol of different concentrations.
2. Specific gravity.
3. Optical rotation.
4. Refractive index (R.I.).
5. Congealing point.
6. Distillation range.

Determination of selected physical constants

1. Determination of specific gravity (Sp. Gr.)

The specific gravity of a volatile oil could be deduced from the following ratio:

Sp. Gr. = wt. of a certain volume of the oil / wt. of an equal volume of water

It is determined by the use of a **pycnometer** as follows :

Sp. Gr. = [c – a] / [b – a], where

a = Wt. of empty dry pycnometer

b = Wt. of pycnometer filled with H_2O

c= Wt. of pycnometer filled with oil.

For the official volatile oils Sp. Gr. varies in the range of 0.84-1.2.

- **Oils lighter than water** as oil of orange, caraway and coriander .... etc. are usually rich in hydrocarbons, alcohols, esters and ketones.
- **Oils heavier than water** as oil of cinnamon and clove usually contain aldehydes , phenols and phenolic derivatives.

2. Determination of refractive index (R.I.)

The refractive index of a volatile oil is measured in a refractometer such as Abbé **refractometer** and is calculated as follows:

R. I. = sin of angle of incidence light / sin of angle of refraction light

3. Determination of specific rotation

Optical rotation is defined as "the angle through which the plane polarized light is rotated by an optically active compound in solution". It can be measured by a **polarimeter**. Optically active substances are either levo- [l, (-)] or dextro-rotatory [d, (+)].

Specific rotation $[\alpha]^t_\lambda$

The optical rotatory power of a substance is generally expressed as the **specific rotation $[\alpha]^t_\lambda$** as follows:

$[\alpha]^t_\lambda$ = rotation per decimeter of solution / concentration of the solution.

It depends on the concentration and temperature of the substance and on the wave length of plane polarized light.

e.g. Specific rotation at 20 ^oC using the sodium D line is expressed as:

$$[\alpha]^{20}_D = [100 \times a] / [l \times c]$$, where

a = observed angle of rotation

l = length of the tube in dm

c = concentration g / 100 ml

4. Determination of congealing point

Some essential oils solidify when cooled at a low temperature. Examples are oils of anise, fennel and eucalyptus which contain high percentage of the readily crystallizable constituents anethole and cineole.

The congealing temperature of a volatile oil is one of the criteria for its genuinity. Low congealing temperature may indicate the partial removal of the characteristic constituents for which the oil is valued or addition of extraneous matter as alcohol.

II- Qualitative tests for selected volatile oils constituents

A. Menthol (Major constituent of oil of peppermint)

1. **Vanillin / sulfuric acid test**:

   To a solution of menthol in sulfuric acid, add a drop of **vanillin / sulfuric acid** reagent, an **orange yellow color** is developed, and on addition of **H_2O**, **a violet color** is produced.

2. **Sulfuric acid / nitric acid test**:

   To a solution of menthol in glacial acetic acid add 3 drops of **sulfuric acid** and a drop of **nitric acid**, **no green or bluish green** color is produced (**c.f. thymol**).

B. Thymol (Major constituent of oil of thyme)

1. **Sulfuric acid / nitric acid test**:

   To solution of thymol in glacial acetic acid, add 3 drops of **sulfuric acid**, followed by 1 drop of **nitric acid** , a **bluish green color** is produced (**c.f. menthol**).

2. Heat few crystals of thymol with NaOH solution on a water bath, a clear **colorless or pale red** solution is formed, which darkens on standing. Add few drops of **$CHCl_3$** and warm gently with continuous shaking, a **violet color** is produced.

3. Dissolve few crystals of thymol in about 2 ml of **$CHCl_3$** add a small amount of solid **KOH** and warm on a water bath, a **purple red color** is produced.

Camphor (Major constituent of oil of *Cinnamomum camphora*)

1. Natural **camphor** may be dextro- or levorotatory while **synthetic camphor** is optically inactive (being a racemic mixture) and usually contains **organic chlorocompounds.**

2. **Test for synthetic camphor (test for organic chlorocompounds) :**

   Place 0.1g of the camphor on a thin copper plate, about 4 cm square, in a porcelain dish and ignite it while covered with an inverted beaker of about one liter capacity the inner surface of which is moistened with distilled water. Set aside for 5 min., then rinse the beaker with 10 ml of distilled water and filter . To the filterate add few drops of nitric acid (T.S.) and 0.5 ml of N/10 silver nitrate.

   - If no opalescence is observed the sample is pure natural camphor.
   - If a slight opalescence is produced within 5 min (chloride ion) the sample is synthetic camphor or adulterated natural camphor.

II- Quantitative Determination of Selected Volatile Oil Constituents

1- Determination of Oxides in volatile Oils

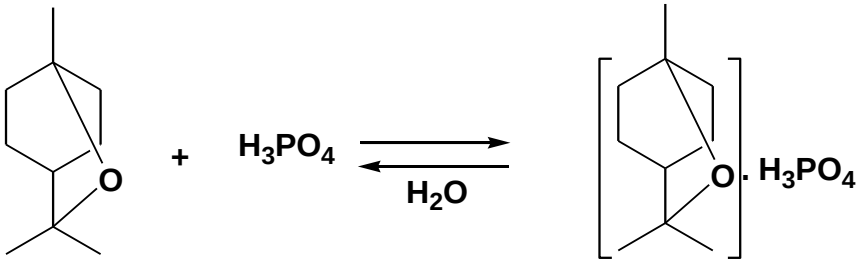
Determination of Cineole in oil of Eucalyptus

Occurrence :

Cineole is the major constituent of oils of cajuput and *Eucalyptus* (60 - 80%).

A. Volumetric method

Principle : This method depends on that cineole at low temperature reacts with syrupy phosphoric acid (Sp. Gr. 1.74) forming an additive solid product.



Cineole **Cineole phosphoric acid complex**

The oily non-cineole portion is separated from the solid mass by pressing between sheets of filter paper. The solid mass is then decomposed by hot water, the liquid obtained is transferred to a Cassia flask and separates into two layers. The volume of the oily layer is measured and represents the amount of cineole in the oil sample.

Procedure

1. In a clean and dry evaporating dish, put 5 ml of oil of *Eucalyptus* **accurately measured by a pipette** and place the dish in a freezing mixture (ice + NaCI) for 15 min.
2. In a **clean and dry test tube** take 1.5 - 3 ml of syrupy phosphoric acid (sp. gr.=1.74) and place the tube in the cooling mixture.
3. When both the oil and phosphoric acid are sufficiently cooled, add phosphoric acid **dropwise** to the oil in the dish and stir with a glass rod till formation of a solid friable mass.
4. Transfer this mass to a piece of lint, fold well and place it between two sheets of filter paper. Press the cake in a compressor for 5 min. Then replace the filter paper by another clean sheet and press again for 2 min .

5. Transfer quantitatively the solid mass into a clean **Cassia flask** by the aid of hot water (50 ml) and heat on a hot water bath for few min (to ensure complete breakdown of cineole / H_3P0_4 complex . Pure cineole appears as a clear upper oily layer).
6. Raise the oily layer into the graduated neck of the flask by addition of hot water (on the wall of the flask).
7. Leave at room temperature then measure the amount of the oily layer (in ml).

Calculation:

Percentage of cineole = [(volume of oily layer) x 100] / 5 = %v/v

Report	**Date:**
ml of cineole=	
Percentage of cineole =	= % v/v

B. Colorimetric method

Colorimetric methods are sensitive and used to estimate pure substances when present in very low concentrations. The method depends on the reaction between the tested substance and a specific reagent giving a stable blue color. The intensity of the color is directly proportional to the concentration (i.e. obeys Beer - Lambert's law).

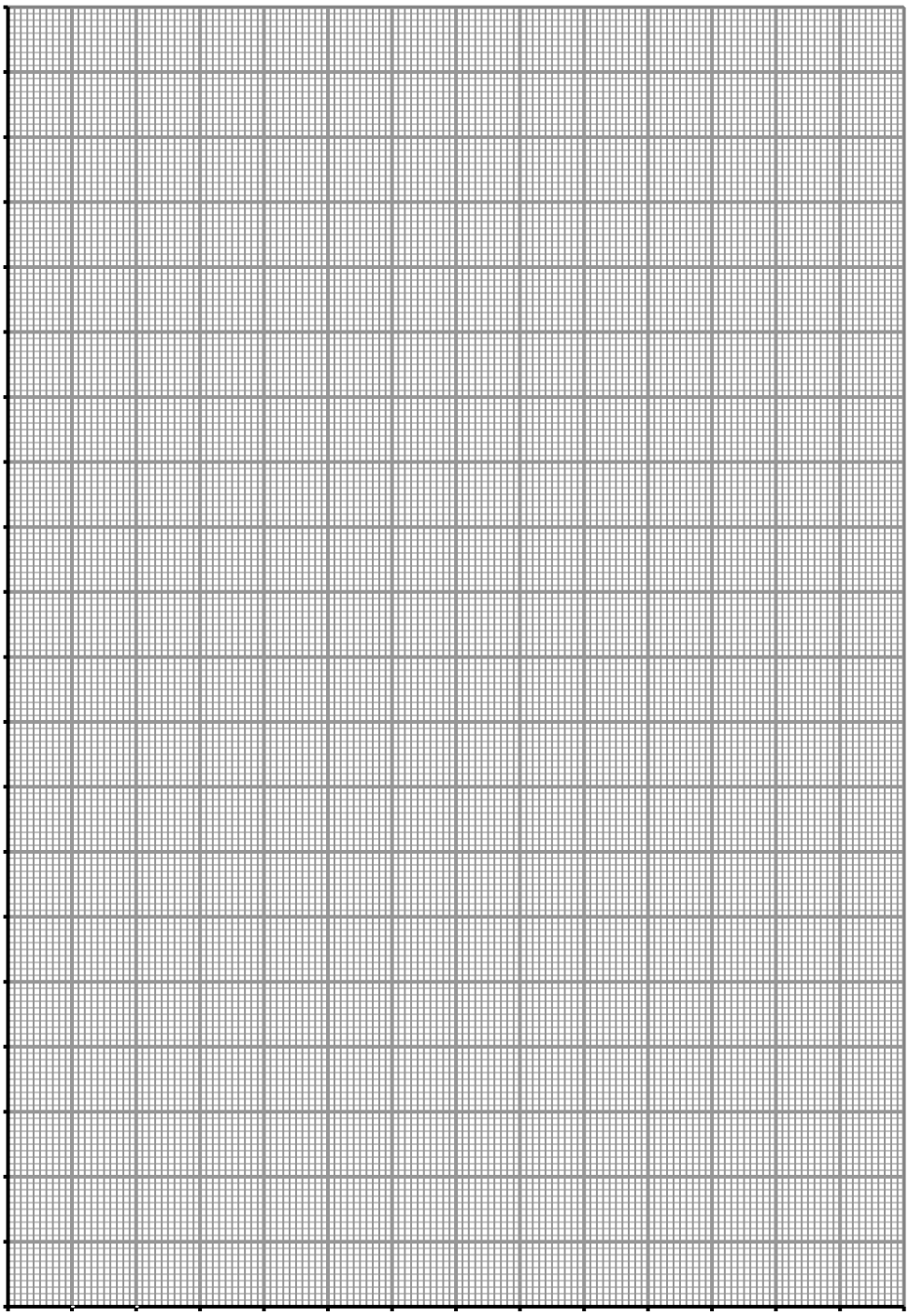
Principle

Cineole gives a blue color with vanillin/ sulfuric acid reagent which is proportional to its concentration. The absorbance of the color produced is measured at λ_{max} 610 nm (using filter No.5) against a blank.

Procedure

1. In a clean dry test tube which contains 2 ml ethyl alcohol (measured by pipette), transfer 1 ml of cineole solution in ethyl alcohol + 0.4 ml vanillin / H_2SO_4 reagent.
2. Carry out a blank experiment by using 3 ml ethyl alcohol + 0.4 ml vanillin / H_2SO_4 reagent.
3. Measure the color intensity (A) at λ_{max} 610 nm (using filter No.5) against the blank.
4. Determine the cineole concentration (C) from the standard curve established using different concentration of cineole as shown in the following table:

Concentration (μg)	**Absorbance**
50	0.125
200	0.31
300	0.44
500	0.62
650	0.87



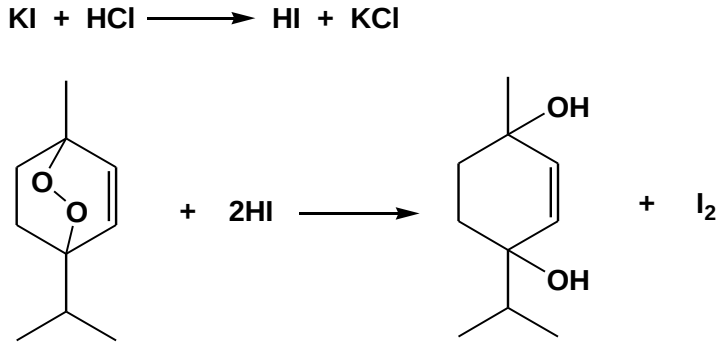
2- Determination of Peroxides in Volatile Oils

Determination of Ascaridole in Oil of Chenopodium

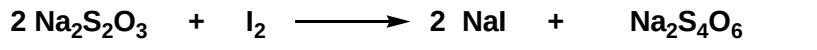
Occurrence: Major constituent of oil of Chenopodium (65 - 70%)

Principle

It depends on the oxidizing action of the peroxide (peroxide bond) present on the molecule of KI in strong acidic medium (HCI and gl. acetic acid) and at low temperature which leads to liberation of an equivalent amount of I_2 which can be titrated against $Na_2S_2O_3$ using starch as indicator.



Ascaridole



Sod. Thiosulphate **Sod. tetrathionate**

Procedure

1. In each of two stoppered tubes, transfer 1 ml 42% KI by pipette, 5 ml concentrated HCl and 10 ml glacial acetic acid. Immerse the tubes in a cooling mixture for 15 min.
2. To one of the stoppered tubes add by a burette 5 ml of the oil sample in glacial acetic acid, and keep the tube in the dark for 5 min (to prevent oxidation of KI to I_2 by light and to allow the reaction to complete).
3. Titrate the liberated I_2 with N/10 $Na_2S_2O_3$ using starch solution as indicator near the end point (straw yellow color).

E = ml of N/10 $Na_2S_2O_3$ consumed in the experiment

4. The other stoppered tube (free from the oil) is used as a blank. The reaction mixture is diluted with 20 ml H_20, and titrated as above.

B = ml of N/10 $Na_2S_2O_3$ consumed in the blank

Calculation:

1 ml N/10 $Na_2S_2O_3$ ≡ 0.00665 g ascaridole (**F**= 0.00665)

Percentage of ascaridole = [(**E - B**) x **F** x 100] / 5 = g % w/v

Remember:

1. **Cooling is essential in step "1"** because:
   - The reaction is vigorous at room temperature.
   - The reaction is exothermic and may result in volatilisation of I_2.
   - Heat of reaction may initiate consumption of I_2 in side reactions (e.g. addition on the double bond to form diodo derivative).
2. Water is added only in the blank (not in the experiment) as the oil is miscible only in strong acidic medium (glacial acetic acid / concentrated HCl) and separates on dilution with H_20.
3. Starch solution is added near the end point to avoid hydrolysis of starch in the strong acidic medium.
4. A blank experiment is essential to overcome the action of oxidising impurities (e.g. Cl_2 and KIO_3) in the reagent which may result in liberation of I_2 as follows:

a- $KIO_3 + HCl \longrightarrow HIO_3 + KCl$

$KI + HCl \longrightarrow HI + KCl$

$HI + HIO_3 \longrightarrow I_2$

b- $Cl_2 + 2\ KI \longrightarrow I_2 + 2\ KCl$

Report	**Date:**
E=	ml N/10 $Na_2S_2O_3$
B=	ml N/10 $Na_2S_2O_3$
Percentage of ascaridole =	= g% w/v

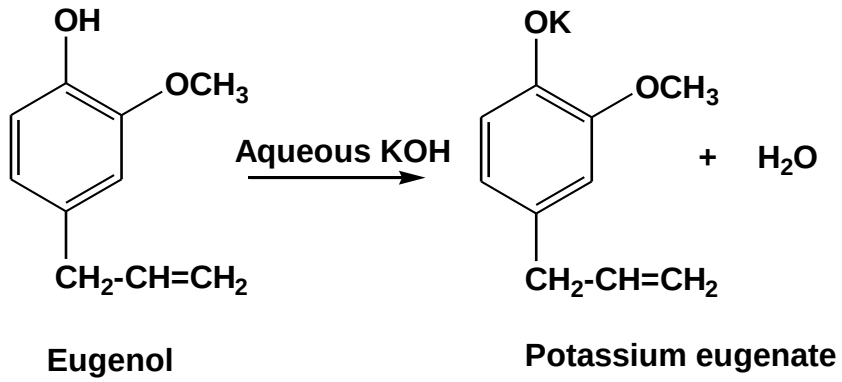
3- Determination of Phenols in Volatile Oils

Volumetric Determination of Eugenol in Oil of Clove

Occurrence : Eugenol occurs in the free form as the major constituent of oil of clove and combined as the glycoside gein in *Geum urbanum.*

Principle

It depends on that the volume of a phenol-containing volatile oil decreases on treatment with aqueous solution of strong alkali (e.g. KOH) due to solubility of its phenolic constituents in KOH to form K phenates. While the non-phenolic portion of the oil remains undissolved. By using a Cassia flask the non-phenolic portion of the oil can be determined and the volume of phenolic constituents is calculated by difference.



Eugenol **Potassium eugenate**

Procedure

1. Using a burette, transfer 5 ml of labelled oil of clove into a Cassia flask.
2. Add 50 ml of 5% aqueous KOH solution .
3. Heat on a boiling water bath for 15 min with occasional shaking, cool, and set aside till a clear oily layer separates.
4. Raise the oily layer to the graduated part of the flask using 5% aqueous KOH solution.
5. Determine the volume of the non-phenolic portion and calculate the volume of phenolic constituents.

Calculation:

Percentage of eugenol = [5 - (volume of non-phenolic portion) x 100] / 5 = % v/v

Notes:

1. Heating on a boiling water bath allows saponification of any eugenyl acetate present in oil of clove. Accordingly, the difference in volume will represent both eugenol and eugenyl acetate. Therefore, to determine eugenol only the reaction mixture is not heated.
2. KOH is preferred to NaOH as alkali because of the higher solubility of the potassium phenate salts in water.
3. Alcoholic KOH is not used due to solubility of both the phenolic and non phenolic portions of the oil in alcohol.
4. KOH is used in a 5% concentration , since higher concentrations of aqueous alkali may dissolve all the oil constituents and not only the phenolic.
5. This method is applied for estimation of high phenol concentrations in volatile oils . Low concentrations are determined by colorimetric or chromatographic methods.

Report **Date:**

Volume of non-phenolic portion = ml

Volume of phenolic portion = ml

Percentage of Eugenol = = % v / v

Colorimetric Determination of Thymol in Oil of Thyme

Occurrence: Thymol is the major constituent of oil of thyme (up to 80%)

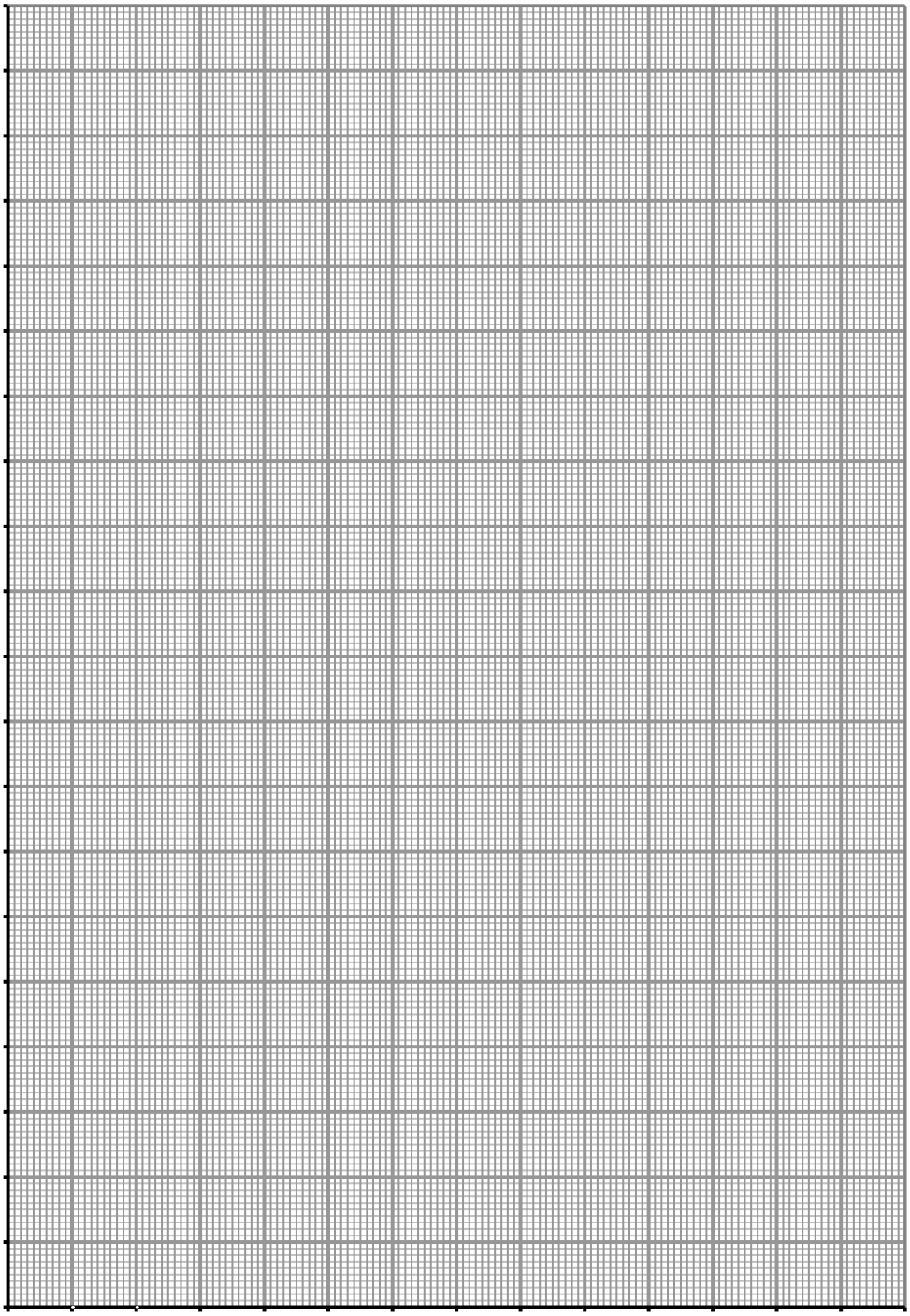
Principle

Thymol reacts with chloroform in alkaline medium to give a crimson red color, which is gradually developed, reaches its maximum intensity after 1 hr., and then fades gradually. The intensity of the color produced is proportional to concentration.

Procedure

1. **In a clean dry test tube, pipette 3 ml of thymol solution in anhydrous chloroform.**
2. **Add 1 ml 10% alcoholic KOH (in absolute alcohol).**
3. **Shake well and keep for 1 hr with frequent shaking.**
4. **Carry out a blank experiment using 3 ml of pure anhydrous $CHCl_3$.**
5. **Filter, under dry conditions (to avoid fading of the developed color and to prevent separation of $CHCl_3$ from alcoholic KOH).**
6. **Determine the color intensity (A) of the solution against the blank at λ_{max} 510 nm, (use filter No. 5).**
7. **From a pre-established standard curve of thymol, deduce the concentration (C) of thymol in the oil sample.**

Concentration (mg)	Absorbance
4	0.07
6	0.10
8	0.13
12	0.21



Report **Date:**

Absorbance of the solution: A =

Concentration of thymol in the oil: C =

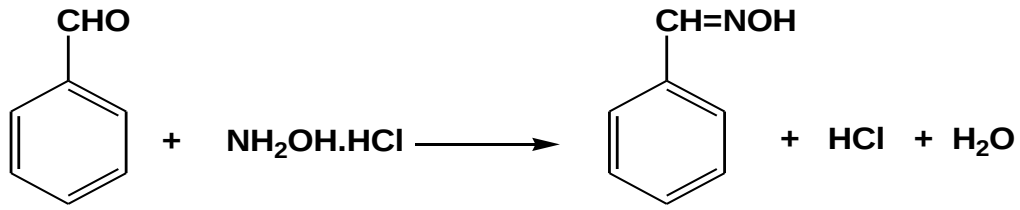
4 - Determination of Aldehydes in Volatile Oils

Determination of Benzaldehyde in Oil of Bitter Almond

Occurrence: Benzaldehyde occurs combined as the cyanophore glycoside, amygdalin in the kernels of bitter almond seeds; and as traces in the free state in oils of cinnamon, cassia and neroli.

Principle

Determination of aldehydes by **hydroxylamine method** is based on the fact that hydroxylamine hydrochloride reacts quantitatively with aldehydes to form aldoximes, with the liberation of equivalent amount of HCl, which is titrated against standard alkali, using methyl orange as indicator.



Benzaldehyde **Benzoxime**

Procedure

1. Transfer 5 ml of the oil solution (5% in alcohol) into a glass stoppered tube followed by 10 ml of hydroxylamine HCl in **alcohol 60%.**
2. Add 2 drops of **methyl orange indicator** (the solution becomes red).
3. Shake and neutralize the liberated acid with a solution of KOH (N/2 in **alcohol 60%)** until the red color turns yellow.
4. Continue titration with occasional shaking until the full yellow color remains permanent after shaking for 2 min. The reaction is completed in about **15 min**.
5. Add an additional amount (0.5 ml) of N/2 KOH (in 60% alcohol). Shake and set aside as color standard. (not more than 0.5 ml should be added, otherwise the solution becomes diluted).
6. Carry out a second determination following exactly the same procedure and match the color produced with that obtained in the previous experiment.
7. **Calculation:** Since 1 mole of benzaldehyde (M.Wt. 106) will liberate 1 mole of HCl from 1 mole of hydroxylamine hydrochloride and 1 mole of HCl ≡1 mole of KOH, therefore:

$$1 \text{ ml N / 2 KOH} = \frac{106}{2 \times 1000} = 0.05305 \text{ g benzaldehyde}$$

$$\text{Percentage of benzaldehyde} = \frac{\text{ml N / 2 KOH} \times 0.05305 \times 1.008 \times 100}{5} = \% \text{ w/v}$$

Notes on the procedure

1. The correction factor (1.008) is obtained experimentally and is due to the fact that the end point of the titration occurs at a pH different from that of the normal hydroxylamine HCl (end point occurs at pH 3.7-4, while the pH of end point should be at 7).
2. The change in color of methyl orange occurs at pH 3.5 - 5.0

Report	**Date:**
End point = mls N / 2 KOH	
Percentage of Benzaldehyde =	= % w / v

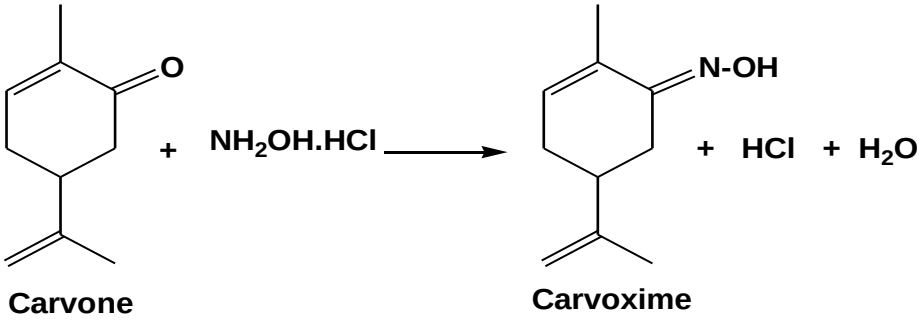
5- Determination of Ketones in Volatile Oils

Determination of Carvone in Oil of Caraway

Occurrence : Carvone occurs as the *d*-form in oils of caraway and dill fruit (50 - 60%) and in the *l*-form in oil of spearmint (70%).

Principle

Similar to aldehydes, ketones react quantitatively with hydroxylamine hydrochloride to form ketoximes with the liberation of HCl. They need more **drastic conditions**. The rate of the reaction is slower than that of aldehydes and is enhanced by heating on a water bath at 75–80 ^{o}C for 35 min to ensure reaction completion.



Procedure

1. Transfer 5 ml of the oil (5% solution in alcohol) into a stoppered tube.
2. Add 10 ml of NH_2OH. HCl in **alcohol 90%.**
3. Place the tube in a **water bath** kept at **75 -80 ^{o}C**.
4. Titrate the liberated acid with **N/1 KOH in alcohol 90%** until the red color of the dimethyl yellow indicator (**internal indicator** dissolved in the hydroxylamine HCl solution) turns to yellow.
5. Continue the addition of N/1 KOH, while heating in the water bath until the full yellow color of the indicator is reached and remains permanent after further heating for 5 min. The reaction is completed in about 35 min.
6. Add an additional amount of N/1 KOH (0.5 ml, to ensure that the reaction is complete), observe the yellow color produced and keep it as standard.
7. Carry out a second experiment following the same procedure until the yellow color obtained matches with that of the previous experiment.

Calculation:

1 ml N/1 KOH ≡ 0.1501 g carvone

$$\text{Percentage of Carvone} = \frac{\text{ml of N/1 KOH} \times 0.1501 \times 1.008 \times 100}{5} = \text{\% w/v}$$

Notes on the procedure

1. Heating at 75 - 80 ^{o}C is necessary in order to enhance the rate of the reaction (c.f. aldehydes)
2. The precipitate formed during titration is due to the formation of KCl which is sparingly soluble in alcohol 90%.

$$HCl + KOH \longrightarrow KCl + H_2O$$

3. The main differences between the reaction conditions required for aldehydes and ketones with $NH_2OH.HCl$ are summarized in the following table:

Aldehydes	Ketones
1. Reaction is carried out at room temperature **2.** The reaction is rapid and completed in about 15 min. **3.** $NH_2OH.HCl$ is dissolved in alcohol 60%. **4.** N/2 KOH is used in alcohol 60%. **5.** Methyl orange is used as external indicator.	**1.** Reaction is carried out at 75-85 ^{o}C. **2.** The reaction is slow and completed in about 35 min. **3.** $NH_2OH.HCl$ dissolved in alcohol 90%. **4.** N/1 KOH is used in alcohol 90%. **5.** Dimethyl yellow is used as internal indicator.

Report **Date:**

End point= mls N/1 KOH

Percentage of Carvone= = % w/v

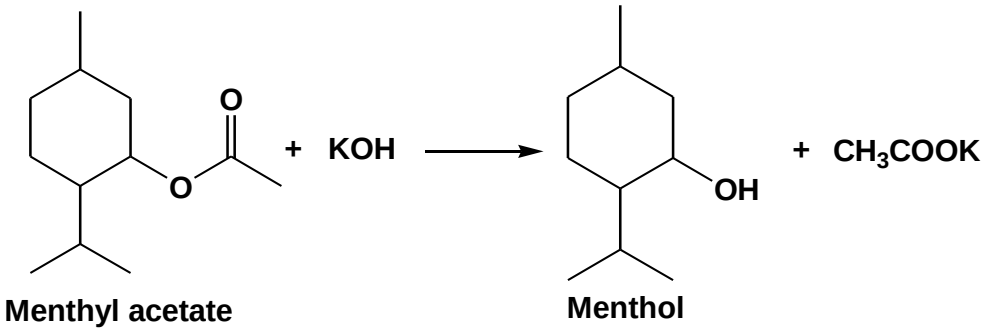
6 - Determination of Alcohols in Volatile Oils

Determination of Menthol in Oil of Peppermint

Occurrence: Oil of peppermint (50 - 65%) and Japanese mint oil (75 - 90%).

Principle

For volumetric determination of alcohols they should be **first converted to their corresponding esters**. The latter are hydrolyzed (saponified) when refluxed with alcoholic KOH, with the production of free alcohols & potassium salts of the acid components of the ester.



Menthyl acetate **Menthol**

Procedure

This includes 3 steps:

Step 1. Determination of the **ester value (a)** of the oil sample (this indicates the original ester content).

Step 2. Acetylation of the oil with acetic anhydride in the presence of anhydrous sodium acetate (in order to esterify the free alcohols)

Step 3. Determination of the **ester value (b)** of the acetylated oil (the increase observed in the ester value corresponds to the free alcohol content of the oil).

Calculation of free alcohol percentage (e.g. % of total alcohols calculated as menthol) is deduced from the following equation:

$$\text{Percentage of free alcohols} = \frac{(b\text{-}a) \times M}{0.42 \times (1335 - b)} = \text{g\% calculated as menthol}$$

Where, M = **mol. wt. of the alcohol (for menthol =156.3).**

a = **ester value of the original oil.**

b = **ester value of the acetylated oil.**

Notes:

- **The "**ester value**" of a sample of oil is the number of mgs of KOH required to saponify the esters present in 1 g of the oil.**
- **Determination of esters in terms of "ester value'" is convenient when the esters present in the oil sample are either unknown (since the mol. wt of the alcohol is not involved in the calculation), or when they vary much in their molecular weights.**

Step 1. Determination of the ester value of the original oil

1. In a conical flask, weigh accurately 2 g of the oil sample (using a tared flask).

2. Add 5 ml of neutral alcohol to dissolve the oil, followed by addition of 4 drops of phenol phthalein indicator.

3. Neutralize the free acids present in the oil sample with N/l0 alcoholic KOH (drop-wise till faint pink color is reached).

4. Add accurately 20 ml N/2 alcoholic KOH and reflux on a boiling water bath for 90 min. Cool and add 20 ml of H_20.

5. Titrate the excess alcoholic N/2 KOH against standard N/2 HCl, after addition of extra drops of phenol phthalein indicator **(E)**.

6. Carry out a blank experiment in the same manner (using a volume of neutral alcohol instead of that of the oil sample). The difference between the volume of N/2 KOH added and that N/2 HCl consumed in the titration represents the volume of alkali used for saponification of the esters present in the oil (i.e. representing the **blank, B**).

Calculation

Each 1 ml N/2 alcoholic KOH is equivalent to $\frac{\text{mol. wt of KOH}}{2 \times 1000}$

i.e. to $\frac{56.1}{2 \times 1000}$ = 0.02805 g of KOH

Ester value (a) = $\frac{(B - E) \times 0.02805 \times 1000}{\text{wt of the oil}}$ and

Ester percentage = $\frac{(B-E) \times M \times l00}{\text{wt of the oil}}$

where, **M** is the equivalent of 1 ml of N/2 N KOH corresponding to the ester

Examples: **M** = 0.0991 g of menthyl acetate
= 0.0981 g bornyl or linalyl acetate
= 0.0767 g methyl salicylate

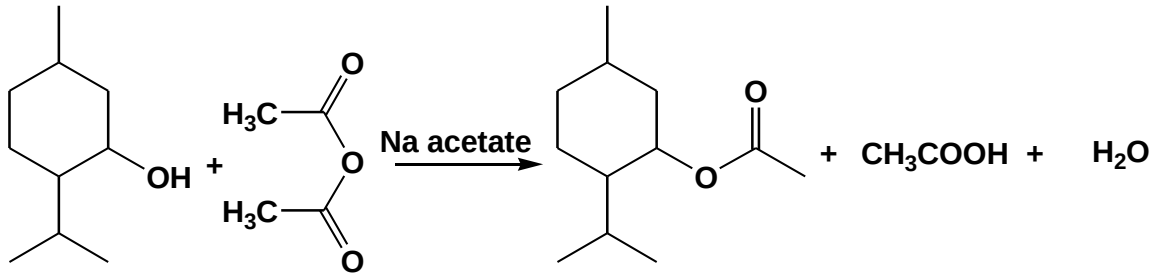
Notes:

1. The amount of volatile oil analyzed should be suitable. To ensure complete saponification the amount of alkali added is at least double that theoretically required. Too large samples or oils rich in esters should be treated with additional amounts of alkali.
2. Neutralization of free acids present in the oil before carrying out the ester value is necessary to prevent their interference during determination of the total esters.
3. Alcoholic KOH (not aqueous) is used for saponification because the oil is soluble in alcohol.
4. A blank experiment is carried out to determine the amount of alkali consumed in saponification process.

Report	**Date:**
Blank (**B**)=	mls N/2 HCl
End point (**E**) =	mls N/2 HCl
Ester value (**a**) =	mg KOH

Step 2. Acetylation of the free alcohols (major alcohol menthol)

Acetylation (e.g. of menthol) is carried out using **acetic anhydride** in presence of **anhydrous sodium acetate** which accelerates the acetylation process (by absorbing the H_20 liberated from the reaction and increasing the acetate ions).



Menthol **Menthyl acetate**

1. Transfer 10 ml of the oil by a pipette into an acetylation flask.
2. Add 20 ml acetic anhydride and 2 g of fused sodium acetate.
3. Attach the flask to an air condenser (not less than 75 cm long) and put on a small flame for about 2 hr (Add pumice to regulate the boiling).
4. Add 50 ml H_2O, heat on a boiling water bath for 15 min then cool (to decompose the excess acetic anhydride to acetic acid which is soluble in H_2O).
5. Transfer the content of the flask to a separating funnel and reject the lower aqueous layer.
6. Wash the oily layer by shaking with 50 ml brine solution (to minimize the solubility of the oil) and remove the aqueous layer.
7. Add 50 ml brine solution containing Na_2CO_3 (to neutralize the acetic acid resulting from the decomposition of acetic anhydride), then reject the brine solution (lower layer).
8. Wash the oil again with 50 ml brine solution and reject the lower layer.
9. Finally wash the oil with 50 ml of H_2O and remove the lower aqueous layer.
10. Transfer the acetylated oil to a clear bottle containing about 2 g of anhydrous sodium sulfate to avoid decomposition of esters due to presence of moisture.

Step 3. Determination of the ester value (b) of the acetylated oil

Repeat the procedure previously described in **step 1** using **1.0 g of the acetylated oil** and calculate in the same way the ester value **(b)** of the acetylated oil.

$$\textbf{Ester value (b)} = \frac{(B - E) \times 0.02805 \times 1000}{\text{wt of the acetylated oil}}$$

Report	**Date:**
Blank (**B**) =	mls N/2 HCl
End point (**E**) =	mls N/2 HCl
Ester value (b) =	mg KOH

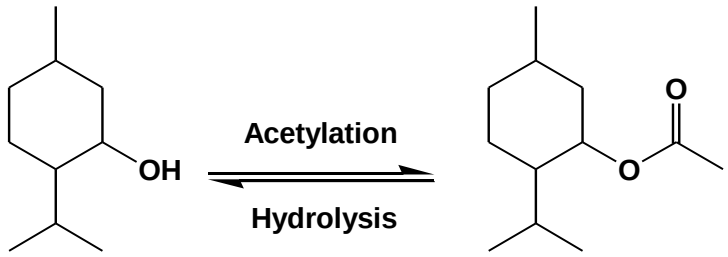
Calculation of alcohol content in the oil

The free alcohol content of oil of mentha calculated as menthol is deduced from the following equation:

$$\textbf{Percentage of free alcohols} = \frac{\textbf{(b-a) X M}}{\textbf{0.42 X (1335 - b)}} = \textbf{g\% calculated as menthol}$$

Explanation

1. Acetylation of menthol to menthyl acetate results in an increase in its molecular weight by 42 g (Mol wt of menthol = 156 g, Mol wt of menthyl acetate = 198g)



Menthol **Menthyl acetate**

- If 156 g of menthol are increased by 42g, therefore
- each **1 g** of menthol on acetylation will be increased by **42 / 156** and consequently
- **X g** of menthol will be increased by **42 X / 156**

2. Suppose that **1 g of the oil before acetylation** contains **X g menthol, therefore**

1 g of original oil on acetylation will yield 1 + (42X / 156) of acetylated oil.

3. The ester value **b** of the acetylated oil is calculated for 1 g of this oil (acetylated).

The ester value of an amount of the acetylated oil equivalent to 1 g of the original oil **will be equal to** b [1 + (42X / 156)]**.**

4. The **amount of mg of KOH required for saponification of menthyl acetate produced from X g menthol by acetylation will thus be:**

$$\frac{[\mathbf{b}\ (1 + (42X / 156)] - \mathbf{a}}{1000} = \text{mg KOH} \qquad (\textbf{equation 1})$$

Where, a = **ester value of the original oil and** b = **ester value of the acetylated oil.**

5. As 1 mole menthol ≡ 1 mole KOH

i.e. 156 g menthol ≡ 56.1 g KOH

Then, ? g menthol ≡ **1 g KOH**

1 g KOH ≡ 156 / 56.1 g menthol **(**equation 2**)**

6. From equations (1) and (2) we can conclude that:

$$\mathbf{X}\ (\text{g menthol in the oil}) = \frac{[\mathbf{b}\ (1 + (42\mathbf{X} / 156)] - \mathbf{a}}{1000} \times \frac{156}{56{,}1}$$

or $$\mathbf{X} = \frac{156\ (b - a)}{56100 - 42\ b}$$

7. The percentage of free alcohol calculated as menthol is thus deduced as follows:

$$\textbf{Percentage of menthol} = \frac{156\ (b - a)}{561 - 0.42\ b} = \frac{156\ (b - a)}{0.42\ (1335 - b)} = \%\ \text{w/w}$$

8. For determination of alcohols other than menthol, 156 is replaced by their corresponding mol. wt. (M)

Report **Date:**

Ester value of original oil **a** = mg of KOH

Ester value of acetylated oil **b** = mg of KOH

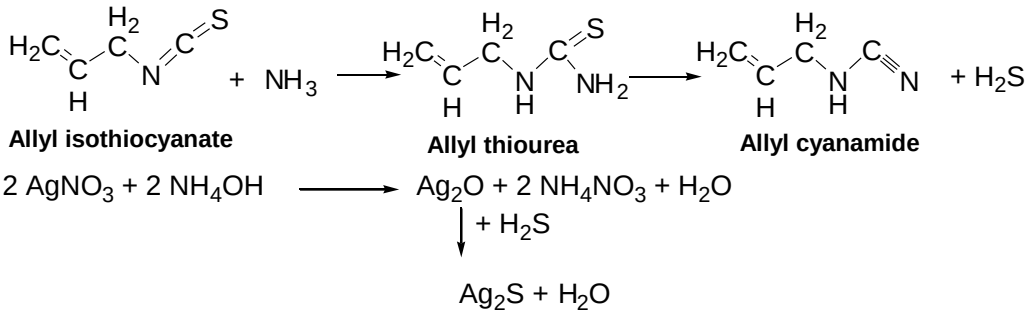
Percentage (% w/w) of alcohols calculated as menthol:

7 - Determination of Allyl Isothiocyanate in Volatile Oil of Black Mustard

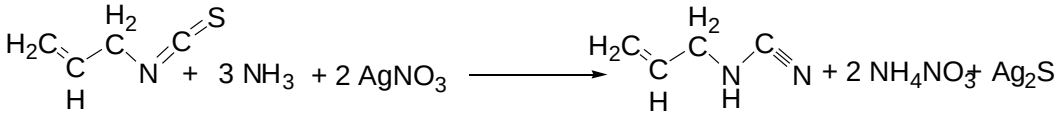
Occurrence: It occurs combined as the thioglycoside sinigrin in black mustard seeds.

Principle

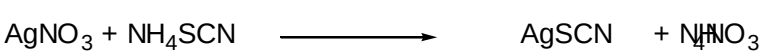
- The assay is based on that allyl isothiocyanate reacts quantitatively with $AgNO_3$ in ammoniacal solution to give allyl cyanamide and silver sulfide (Ag_2S).
- The precipitated Ag_2S is removed by filtration.
- The unreacted $AgNO_3$ is obtained in the filtrate (in the form of silver amine complex) and can be determined, after acidification with concentrated HNO_3 , by titration with ammonium thiocyanate using ferric alum (ferric ammonium sulfate) as indicator.



The overall reaction may be represented as follows:



The remaining $AgNO_3$ liberated from its comlex with ammonia reacts with NH_4SCN as follows:



Procedure

1. Transfer 20 ml of N/20 $AgNO_3$ (by pipette) to a 100 ml measuring flask and add 5 ml of the oil sample (by pipette) and 5 ml concentrated ammonia.
2. Stopper the flask, heat in a water bath at 60-80 ^{O}C for 30 min with frequent shaking. Cool to 25 ^{O}C and complete to the mark with distilled water.
3. Mix well and filter the black precipitate formed (Ag_2S) through dry filter paper into a dry flask. Reject the first 10 ml of the filtrate.
4. Take 20 ml of the filtrate (by pipette) into a conical flask, add 4 ml of concentrated HNO_3 and 3-4 drops of ferric alum solution as indicator.

5. Titrate against N/20 ammonium thiocyanate until the first permanent red color is obtained in the supernatant solution (notice that near the end point, flocculation of the white precipitate of AgSCN occurs).
6. Carry out a blank experiment using 20 ml of pure alcohol instead of the oil sample.

Calculation:

Since, 1 mole of allyl isothiocyanate ≡ 2 moles $AgNO_3$ ≡ 1 mole Ag_2O

Therefore, 1 ml N/20 $AgNO_3$ ≡ m. wt of C_3H_5NCS / 2 x 20 x 1000

i.e. 1 ml N/20 $AgNO_3$ ≡ 0.002479 g allyl isothiocyanate

$$\textbf{Percentage (w/v) of allyl isothiocyanate} = \frac{[\text{ml of } AgNO_3 - (\text{ml of } NH_4SCN \times 5)] \times 0.002479 \times 100}{5}$$

Notes:

1. The black precipitate formed on addition of ammonia is Ag_2O. However, on heating the most stable (Ag_2S) ppt is formed.
2. Heating for coagulation of the precipitate is carried at a temperature not exeeding 80 ^oC to avoid volatilization of the oil and decomposition of allyl thiourea.
3. Acidification of the filtrate with concentrated HNO_3 is essential to transform the unreacted silver present as silver amine complex into silver nitrate which is then titrated against ammonium thiocyanate.

Report **Date:**

End point = mls NH_4SCN

Percentage of allyl isothiocyanate = = %w/v

Quantitative estimation of atropine using colorimetric method

Principle:

The development of specific colour of alkaloids and particular reagents is utilized in the quantitative estimation of alkaloids in the extracts and/ or pharmaceutical preparations.

This technique required:

- Stable colour with this reagent.
- Linear measurement over a wide range of concentrations.

In this respect atropine as a father of Solanaceous alkaloids gives stable pink colour with para-dimethylaminobenzaldehyde suitable for measurement colourmetrically at λ 550 nm with sensitivity range 20-1500 μg/ml, against blank experiment. Final concentration of the alkaloid is obtained from previously prepared standard curve.

Procedure:

- Known weight of powdered drug or solid pharmaceutical product (known volume in case of ampoules or solution) is extracted for isolation of atropine.
- The chloroformic extract of atropine is evaporated in porcelain dish on boiling water bath till dryness.
- Five ml of colour reagent is added to the residue.
- Transfer the solution to test tube and heating is continued for 30 minutes on boiling water bath.
- The solution is left to cool to room temperature.
- Then five ml distilled water is added, mixed thoroughly and measured in spectrophotometer at λ 550 nm against blank prepared simultaneously.

Colour reagent:

Para-dimethylaminobenzaldehyde reagent 1.25 % (w/v) in sulphuric acid 65%, freshly prepared every 3-4 days.

Preparation of blank:

The blank is prepared by the previous mentioned procedure without using atropine.

Standard calibration curve:

The standard calibration curve is made as the following

- Different known concentrations of standard atropine are prepared.
- Complete the steps from 2-6 as mentioned before.
- The absorbance of the colour corresponding to each concentration of standard atropine is recorded.
- Construct a relationship between the absorbance and the concentration of standard atropine.

Calculation:

The alkaloidal content is expressed in a percentage value from the original weight of powdered drug or pharmaceutical product.

% Atropine content = (Atropine in μg x 100) / (Original weight of drug)

Results and comments:

Lab. report sheet

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Subject: .....

Report: …….. *Date:……*

Lab. report sheet

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