# Biochemistry

# 4th Year students

# By


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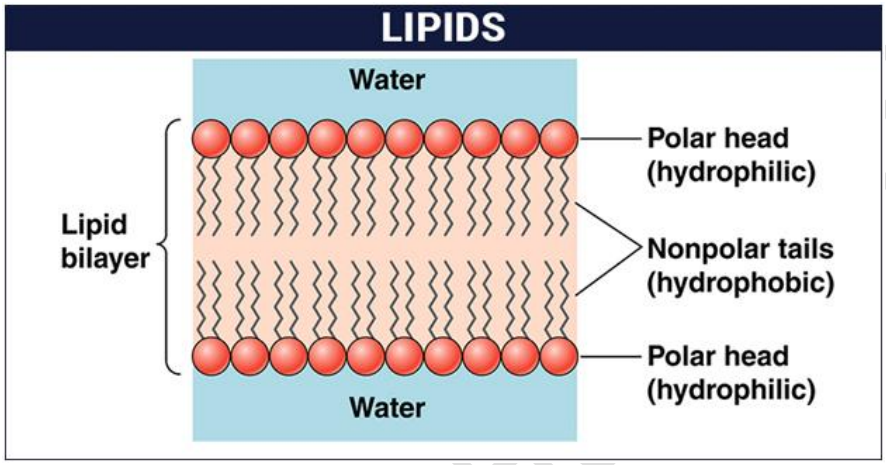
## Lipid

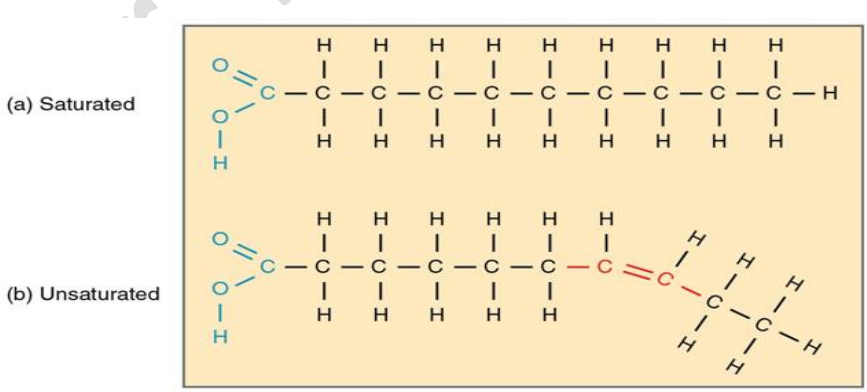
Lipids are organic compounds that contain hydrogen, carbon, and oxygen atoms, which form the framework for the structure and function of living cells

These organic compounds are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water because water is a polar molecule. In the human body, these molecules can be synthesized in the liver and are found in oil, butter, whole milk, cheese, fried foods and also in some red meats.




## Lipid structure

Lipids are the polymers of fatty acids that contain a long, non-polar hydrocarbon chain with a small polar region containing oxygen. The lipid structure is explained in the diagram below:



## Types of Lipids

There are numerous specific types of lipids, which are important to life, including **fatty acids, triglycerides, glycerophospholipids, sphingolipids and steroids**. These are broadly classified as simple lipids and complex lipids.

## Simple Lipids

Esters of fatty acids with various alcohols.

1. **Fats:** Esters of fatty acids with glycerol. Oils are fats in the liquid state
2. **Waxes**: Esters of fatty acids with higher molecular weight monohydric alcohols

## Complex Lipids

Esters of fatty acids containing groups in addition to alcohol and fatty acid.

1. Phospholipids: These are lipids containing, in addition to fatty acids and alcohol, phosphate group. They frequently have nitrogen-containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
2. Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine and carbohydrate.
3. Other complex lipids: Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category.

### Fatty acids

Fatty acids are carboxylic acids (or organic acid), usually with long aliphatic tails (long chains), either unsaturated or saturated.

### Saturated fatty acids

Lack of carbon-carbon double bonds indicate that the fatty acid is saturated. The saturated fatty acids have higher melting points compared to unsaturated acids of the corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape.

### Unsaturated fatty acids

Unsaturated fatty acid is indicated when a fatty acid has more than one double bond.

"Often, naturally occurring fatty acids possesses an even number of carbon atoms and are unbranched."

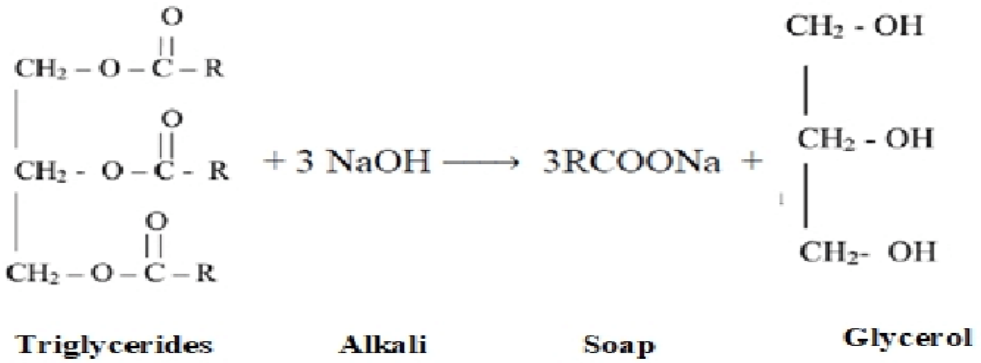
On the other hand, unsaturated fatty acids contain a cis-double bond(s) which create a structural kink that disables them to group their molecules in straight rod-like shape.

## Chemical properties of lipids

### Hydrolysis

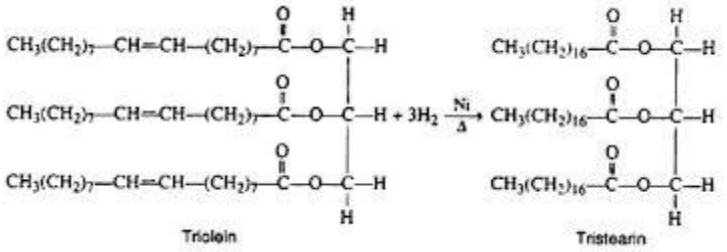
Fats undergo hydrolysis when they are treated with mineral acids , the alkalies of fat splitting eznyme lipase to yield glycerol and fatty acids.

Hydrolysis by alkalies such as NaOH or KOH leads to the formation of sodium or potassium salts of fatty acids. the salts are known as soaps and process of its formation is known as saponification.



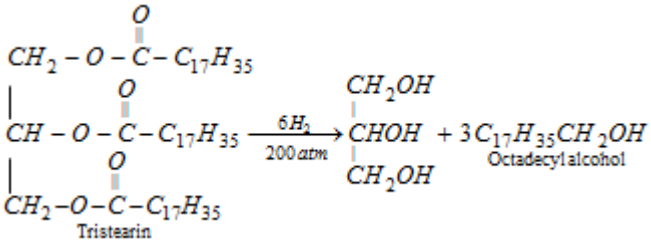

## Hydrogenation

Oil containing unsaturated fatty acids can be hydrogenated in the presence of high temperature, pressure and finely divided nickel. By this process the oils are converted to solid fats. this reaction forms the basis of the industrial production of hydrogenated oil (vegetable ghee or margarine).




## Hydrogenolysis

Oils and fats are converted in to glycerol and long chain aliphatic alcohol when excess hydrogen passed through them in the presence of copper-chromium catalyst. This splitting of fats by hydrogen is called hydrogenolysis.



## Halogenation

When unsaturated fatty acids are treated with halogen such as iodine and chlorine, they take up iodine or other halogens at their double bond site.

This process of taking of iodine is known as halogenation and it is indication of unsaturation . Iodine number is the percentage of iodine absorbed by a fat.

## Rancidity

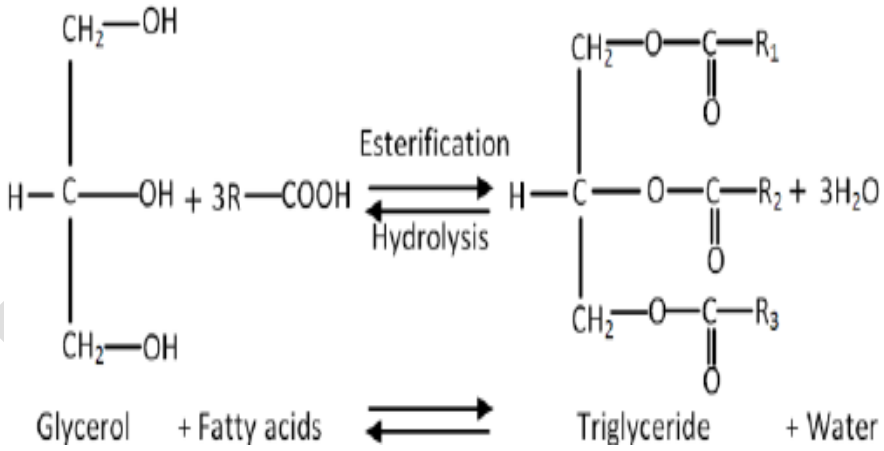
Oils and fats, on long storage in contact with heat, light, air and moisture, develop an unpleasant odour. Such oil and fats are known as rancid oils and fats. the rancidity develops due to certain chemical changes taking place in the fat.

The changes include:

**A-enzymatic hydrolysis**

**B-air oxidation of unsaturated fatty acids.**

**C-β-oxidation of saturated fatty acids.**



## Emulsification

This process of breaking of large-sized fat molecules into smaller ones is known as emulsification. In animals, it is brought about by bile juice liberated from liver. Other emulsifying agents are water, soaps, proteins and gums.

## 3-Derived lipids ( steroids)

- Are manufactured from lipid precursors in the cells
- Each steroid molecule possesses a fused four ring skeleton known as cyclopentanoperhydrophenanthrene.
- Steroids are the members of the group triterpenoids.
- Cholesterol is the most important and common sterol found in the mammals.
- Sterol glycosides are present in small amount in higher plants. They can be divided into three main group sterolins- saponins – cardiac glycosides.
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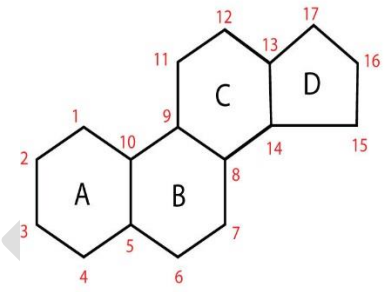
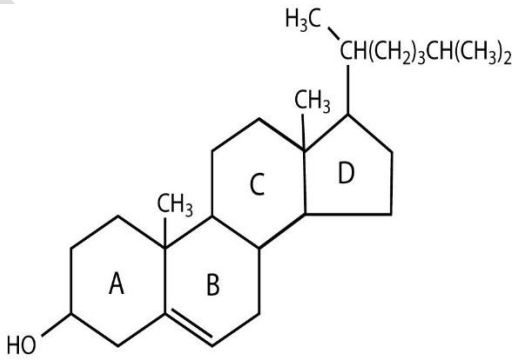



(a) Steroid skeleton




(b) Cholesterol

## Importance of lipids

- Fats serve as reserve food.
- Oils are used by human for various purposes

- Lipids provide the structural frame-work to the living tissues of plants and animals.
- Lipids serve as the prime fuel reserve for metabolism and provide more energy than carbohydrates and proteins.
- Waxes give a protective covering on the upper surface of leaves, stems and fruits and this covering also provides resistance to water, insects and bacteria.
- Certain oils, like castor oil, mustard oil, clove oil and coconut oil are used medicinally.
- Other oils are used in the preparation of soaps and vegetable ghee.

## Lipid metabolism

Biosynthesis of fats

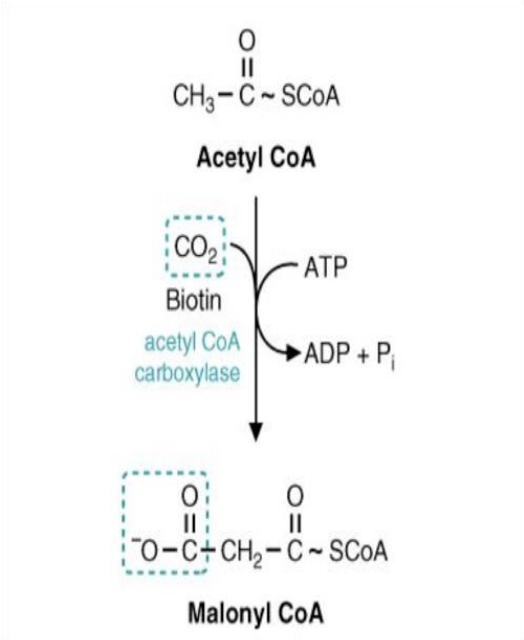
1-Synthesis of fatty acids

2- Synthesis of glycerol

3-condensation of fatty acids and glycerol

### Synthesis of fatty acids

in biochemistry, **fatty acid synthesis** is the creation of **fatty acid** from acetyl-CoA and NADPH through the action of enzymes called fatty acid synthases. This process takes place in the cytoplasm of the cell. Most of the acetyl-CoA which is converted into fatty acids is derived from carbohydrates via the glycolytic pathway. The glycolytic pathway also provides the glycerol with which three fatty acids can combine (by means of ester bonds) to form triglycerides (also known as "triacylglycerols" – to distinguish them from fatty "acids" – or simply as "fat").






## Secondary Metabolites

The sum of all of the chemical reactions that take place in an organism is called metabolism. Some aspects of metabolism, such as the metabolism of carbon and nitrogen assimilation and energy conversions. Most of that carbon, nitrogen, and

energy ends up in molecules that are common to all cells and are required for the proper functioning of cells and organisms. These molecules, e.g., lipids, proteins, nucleic acids, and carbohydrates, are called primary metabolites. Unlike animals, however, most plants divert a significant proportion of assimilated carbon and energy to the synthesis of organic molecules that may have no obvious role in normal cell function. These molecules are known as secondary metabolites.

- Terpenes, including hormones, pigments, essential oils, steroids, and rubbe

• Phenolic compounds, including coumarins, flavonoids, lignin, and tannins

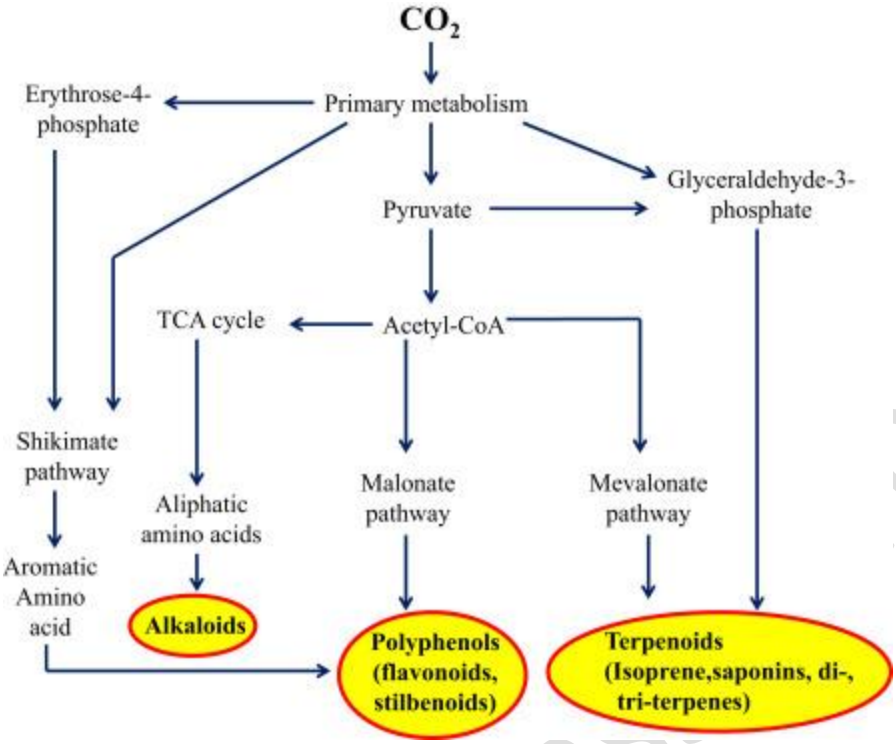
• Glycosides, including saponins, cardiac glycosides, cyanogenic glycosides, and glucosinolates.

• Alkaloids.

Some secondary metabolites are also involved in defense against invading pathogens, a subject that is also addressed in this chapter.

At the biosynthetic level, primary and secondary metabolites share many of the same intermediates and are derived from the same core metabolic pathways (Figure 27.1). In the strictest sense, however, secondary metabolites are not part of the essential molecular structure or function of the cell. Secondary metabolites generally, but not always, occur in relatively low quantities and their production may be widespread or restricted to particular families, genera, or even species. Also known as natural products, these novel phytochemicals were of little interest to biologists because of their apparent lack of biological significance. They were known, however, to have significant economic and medicinal value and were thus of more than a passing interest to natural products chemists. They include drugs and other medicinal products, dyestuffs, feedstocks for chemical industries (gums, resins, rubber), and a variety of substances used to flavor food and drink. In recent years, however, it has

become increasingly evident that many natural products do have significant ecological functions, such as protection against microbial or insect attack.






## Terpenoids

With nearly 15,000 structures known, terpenoids are probably the largest and most diverse class of organic compounds found in plants. As discussed earlier in Chapter 19, the unifying feature of terpenes is that they are generally lipophilic polymers

based on the simple 5-carbon unit 2-methyl-1,3-butadiene, or isoprene, which is derived via either the mevalonic acid pathway or the MEP pathway (Chapter 19, Section 19.3). Terpenes can be grouped into several classes, based on the number of carbon atoms (Figure 27.2). This large chemical diversity arises from the number of basic units in the chain and the various ways in which they are assembled. Formation of cyclic structures, addition of oxygen-containing functions, and conjugation with sugars or other molecules all add to the possible complexity. The name terpenoid derives from the fact that the first compounds in the group were isolated from turpentine (Ger. terpentin), an essential oil (chiefly pinene) distilled from the resins of several coniferous trees.

The terpene family includes hormones (gibberellins and abscisic acid): the carotenoid pigments (carotene and xanthophyll); sterols (e.g., ergosterol, sitosterol, cholesterol) and sterol derivatives (e.g., cardiac glycosides); latex (the basis for natural rubber); and many of the essential oils that give plants their distinctive odors and flavors. Cytokinin hormones and chlorophyll, although not terpenes per se, do contain terpenoid side chains. It is apparent from this list that many terpenes have significant commercial value as well as important physiological roles. Many terpenes and terpene derivatives may be considered primary metabolites. The hormones abscisic acid and gibberellin, the carotenoid and chlorophyll pigments, and sterols (steroid alcohols) all play significant roles in plant growth and development. The vast majority of terpenes, however, are secondary metabolites, many of which appear to act as toxins or feeding deterrents to herbivorous insects.

## TERPENES ARE CONSTITUENTS OF ESSENTIAL OILS

Many plants, such as citrus, mint, Eucalyptus, and various herbs (sage, thyme, etc.), produce complex mixtures of alcohols, aldehydes, ketones, and terpenoids, known generally as essential oils (essence, as in perfume). Essential oils are responsible for

the characteristic odors and flavors of these plants but they are also known to have insect-repellant properties. The terpenes and terpene derivatives found in essential oils are predominantly hemi-, mono-, and sesquiterpenes, which can be moderately to highly volatile. Several examples are shown in Figure 6. In most plants, the essential oils are synthesized in special glandular trichomes (hairs) on the leaf surface (Figure 27.4), although the essential oils of citrus are produced by glands in the peel. The resins of certain conifers, for example, also accumulate mixtures of terpenes, including the monoterpenes, α- and β-pinene, and myrcene (Figure 27.5).

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

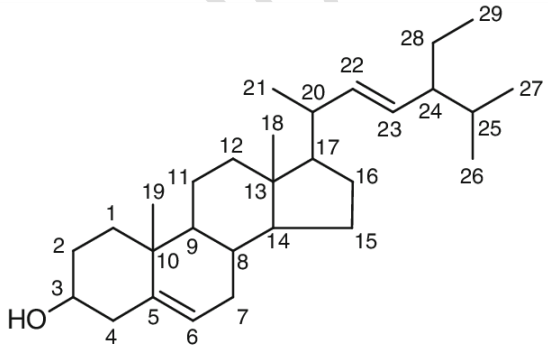
| S.No. | No of C atoms | Isoprene units, n | Class | Examples |
|---|---|---|---|---|
| 1 | 5 | 1 | Hemiterpenes $C_5H_8$ | Prenol; isovaleric acid (O containing derivatives) |
| 2 | 10 | 2 | Monoterpenoids ($C_{10}H_{16}$) | Myrcene; Limonene; pinene |
| 3 | 15 | 3 | Sesquiterpenoinds ($C_{15}H_{24}$) | Farnesene; humulene; cadinenes; Copaene |
| 4 | 20 | 4 | Diterpenoids ($C_{20}H_{32}$) | retinol, retinal, phytol |
| 5 | 25 | 5 | Sesterpenoids ($C_{25}H_{40}$) | ceroplastol |
| 6 | 30 | 6 | Triterpenoids($C_{30}H_{48}$) | squalene |
| 7 | 40 | 8 | Tetraterpenoids($C_{40}H_{64}$) | Carotenes, carotenoids |
| 8 | >40 | >8 | Polyterpenoids$(C_5H_8)_n$ | Natural Rubber |

**Fig. 6**

## STEROIDS AND STEROLS ARE TETRACYCLIC TRITERPENOIDS

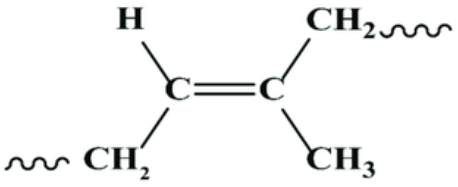
Steroids and sterols are synthesized from the acyclic triterpene squalene, although they generally are modified and have fewer than 30 carbon atoms. Steroids with an alcohol group, which is the case with practically all plant steroids, are known as

sterols. The most abundant sterols in higher plants are stigmasterol and sitosterol (Figure 27.6), which often make up more than 70 percent of the total sterols. However, plants also contain a large number of the more than 150 other sterols known to occur in nature. Plant sterols include cholesterol which, although widespread in occurrence, is present in only trace quantities. The extremely low level of cholesterol allows plant oils to be marketed as ‘‘cholesterol-free.’’ Sterols are constituents of plant membranes, which is perhaps their most important known function in plants. Because sterols are planar molecules, their packing properties are different from phospholipids that make up the bulk of the membrane bilayer. Sterols pack more tightly than phospholipids and therefore tend to increase the viscosity and enhance the stability of membranes. Otherwise, little is known about the function of sterols in plants. Unlike the steroid hormones in animals, there is no known hormonal role for sterols in plant development. Some sterols may have a protective function, such as the phytoecdysones, which have a structure similar to the insect molting hormones. When ingested by insect herbivores, phytoecdysones disrupt the insect’s molting cycle. Other sterols are present as glycosides, which give rise to a number of interesting and economically significant secondary products. Steroid glycosides are discussed below.

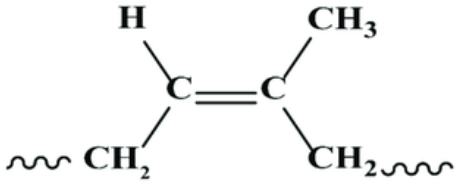



## POLYTERPENES INCLUDE THE CAROTENOID PIGMENTS AND NATURAL RUBBER

Larger terpenes include the tetraterpenes (40-carbon) and the polyterpenes. The principal tetraterpenes are the carotenoid family of pigments. The only important isoprene derivatives with a greater molecular mass than the tetraterpenes are rubber and gutta. Rubber is a polymer consisting of up to 15,000 isopentenyl units. The polymer may be linear, as shown in Figure 7, or cross-linked into more complex configurations. The only difference between rubber and gutta is the configuration of the double bonds. In rubber the double bonds are all cis configurations, while in gutta the double bonds are all trans.




Gutta-percha ( *trans*-1,4-polyisoprene)




Natural rubber ( *cis*-1,4-polyisoprene)

**Fig.7**

In the plant, rubber occurs as small particles suspended in a milky-white emulsion called latex. Latex production is widespread in plants, with estimates ranging from a few hundred to several thousand species that produce latex in some form. The principal commercial source is *Hevea brasiliensis*, a rubber tree native to the Amazon rainforest. Others include the ornamental rubber tree (*Ficus elastica*), milkweed (Asclepias), and the Russian dandelion (Taraxacum Kok-saghyz). Latex contains about 30 to 40 percent rubber and 50 percent water. The balance is a complex mixture of resins, terpenes, proteins, and sugars. In most plants, latex is produced in the phloem, accumulating in a series of long, interconnected vessels called laticifers. The best-known source of gutta is a desert shrub, Parthenium argentatum, which grows in northern Mexico and southwestern United States. Parthenium (commonly known as guayule) may contain as much as 20 percent latex

by weight, which is stored not in laticifers but in the vacuoles of stem and root cells. Guayule was at one time a significant commercial source of gutta for use in rubber products. However, while a single rubber tree, if properly tapped, can continue to produce for up to 30 years, guayule plants must be harvested (and, of course, replanted) annually.

Finally, there is a connection between terpenes and air pollution. Many of the essential oils, especially hemiterpenes, monoterpenes, and sesquiterpenes, are highly volatile and are given off in large quantities by plants, particularly during warm weather. Known generally as volatile organic carbon (**VOC**), these natural emissions from plants contribute to the formation of haze and cloud, and are involved in the formation of toxic tropospheric ozone.

## GLYCOSIDES

Some of the more interesting, if not important, derivatives synthesized by plants are glycosides. Most glycosides are thought to function as deterrents to herbivores. The term glycoside (Gr. glykys, sweet) refers to the bond formed (called a glycosidic bond) when a sugar molecule condenses with another molecule containing a hydroxyl group. Sugars may form glycosidic bonds with other sugars, such as when linked together to form polysaccharides, or with hydroxyl groups on noncarbohydrate molecules, such as steroids or amino acids. The sugar most commonly found in glycosides is glucose, although specific glycosides often contain rare sugars. Three particularly interesting glycosides are the **saponins**, the **cardiac glycosides (cardenolides)**, and the **cyanogenic glycosides**. A fourth family, the glucosinolates, although technically not glycosides, are a similar structure and so are included here.

## SAPONINS ARE TERPENE GLYCOSIDES WITH DETERGENT PROPERTIES

Saponins may take the form of (1) steroid glycosides, (2) steroid-alkaloid glycosides, or (3) triterpene glycosides (Figure 27.8). Saponins may also occur as a glycones (e.g., the terpene without the sugar), which are known as sapogenins. In much the same way as soap, which is the sodium salt of a fatty acid, the combination of a relatively hydrophobic triterpene with a hydrophilic sugar gives saponins the properties of a surfactant or detergent. When agitated in water, saponins form a stable soapy foam. The name saponin is in fact derived from Saponaria (soapwort), which at one time was employed as a soap substitute.

The principal role of saponins appears to be as a preformed defense against attack by fungi. Evidence indicates that saponins form complexes with sterols containing an unsubstituted 3-β-hydroxyl group. When the saponins react with sterols in the membranes of invading fungal hyphae, the result is a loss of membrane integrity. In a classic example of one-upmanship, however, many pathogenic fungi have developed strategies, such as the development of detoxifying enzymes, for circumventing this defense mechanism. Oat (Avena), for example, produces a triterpenoid saponin, avenacin A-1, which is localized in the root epidermal cells and effectively protects against an invasion by a fungal pathogen (Gaeumannomyces graminis var. tritici) that infects the roots of both wheat (Triticum) and barley (Hordeum). However, another strain of G. graminis (var. avenae) produces an enzyme, avenacinase, that detoxifies avenacin A-1 and allows the pathogen to invade oats as well as wheat and rye. The effect of saponins on eukaryotic membranes is highly nonspecific and it is not clear how plants protect their own membranes against the deleterious effects of their own saponins. One possibility is that the saponins are stored in the form of a biologically inactive molecule, called a

bisdesmosidic saponin, which has two sugar chains rather than one. When under attack, the inactive form may be converted to the active monodesmosidic form by hydrolytic removal of the second sugar chain. Alternatively, biologically active, monodesmosidic saponins may be sequestered in vacuoles or organelles whose membranes containahigh proportion of sterols with a protected 3-β-hydroxyl position. The effect of saponins on animals is somewhat variable. While not significantly toxic to mammals, saponins do have a bitter, acrid taste and will cause severe gastric irritation if ingested. Saponins will hemolyze red blood cells, however, if injected into the bloodstream. This action is presumably because of their detergent properties and their ability to disrupt membranes generally. On the other hand, saponins are highly toxic to fish and have been used as fish poisons. Saponins have also been implicated in reports of livestock poisoning. Alfalfa saponins, for example, can cause digestive problems and bloating in cattle. At the same time, there are reports that saponins contained in alfalfa sprouts will lower serum cholesterol levels. Commercially, saponins from the bark of Quillaja saponaria have been used as surfactants in photographic film, in shampoos, liquid detergents, toothpastes, and beverages (as emulsifiers). The saponin glyscyrrhizin from licorice (Glyscyrrhiza glabra) has been used in medicines and as a sweetener and flavor-enhancer in foods and cigarettes.

## CARDIAC GLYCOSIDES ARE HIGHLY TOXIC STEROID GLYCOSIDES

The cardiac glycosides (or, cardenolides) are structurally similar to the steroid saponins and have similar detergent properties. They are distinguished from other steroid glycosides by the presence of a lactone ring (attached at C17) and the rare sugars (found almost exclusively in this group of steroids) that form the glycoside (Figure 27.9). Like the saponins, cardenolides occur naturally as either the glycoside or the aglycone (or genic). The cardenolides have a wide distribution; they have been

recorded in more than 200 species representing 55 genera and 12 families and are a principal agent in accidental poisonings of humans. Perhaps the best known is digitalis,amixture of cardenolides extracted from the seeds, leaves, and roots of purple foxglove, Digitalis purpurea or Grecian foxglove, D. lanata. The two principal cardenolides in digitalis are digitoxin and its close analog digoxin. Digitalis is also the source of a saponin, digitonin. Since the late eighteenth century, digitalis has been used for its therapeutic value in treating heart conditions such as atherosclerosis. Because they disrupt the heart muscle Na+/K+-ATPase pumps (hence the appellation cardiac), cardenolides are highly toxic to vertebrates. The extreme toxicity of cardenolides has long been exploited by African hunters, who coated their arrows and spears with cardenolide-rich extract from plants. In therapeutic use, however, carefully regulated doses can both slow and strengthen the heartbeat. Unfortunately, the lethal and therapeutic doses are very close, so the therapy must be carefully monitored. Other common sources of cardenolides are the milkweeds, Asclepias and Calotropis. These two species are known as ‘‘milkweeds’’ because they produce a milky-white, cardenolide-rich latex. The milkweeds are particularly interesting because they are the principal host for ovipositing monarch butterflies. The emerging larvae feed on the milkweed leaves and sequester the cardenolides without ill effect. The cardenolides are retained through metamorphosis and are present in the adult monarchs. When birds, such as blue jays, attempt to feed on monarchs, the accumulated caredenolides induce an emetic reaction that forces the bird to vomit. The bird then wisely avoids attempting to feed on monarch larvae for some time.

## CYANOGENIC GLYCOSIDES ARE A NATURAL SOURCE OF HYDROGEN CYANIDE

It might seem odd that plants synthesize chemicals capable of releasing deadly hydrogen cyanide or prussic acid (HCN), but more than 60 different cyanogenic compounds of plant origin have been described from more than a dozen plant families. Predominant among these are the cyanogenic glycosides. A common cyanogenic glycoside is amygdalin (Figure 27.10), which occurs in many representatives of the family Rosaceae. It is found in the seeds of apples and pears and in the bark, leaves, and seed of the stone fruits (apricot, peaches, plums, cherries). Most cyanogenic glycosides appear to be derived from one of four amino acids (phenylalanine, tyrosine, valine, and isoleucine) or from nicotinic acid. Intact cyanogenic glycosides are not themselves toxic, but when the plant is damaged by a herbivore, the glycoside undergoes an enzymatic breakdown and cyanide is released. Cyanide, a noncompetitive inhibitor of cytochrome oxidase, is acutely toxic. The enzymatic breakdown of cyanogenic glycosides is a two-step process (Figure 27.10). First, the sugars are released by the enzyme β-glycosidase. The resulting hydroxynitrile is moderately unstable and will slowly decompose, liberating HCN in the process. Normally, however, decomposition is accelerated by a second enzyme, hydroxynitrile lyase. Enzymatic release of cyanide does not normally occur in intact plants because the enzymes and the substrate are spatially separated. In some cases, separation is maintained within the cell, but in others, the enzymes are in one cell and the cyanogenic glycosides in another. In Sorghum, for example, the cyanogenic glycoside dhurrin is synthesized and stored in epidermal cells, while the glycosidase and lyase enzymes are found in the mesophyll cells. Only when the tissue is crushed and the contents of the two cells are mixed will cyanogenesis occur. There is some evidence that the presence of cyanogenic glycosides deters feeding by insects and other herbivores, although most animals have the ability to detoxify small quantities of cyanide. Clearly the effectiveness of cyanogenic glycosides as a deterrent depends on many factors, such as the amount present, the rate of release of

cyanide, and the ability of the animal to detoxify. The level of cyanogenic glycosides in plants is highly variable, influenced by both genetic control and environmental stress. The latter is a concern when using Sorghum for livestock forage. Dhurrin accumulates rapidly and can cause livestock poisoning when Sorghum plants are stressed by drought or frost. Many common food plants naturally contain cyanogenic glycosides in concentration sufficiently low that they are not normally a health hazard. These include soy and other beans (Fabaceae); apples, apricots, peaches, plums, and other fruits in the family Rosaceae; and flax seed (Linum), which is a popular health food. One food source that contains large amounts of cyanogenic glycosides is cassava, a potato-like root of the tropical plant Manihot esculenta. Cassava, also known as manioc or, in North America, tapioca, is a major source of starch for millions of people in tropical countries. However, poisoning is avoided by careful preparation of the plant. This includes grinding the root and expressing the fluids, or boiling the root in several changes of water.

## GLUCOSINOLATES ARE SULFUR-CONTAINING PRECURSORS TO MUSTARD OIL.

Glucosinolates are found primarily in the mustard family (Brassicaceae) and related families in the order Capparales. They are precursors to the mustard oils, an economically important class of flavor constituents hat gives the pungent taste to condiments such as mustards and horseradish as well as the distinctive flavor of cabbages, broccoli, and cauliflower. All glucosinolates are thioglucosides (thio, sulfur) with the general structure shown in Figure 27.11A. The sugar is always glucose. The diversity encountered in structure and properties is due to the R group, which may range from a simple methyl group to large linear or branched chains containing aromatic or heterocyclic structures. The biological activity of glucosinolates depends primarily on their hydrolysis to mustard oils (Figure

27.11B). Hydrolysis of glucosinolates is catalyzed by an enzyme called myrosinase (a thioglucosidase). The hydrolysis product is unstable and immediately undergoes a rearrangement to form a thiocyanate or isothiocyanate. Like the cyanogenic glyosides, glucosinolates are spatially separated from the hydrolytic enzymes so that the mustard oils are normally formed only when the cells are disrupted, allowing the enzyme and substrate to come together. As with other defense compounds, some herbivores are deterred or repelled by the presence of glucosinolates in a plant, while others have adapted to use the glucosinolates or mustard oils as attractants to stimulate feeding and ovipositing. Glucosinolates, or rather their absence, have had a significant impact on the oilseed industry. Rape seed (principally *Brassica napus*) is a good source of vegetable oil, but its high content of glucosinolate together with high erucic acid (a 22-carbon fatty acid) gives the oil undesirable taste and poor storage properties. New strains have been bred with low glucosinolates and erucic acid. These strains, called canola in order to distinguish them from normal rape, are now an economically important oil source.

## PHENYLPROPANOIDS

Aromatic amino acids may be directed toward either primary or secondary metabolism. Also known as phenolics, or polyphenols, phenylpropanoids are a l

## . Alkaloids

Alkaloids are organic compounds with at least one nitrogen atom in a heterocyclic ring. Their definition is problematic, as they do not represent a homogeneous group of compounds from any standpoint, whether chemical, biochemical, or physiological. Except for the fact that they are all nitrogen-containing compounds, no general definition fits all alkaloids. Alkaloids can be divided according to their basic chemical structure into different types. The following are basic types of alkaloids: acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolines, quinozolines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrrolidines, pyrrolizidines, pyrroloindoles, pyridines and simple tetrahydroisoquinolines [28].

Although plants containing alkaloids have been used by man for at least 3000 years as medicines, teas and potions, the compounds responsible for activity were not isolated and characterized until the nineteenth century [1]. Alkaloids are not common in lower plants. Lysergic acid derivatives and sulfur-containing alkaloids, e.g., the gliotoxins, are detected in fungi. Concerning the pteridophytes and gymnosperms alkaloids reported for their medicinal uses include the lycopodium, ephedra and *Taxus* alkaloids. Alkaloids are unevenly distributed among the angiosperms. The following are the orders reported to be rich in alkaloids: Centrospermae (Chenopodiaceae), Magnoliales (Lauraceae, Magnoliaceae), Ranunculales (Berberidaceae, Menispermaceae, Ranunculaceae), Papaverales (Papaveraceae, Fumariaceae), Rosales (Leguminosae, subfamily Papilionaceae), Rutales (Rutaceae), Gentiales (Apocynaceae, Loganiaceae, Rubiaceae), Tubiflorae (Boraginaceae, Convolvulaceae, Solanaceae) and Campanulales (Campanulaceae, sub-family Lobelioideae;

Compositae, subfamily Senecioneae). However, there is no report for the presence of alkaloids in Salicales, Fagales, Cucurbitales and Oleales dicot orders till the present time [7].

Alkaloids demonstrate a diverse array of pharmacological actions including analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation and toxicity, as well as antineoplastic, hypertensive and hypotensive properties. The activity of alkaloids against herbivores, toxicity in vertebrates, cytotoxic activity, the molecular targets of alkaloids, mutagenic or carcinogenic activity, antibacterial, antifungal, antiviral and allelopathic properties have been reported in literature. Many alkaloids are sufficiently toxic to animals to cause death if eaten. Several (e.g., nicotine and anabasine) are used as insecticides [1, 8].

Examples of some alkaloids:

### 3.1. Nicotine

Nicotine is found in the tobacco plant (*Nicotiana tabacum*) and other *Nicotiana* species; it has tranquilizing properties and is the addictive component of tobacco. It is also extremely toxic, causing respiratory paralysis at high doses (Figure 7). Nicotine is a ganglion cholinergic-receptor agonist with complex pharmacological actions, including effects mediated by binding to receptors in the autonomic ganglia, the adrenal medulla, the neuromuscular junction and the brain [29].

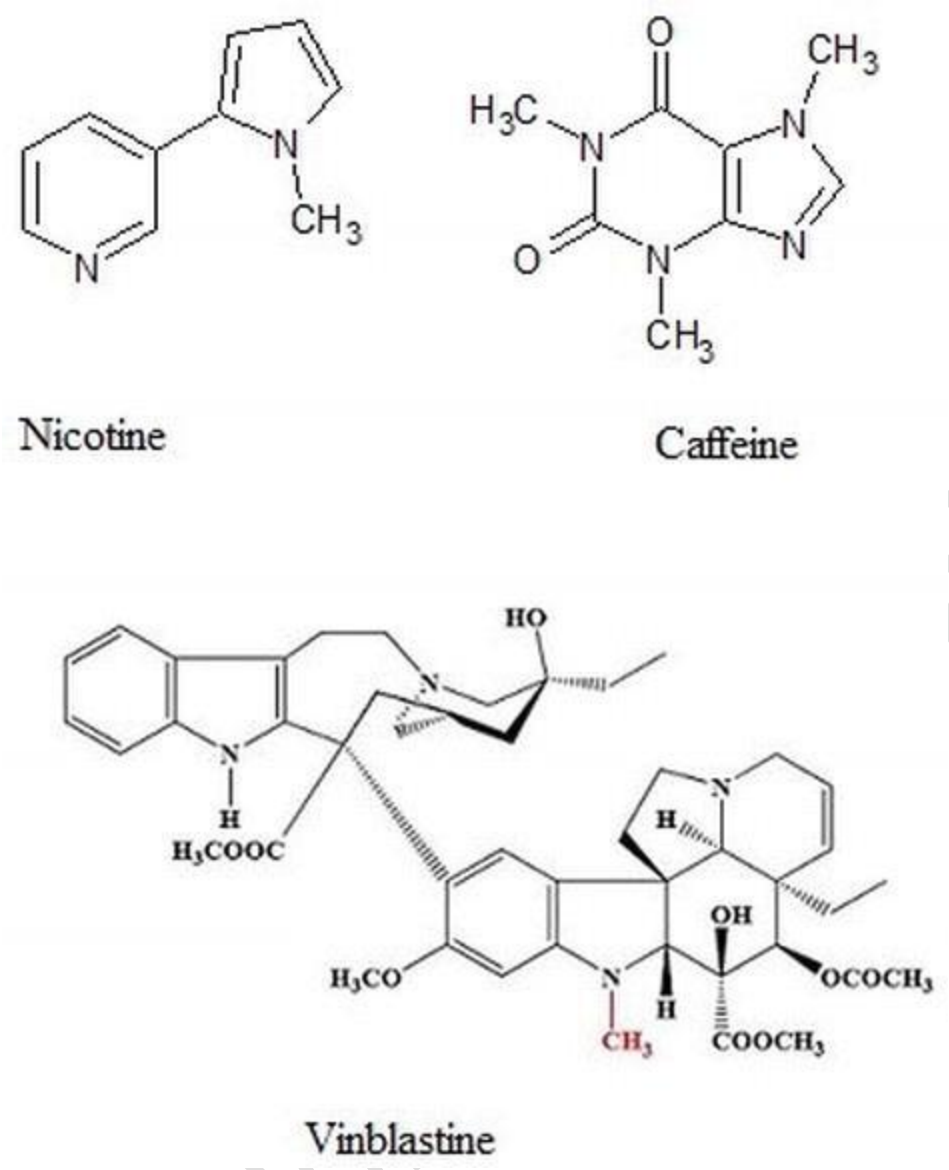



*Figure 7.*

Examples of alkaloids.

### 3.2. Caffeine

Caffeine occurs in a number of botanically unrelated species, including coffee (*Coffea* spp.), tea (*Camellia sinensis*), mate (*Ilex paraguariensis*), guarana (*Paullinia cupana*) and kola (*Cola acuminata*) (Figure 7). Caffeine is bound to chlorogenic acid in raw coffee beans. The roasting process liberates the caffeine and

other compounds that contribute to the aroma of coffee. Caffeine is a diuretic and has stimulant effects on the respiratory, cardiovascular and central nervous systems [30].

### 3.3. Vinblastine

Vinblastine is isolated from *Catharanthus roseus* G. (Figure 7) and has been used to treat diabetes and high blood pressure and as disinfectant. Nevertheless, Vinblastine is so important for being cancer fighters. It is used along with the other vinca alkaloids vinorelbine, vincristine and vindesine, which are in clinical use in the United States and Europe [31].

## 4. Saponins

Saponins are compounds that possess a polycyclic aglycone moiety with either a steroid (steroidal saponins) or triterpenoid (triterpenoidal saponins) attached to a carbohydrate unit (a monosaccharide or oligosaccharide chain) (examples illustrated in Figures 8 and 9). These sugar units are composed variously of pentoses, hexoses, or uronic acids. This hydrophobic-hydrophilic asymmetry means that these compounds have the ability to lower surface tension and are soap-like. They form foam in aqueous solutions and cause hemolysis of blood erythrocytes in vitro. The aglycone portion of the saponin molecule is called the *genin* or *sapogenin*. Saponins are widespread among plants, having been reported from more than 500 plants from at least 90 different families; these substances have been isolated from all parts of plants: leaves, stems, roots bulbs, flowers and fruits, although they tend to be concentrated in the roots of many species such as *Digitalis purpurea* (*foxglove*), *Dioscorea villosa* (*wild yam*), *Eleutherococcus senticosus* (*Siberian ginseng*), *Gentiana lutea* (*gentian*), *Glycyrrhiza* spp. (*licorice*) and *Panax ginseng* (*Korean ginseng*) [32].

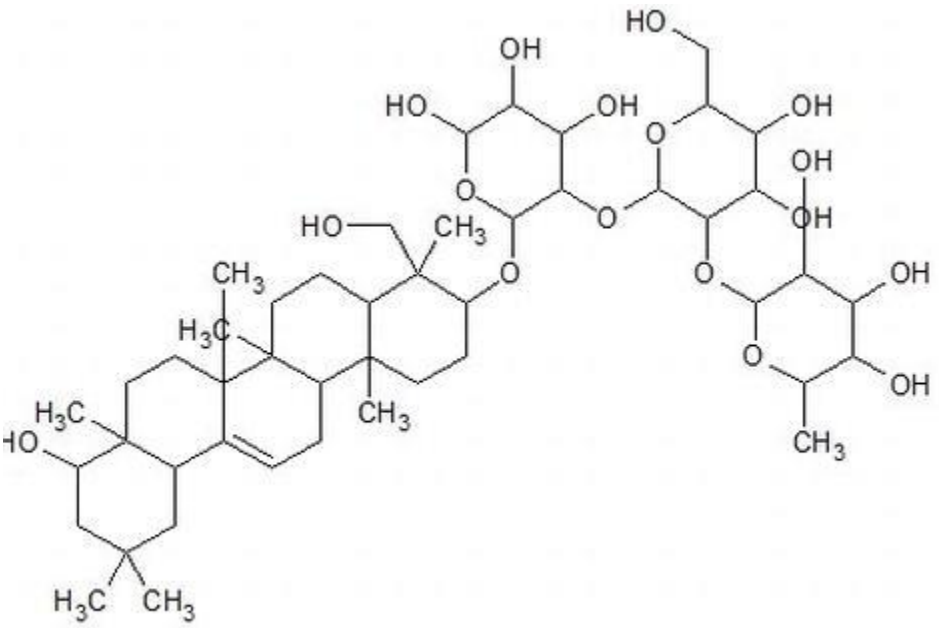



*Figure 8.*

Example of triterpenoidal saponin.

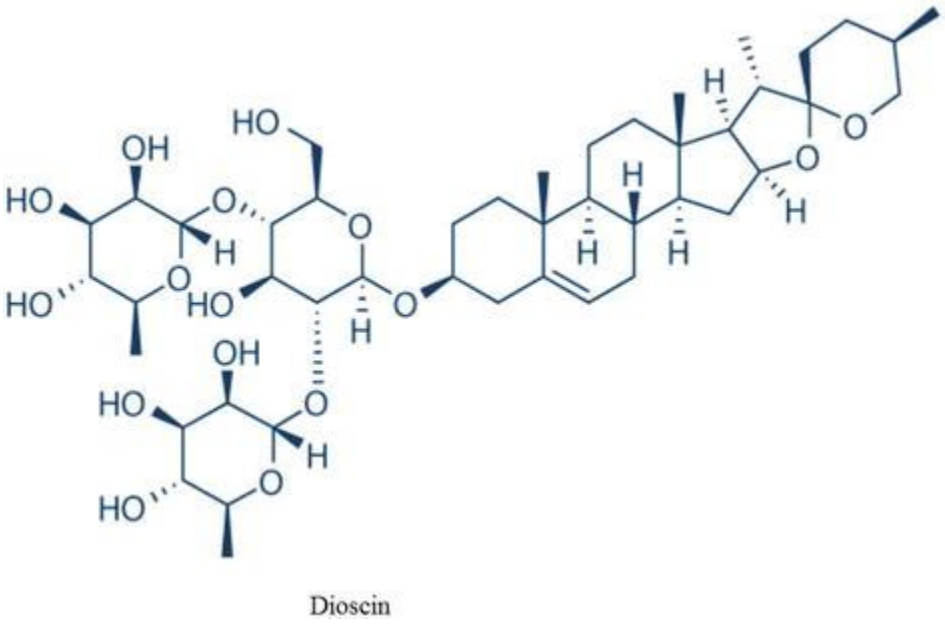



*Figure 9.*

Example of steroidal saponin.

Saponins have demonstrated numerous pharmacological properties. Some saponins have antitumor, piscicidal, molluscicidal, spermicidal, sedative, expectorant and analgesic properties. Glycyrrhizin from *glycyrrhizae radix* (from *Glycyrrhiza glabra*, Fabaceae) is useful as expectorant and antitussive agent. It is also used to treat chronic hepatitis and cirrhosis. Some saponins have anti-inflammatory properties as the saponins from *Bupleurum falcatum* (Apiaceae). *Phytolacca americana* roots are reputed to possess anti-inflammatory properties in Korean medicine. Similar properties have been demonstrated for a number of other saponins, for example aescin, from horse chestnut (*Aesculus hippocastanum*), has been shown to be 600 times more effective than rutin in reducing rat paw edema [33].

## 5. Terpenes

Terpenes are the largest and most diverse group of plant secondary compounds. The name "terpene" is derived from the word "turpentine," which in turn comes from the old French *ter(e)bintb*, meaning "resin." They are all derived chemically from 5-carbon isoprene units assembled in different ways [8]. Terpenes are classified according to the number of isoprene units in the molecule; a prefix in the name indicates the number of terpene units as follows.

### 5.1. Hemiterpenes

They consist of *a single isoprene* unit. Isoprene itself is considered the only hemiterpene, but oxygen-containing derivatives such as angelic acid isolated from *Angelica archangelica* and isovaleric acid from *Vaccinium myrtillus* are hemiterpenoids [1].

5.2. Monoterpenes

They consist of *two* *isoprene* units and have the molecular formula $C_{10}H_{16}$ (see Figure 10). They are important components of plant essential oils or volatile oils. Monoterpenes tend to

occur in members of certain plant families, such as Lamiaceae, Pinaceae, Rutaceae and Apiaceae, from which many essential oils are commercially produced. Some of these compounds, such as geraniol, are almost ubiquitous and can be found in small amounts in the volatile secretions of most plants. Monoterpenes are further classified into unsaturated hydrocarbons (e.g., limonene), alcohols (e.g., linalool), alcohol esters (e.g., linalyl acetate), aldehydes (e.g., citronellal) and ketones (e.g., Carvone). Monoterpenes and other volatile terpenes have a number of widespread medicinal uses. Compounds such as camphor and menthol are used as counterirritants analgesics and anti-itching agents. Many monoterpenes have been used as anthelmintics. A series of monoterpene glycosides appear to have vasodilation effect on coronary vessels and the femoral vascular bed [16].

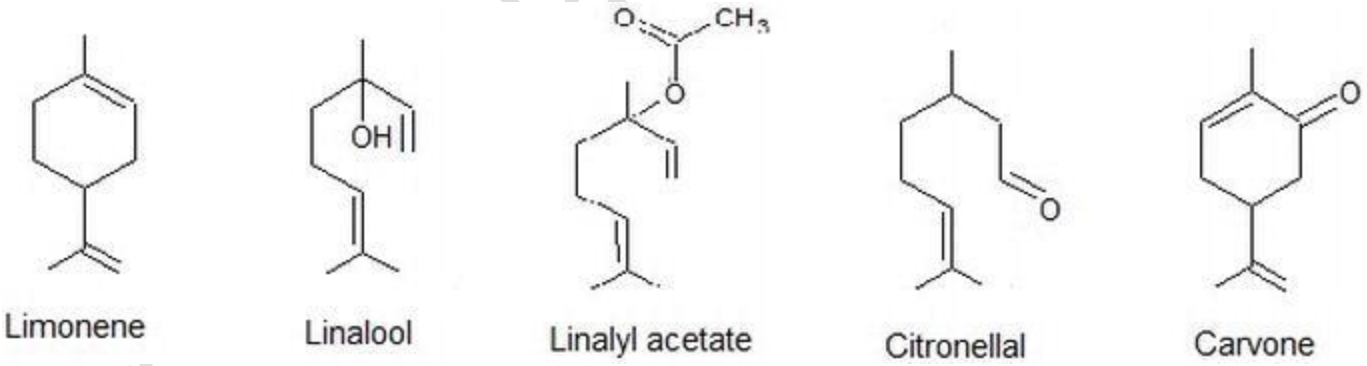



*Figure 10.*

Examples of monoterpenes.

### 5.3. Sesquiterpenes

They consist of *three* *isoprene* units and have the molecular formula $C_{15}H_{24}$ (see Figure 11). Based on biogenetic origin, there are more than 200 different

structural types of sesquiterpenes, and several thousand such compounds are known. These compounds can be conveniently classified into three main groups according to structure: acyclic (e.g., farnesol), monocyclic (e.g., bisabolol) and bicyclic (e.g., caryophyllene). A number of sesquiterpene lactones show antibacterial, antifungal and antiprotozoan activities. Sesquiterpenes from *Vernonia colorata* inhibit *Entamoeba histolytica* at concentrations comparable to metronidazole, an antiamoebic drug. Helenalin and a series of related compounds are responsible for the cardiotonic properties of *Arnica montana* flowers. *Atractylodis rhizoma*, from *Atractylodis macrocephala* (Asteraceae), is clinically used as diuretic, analgesic and anti-inflammatory. The activity is related to the presence of active compounds including eudesma-4(14)-7(1 l)-dien-8-one and atractylenolide I. Several related medicinal plants are also used for the same purposes due to the presence of sesquiterpenes [1, 34].

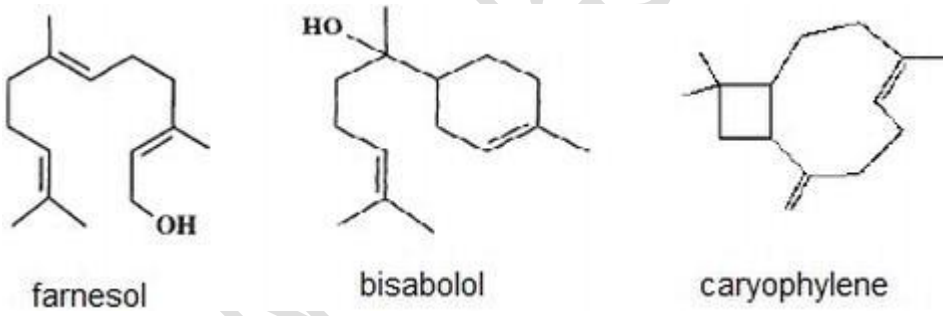



*Figure 11.*

Examples of sesquiterpenes

### 5.4. Diterpenes

They are composed of *four isoprene* units and have the molecular formula $C_{20}H_{32}$ (see Figure 12). Diterpenes are classified into acyclic and macrocyclic compounds. Moreover, macrocyclic diterpenes are classified according to the

number of ring systems present. Diterpenes may be 6-membered ringed structures or they may have fused 5- and 7-membered ringed structures. In addition, many diterpenes have additional ring systems. These occur as side substitutions as esters or epoxides [8]. Diterpenoids constitute the active constituents of a number of medicinal plants. Vitamin K1, an antihemorrhagic compound, first discovered in plants in 1929, is a diterpene. Vitamin A, a diterpenoid, is referred to, together with the related compounds, as "carotenes." The bitter principles of *Jateorhiza palmata* (calumba root) belong to furanoditerpenes. *Teucrium chamaedrys* (wall germander) and *T. scorodonia* (wood sage) family Labiatae, both produce diterpenes of the neoclerodane type. They are used in herbal medicine as diaphoretics and antirheumatics [35]. Like all groups of terpenes, diterpenes have demonstrated a range of pharmacological properties including: analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic and antiprotozoal activities [8]. Some diterpenes from *Kalmia latifolia* (Ericaceae) have antifeedant properties with respect to the gypsy moth. The gibberellins, first obtained from fungi of the genus *Gibberella* but also found in higher plants, are diterpenoid acids, which have a marked effect on growth of seedlings [7].

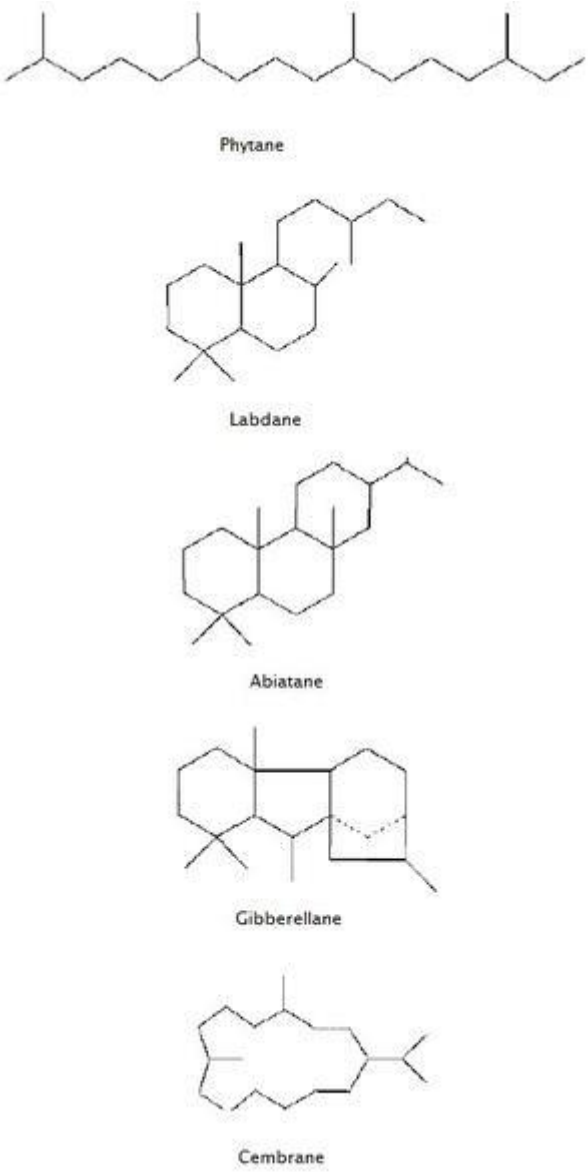



*Figure 12.*

Examples of diterpenes.

## 5.5. Sesterterpenes

Terpenes having 25 carbons and *five isoprene* units are rare relative to the other sizes (the *sester-* prefix means half to three, i.e. two and a half). An example of a sesterterpenoid is geranyl farnesol isolated from seed oils of *Camellia*

*sasanqua* (sasanqua) and *Camellia japonica* (camellia), family Theaceae [36]. Geranyl farnesol showed cytotoxic activity in mouse leukemic M1 cells [37].

### 5.6. Triterpenes

They consist of *six isoprene* units and have the molecular formula $C_{30}H_{48}$ (see Figure 13). The linear triterpene squalene, the major constituent of shark liver oil, is derived from the reductive coupling of two molecules of farnesyl pyrophosphate. Triterpenes constitute a significant portion of the lipid substances of all plants; more than 4000 triterpenoids have been isolated. These compounds are precursors to steroids in both plants and animals. Both triterpenes and steroids occur free, as glycosides or in other combined forms. The structures of triterpenes and steroids may be subdivided into about 40 major types [1]. β-Boswellic acids (ursane-type triterpene) and α-boswellic acids (oleanane-type triterpene) that are isolated from the oleo-gum-resin of *Boswellia carterii* are known for their anti-inflammatory and anti-rheumatic activities [38].

# Practical biochemistry

# Plant Biochemistry

Types of stress

1- **Biotic stress** is **stress** that occurs as a result of damage done to an organism by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants

2- **Abiotic stress** is defined as the negative impact of non-living factors on the living organisms in a specific environment.

Abiotic stress such as cold, drought, salt, and heavy metals largely influences plant development and crop productivity. To cope with abiotic stress, plants can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses.

physiological changes

1-carbohydrates

2-proteins

3-amino acid

4-proline

**Methods Expressing Concentration**

**1- Molarity, M = moles solute/liter of solution**

**2-Normality, N = equivalents of solute/liter of solution**

3-**ppt part per thousand g/l**

4- **Parts per million (ppm) mg/l**

**5-parts per billion (ppb) µg/l**

1PPt =1000 **ppm** = 1000000 **ppb**

**Sterilization**

Sterilization (or sterilisation) refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms, prions, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media.Sterilization can be achieved through various means, including: heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents which are present.

**Methods of seed sterilization**

**1-Ethanol 70%**

70ml absolute ethanol+30ml dist. H2O

To surface-sterilize seeds immersed them in 70% ethanol for 2 min., rinsed with distilled water

**2-$HgCl_2$ mercuric chloride 0 .1%**

Mercuric chloride is highly antimicrobial, with action against both fungi and bacteria, but frequently also kills the seeds/plant materials. At low concentrations (upto 0.1 %) it is perhaps the most effective disinfective agent for seeds with soil-borne and the epiphytic fungi.Treat seeds with 0.1% Mercuric chloride for 2min

Then carefully washed 3-4 times with distilled water in order to ensure safe removal of any sterilizing agent

**3-$H_2O_2$ 30%**

Soak seeds in hydrogen peroxide for five minutes, then rinse them off with water.

**4-sodium hypochlorite (NaOCl).** Surface sterilize seeds with 0.5% sodium hypochlorite (NaOCl) for 4-5minutes. Then carefully washed 3-4 times with distilled water in order to ensure safe removal of any sterilizing agent.

## The role of Acorbic in alleviating salt stress in plants

1-Prepare the following concentration of NaCl by Dilution Law:

0 50 mM 100 mM 150 mM

Prepare 1 M of NaCl as stock solution

$$g = \frac{M*V*M.\ Wt}{1000}$$

M=1 V=1000 ml M. Wt of NaCl = 58.45

g= 58.5 gram of NaCl in 1 liter of dist. Water give 1M OF NaCl

Dilution Law

M*V (before) = M*V (after)

To prepare 0.1 M of NaCl 50 ml from stock solution 1M

1000*V = 50* 1000

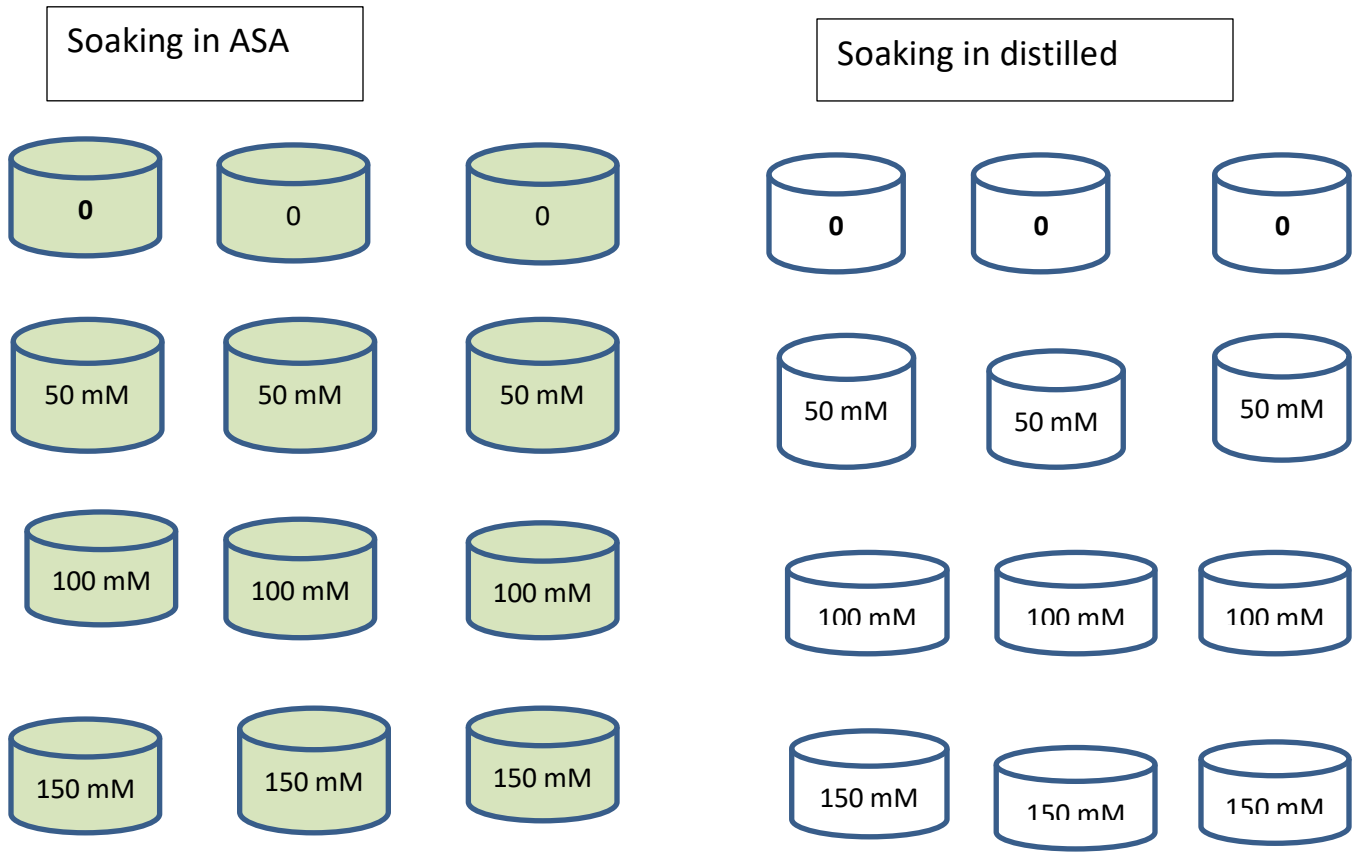
V=50 ml  take 5ml of stock solution and complete it to 1000 ml with dist. Water (950ml)

2- Prepare 50 PPM OF ascorbic acid take 50 mg of IAA in 1Liter of distilled water

3- Divide the seeds into two groups. The first group is soaked in ascorbic acid for 6 hours and the other group is soaked in distilled water for 6 hours.

4- Soaked seeds are taken and placed in petri dishes where a fixed number of seeds are placed in all dishes and irrigated at 30 ml of the appropriate concentration of sodium chloride.

5- Three replicates are made from each concentration



| concentration | NaCl volume | dist. Water volume | Final volume |
|---|---|---|---|
| 0 | 0 ml | 1000ml | 50 ml |
| 50 mM | 50 ml | 950ml | 50 ml |
| 100 mM | 100 ml | 900 ml | 50 ml |
| 150 mM | 150 ml | 850 ml | 50 ml |

6-Petri dishes are covered and germination rate is calculated daily

7-After germination of most seeds, the dishes open to exposed to light.

8-Leave dishes open until seedlings grow and reach a suitable length

9-Seedlings are taken from each dish and the length of the shoot and root are calculated

10-Then the average is calculated for each dish.

11- Root and shoot lengths of each seedling were measured. Fresh weights of seedling organs (roots or shoots) were determined. The dry weights of roots and shoots of seedlings were determined after drying the freshly harvested organs in an aerated oven at 70°C to constant weight for 48 hr. Then water content was calculated as: water content (%) = (fresh weight – dry weight)/ fresh weight ×100).

Germination rate

| Soaking in water | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th |
| 0 | | | | | | | |
| 50 mM | | | | | | | |
| 100 mM | | | | | | | |
| 150 mM | | | | | | | |

| Soaking in ASA | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th |
| 0 | | | | | | | |
| 50 mM | | | | | | | |
| 100 mM | | | | | | | |
| 150 mM | | | | | | | |

g

Effect of different of NaCl on growth parameter

| **treatments** | | **Shoot** | | | | **Root** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Soaking in** | **NaCl (mM)** | **Length (cm)** | **Fresh weight (g)** | **Dry weight (g)** | **Water content (%)** | **Length (cm)** | **Fresh weight (g)** | **Dry weight (g)** | **Water content (%)** |
| **Water** | 0 | | | | | | | | |
| | 50 mM | | | | | | | | |
| | 100 mM | | | | | | | | |
| | 150 mM | | | | | | | | |
| **ASA** | 0 | | | | | | | | |
| | 50 mM | | | | | | | | |
| | 100 mM | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 150 mM | | | | | | | | |

**1- Estimation of photosynthetic pigments**

The photosynthetic pigments were extracted from a known fresh weight of leaves in 85% aqueous acetone. The extract was taken and diluted by 85% aqueous acetone to a certain volume for spectrophotometric measurements, using spectrophotometer.

**-Determination**

The photosynthetic pigments which include (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method recommended by **(Metzner, et al., 1965).**

The pigments extracts were measured against a blank of pure 85% an aqueous acetone at three wavelengths of 663,644 and 452.5 nm. Taking in your mind the dilution factor, it was possible to determine the concentration of pigment fractions (Chl.a, Chl.b and carotenoids) as µg/ml using the following equations:

Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$ = µg/ml

Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$ = µg/ml

$$\text{Carotenoids} = 4.2\ E_{452.5} - \left\{ \begin{array}{c} 0.0264\ \text{Chl.a} \\ + \\ 0.4260\ \text{Chl.b} \end{array} \right\} = \mu g/ml$$

Finally these pigment fractions were calculated as mg/g dry matter.

## 2-Water- soluble Carbohydrates

### Extraction

To estimate water soluble carbohydrates, a known weight of the dried tissue material was put in 10 ml of distilled water which was boiled in water bath at 100°C for 2 hours, after cooling the hydrolyses was filtered and then completed to definite volume.

### Determination

The soluble carbohydrates were determined by the method of anthrone sulphoric acid which was stated by **(Fales, 1951and Schlegel, 1956)** and adopted by **(Badour, 1959)**.

## 3-Reagents

***Anthrone sulphoric acid reagent:***

The anthrone sulphoric acid reagent consists of 0.2 gm anthrone,8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml of concentrated $H_2SO_4$ (D = 1.84).These substances were successively mixed in a beaker under continuous cooling. This reagent must be always freshly prepared.

### Procedures:

**1-** One ml of the plant tissue extract was put in a clean Pyrex test tube of about 16x 160 mm and mixed with anthrone reagent.

**2-** This sample was heated at 100°C in water bath for 7 minutes, and directly cooled under tap water.

**3-** The extinction of developed blue green colour was measured at wavelength of 620 nm against a blank, which contained only distilled water and anthrone reagent, using spectrophotometer.

**4-** A calibration curve using pure glucose was constructed from which it was indicated that one extinction is equivalent to 210 mg glucose. Then 4.5 ml of anthrone reagent was added to 1 ml of the prepared unknown solution in a clean dried test tube.

The carbohydrates content were calculated as mg/g dry weight of the plant organ.

**Estimation of proteins**

- **Water soluble proteins**

**1-Extraction:**

Powdered tissue samples (50 mg) were boiled in 10 ml of distilled water for two hours. After cooling, the water extract was centrifuged and the supernatant was decanted and completed to a definite volume using distilled water.

**2-Determination:**

The soluble proteins were determined according to the method adopted by (**Lowery, et al., 1951)**

**Reagents:**

Reagent A (2 % $Na_2CO_3$ in 0.1 N NaOH)

It was prepared by dissolving 2 g of sodium carbonate in 100 ml of 0.1 N NaOH.

Reagent B (0.5 % $CuSO_4$ in 1 % sodium potassium tartarate )

It was prepared by dissolving 0.5 g CuSO4, $5H_2O$ in 1 % sodium potassium tartarate.

The alkaline reagent solution:

It consists of 50 ml of reagent A and 1 ml of reagent B.

This reagent should be always freshly prepared.

**Procedures:**

**1-** 5 ml of the alkaline reagent solution were added to 0.1 ml of the test solution (water extract) in a clean test tube.

**2-** Both were mixed thoroughly and allowed to stand at room temperature for at least 10 minutes.

**3-** 0.5 ml of the diluted foline-ciocatteau reagent (1:1 v/v) was added to the above mixture and mixed immediately.

**4-** After 30 minutes the extinction against appropriate blank was measured at 700 nm.A calibration curve was constructed using egg albumin and the data were expressed as mg protein/g dry matter.

**◊ Determination of total free amino acids:**

Free amino acids were extracted from plant tissues and determined according to the method of **(Moore and Stein, 1948).** However, in this method traces of proline and hydroxyl proline are encountered. A calibration curve was constructed using glycine. The free amino acids concentration was calculate as mg/g dry matter.

**◊ Determination of proline**

**1- Extraction**

A definite weight of macerated dry matter tissue was homogenized in 5 ml of 3% sulfosalicylic acid, and then filtered through whatman 2 filter paper.

**2-Determination**

Free proline was determined according to **(Bates, et al., 1973)**.

Two ml of the filtrate were mixed with 2 ml glacial acetic acid and 2 ml of acid ninhydrin in a test tube for one hour at 100 °C. The reaction mixture was extracted with 4 ml toluene, mixed vigorously in test tube for one 15-20 sec. The chromophore containing toluene was aspirated from aqueous phase and warmed to room temperature. The absorbance was measured at 520 nm using a standard curve and calculated on a dry weight basis as mg proline/g dry matter.