

**COER PAPER – XI PLANT BIOCHEMISTRY**

**UNIT- 5**

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## **Vitamins - Classification and Functions**

This topic is about Vitamins – classification and functions. It is a known fact that we require energy in order to perform different activities. We get these energies from the food we eat. Apart from the normal food that we take, our body requires a certain number of compounds in small amounts for the proper functioning and deficiency of these compounds may cause diseases. These compounds are known as vitamins.

Vitamins are chemical compounds that are required in small amounts with our regular diet in order to carry out certain biological functions and for the maintenance of our growth.

### Classification of Vitamins

Vitamins are generally classified as water-soluble vitamins and fat-soluble vitamins.

#### 1. Fat-Soluble Vitamins

Vitamin A, D, E and K are fat-soluble. These are stored in adipose tissues and hence are called fat-soluble vitamins.

#### 2. Water-Soluble Vitamins

Vitamins in B-group and vitamin C are water-soluble and cannot be stored in our bodies as they pass with the water in urine. These vitamins must be supplied to our bodies with regular diets.

### Related Topics

- Introduction to Nucleic Acids
- Nucleic Acids: DNA and RNA

### Functions of Vitamins

Based on their role in biological processes and their effect different vitamins have different functions, their function can be best understood by knowing about their deficiency diseases. Given below is the list of vitamins and their deficiency diseases:

1. Vitamin A – Hardening of the cornea in eye, night blindness.
2. Vitamin B1 – Deficiency may cause beriberi, dwarfism.
3. Vitamin B2 – Deficiency can cause disorders in the digestive system, skin burning sensations, cheilosis.
4. Vitamin B6 – Deficiency of B6 causes convulsions, conjunctivitis, and sometimes neurological disorders.
5. Vitamin B12 – Its deficiency can cause pernicious anemia and a decrease in red blood cells in hemoglobin.
6. Vitamin C – It is a water-soluble vitamin, its deficiency causes bleeding in gums and scurvy.
7. Vitamin D – It is obtained by our body when exposed to sunlight. Its deficiency causes improper growth of bones, soft bones in kids, rickets.
8. Vitamin E – Deficiency of vitamin E leads to weakness in muscles and increases the fragility of red blood cells.
9. Vitamin K – It plays an important role in blood clotting. The deficiency of vitamin K increases the time taken by the blood to clot. Severe deficiency may cause death due to excessive blood loss in case of a cut or an injury.

Vitamin C is abundantly found in all citrus fruits. Other sources of Vitamin B and C include:

- **Vitamin B1 or Thiamin:** Found in pork chops, ham, enriched grains and seeds.
- **Vitamin B2 or Riboflavin:** Found in whole grains, enriched grains and dairy products.
- **Vitamin B3 or Niacin:** Found in mushrooms, fish, poultry, and whole grains.
- **Vitamin B5 or Pantothenic Acid:** Found in chicken, broccoli, legumes and whole grains.
- **Vitamin B6 or Pyridoxine:** Found in fortified cereals and soy products.
- **Vitamin B7 or Biotin:** Found in many fruits like fruits and meats.

- **Vitamin B9 or Folic Acid:** Found in leafy vegetables.
- **Vitamin B12:** Found in fish, poultry, meat and dairy products.
- **Vitamin C:** Found in citrus fruits and juices, such as oranges and grapefruits.

## VITAMIN A

Vitamin A has many important functions in your body. It helps regulate your immune system, helps form and maintain healthy teeth, skin, and tissue, it produces the pigments in the retina of the eye, and promotes good vision. A deficiency in this vitamin can lead to problems with any of these. The vitamin A found in plants serves different functions than the vitamin A found in animals. The animal sources of vitamin A are liver, whole milk, and fortified foods, and the plant sources include colorful fruits and vegetables like carrots, spinach, kale, and cantaloupe.

The recommended dietary allowances (RDA) for vitamin A are listed as international units (IU) of retinol activity equivalents (RAE). This is done to account for the different actions of both forms of vitamin A.

The following is the RDA for vitamin A:

Age	Males	Females	Pregnancy	Lactation
1-3 years	1,000 IU	1,000 IU	N/A	N/A
4-8 years	1,320 IU	1,320 IU	N/A	N/A
9-13 years	2,000 IU	2,000 IU	N/A	N/A
14-18 years	3,000 IU	2,310 IU	2,500 IU	4,000 IU
19+ years	3,000 IU	2,310 IU	2,565 IU	4,300 IU

Going above the RDA for vitamin A can initially cause nausea, vomiting, irritability, drowsiness, altered mental status, anorexia, abdominal pain, blurred vision, muscle pain with weakness, and/or headache. Over time, this can lead to hypervitaminosis A or vitamin A toxicity. The harmful effects of this are birth defects, reduced bone density that may result in osteoporosis, central nervous system disorders, and liver abnormalities.

## VITAMIN B1

Vitamin B1 is most commonly known as thiamin. It is involved in numerous body functions, including metabolism of carbohydrates, assisting with muscle functioning, producing hydrochloric acid, and assisting with nervous system functioning. A deficiency can cause weakness, fatigue, nerve damage, and psychosis. When the deficiency becomes severe, it causes a disease known as beriberi. There are many foods that provide thiamin so it's easy to get enough from your diet. Some of these foods are beans, pork, fortified cereal, and enriched rice.

The following is the RDA for thiamin:

Age	Males	Females	Pregnancy	Lactation
1-3 years	0.5 mg	0.5 mg		
4-8 years	0.6 mg	0.6 mg		
9-13 years	0.9 mg	0.9 mg		
14-18 years	1.2 mg	1.0 mg		
19+ years	1.2 mg	1.1 mg		
All ages			1.4 mg	1.4 mg

## VITAMIN B2

Vitamin B2 is most commonly known as riboflavin. It is needed for converting food to energy, to work as an antioxidant by scavenging damaging free radicals, and to convert vitamin B6 and folate into active forms. Most people meet their needs with a balanced diet. Good sources of riboflavin are milk, spinach, fortified cereal, eggs, and green vegetables. You can be deficient in riboflavin when you don't consume enough riboflavin, but other things that can cause a deficiency are malabsorption syndromes, chronic diarrhea, long-term use of barbiturates, peritoneal dialysis, and alcoholism. The symptoms of a riboflavin deficiency are fatigue, skin irritations, cracks and sores around the corner of the mouth, and sensitivity to light.

The following is the RDA for riboflavin:

Age	Males	Females	Pregnancy	Lactation
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1-3 years	0.5 mg	0.5 mg	N/A	N/A
4-8 years	0.6 mg	0.6 mg	N/A	N/A
9-13 years	0.9 mg	0.9 mg	N/A	N/A
14-18 years	1.3 mg	1.0 mg	1.4 mg	1.6 mg
19 + years	1.3 mg	1.1 mg	1.4 mg	1.6 mg

### VITAMIN B3

Vitamin B3 is most commonly known as niacin. It has been shown to help turn carbohydrates into energy, assist with the proper functioning of the nervous and digestive systems, and help maintain healthy skin. There is also evidence that it can help increase HDL or "good" cholesterol levels. Niacin is found in animal products, nuts, green vegetables, legumes, and enriched and fortified cereals

The following is the RDA for niacin:

Age	Males	Females	Pregnancy	Lactation
1-3 years	6 mg	6 mg	N/A	N/A
4-8 years	8 mg	8 mg	N/A	N/A
9-13 years	12 mg	12 mg	N/A	N/A
14-18 years	16 mg	14 mg	18 mg	17 mg
19+ years	16 mg	14 mg	18 mg	17 mg

Pellagra is the disease that occurs due to a severe niacin deficiency. The symptoms of pellagra are known as the four Ds: dermatitis, diarrhea, dementia, and death. Prior to this, the symptoms of a niacin deficiency will involve the digestive system (diarrhea, vomiting, bright red tongue), the skin (dark pigmented rash that develops symmetrically in areas exposed to sunlight, thick and scaly skin), and the nervous system (fatigue, depression, headache, apathy, disorientation, and memory loss).

## VITAMIN B6

Vitamin B6 comes in three forms and plays a role in red blood cell metabolism, making hemoglobin, assisting in the proper functioning of the nervous and immune systems, taking part in protein metabolism, and making the neurotransmitters serotonin and norepinephrine. It is found in many foods so it's easy to consume enough through your diet. Some foods that contain vitamin B6 are bananas, potatoes, chicken breast, garbanzo beans, seeds, and roast beef.

The following is the RDA for vitamin B6:

Age	Males	Females	Pregnancy	Lactation
1-3 years	0.5 mg	0.5 mg	N/A	N/A
4-8 years	0.6 mg	0.6 mg	N/A	N/A
9-13 years	1.0 mg	1.0 mg	N/A	N/A
14-18 years	1.3 mg	1.2 mg	1.9 mg	2.0 mg
19-50 years	1.3 mg	1.3 mg	1.9 mg	2.0 mg
51+ years	1.7 mg	1.5 mg		

Symptoms of a B6 deficiency are depression, confusion, sores or ulcers on the mouth, ulcers at the corners of the mouth, confusion, and irritability. Excess amounts can cause problems as well. Taking a supplement with over 1,000 mg/day has been shown to cause sensory neuropathy. Symptoms of this include difficulty walking and pain and numbness of the extremities.

## VITAMIN B12

Vitamin B12 is a very important water-soluble vitamin. It is needed for producing and maintaining new cells (nerve and red blood cells) and for making DNA. Without enough vitamin B12, you are at risk for pernicious anemia. The symptoms of B12 deficiency are fatigue, constipation, weakness, loss of appetite, weight loss, and numbness and tingling in the hands and feet. You may also experience a difficulty in maintaining balance, confusion, dementia, depression, and poor memory. Fortunately, you can get enough in your diet by consuming animal foods like beef, salmon, trout, tuna, chicken, eggs, and yogurt. You can also get it from fortified cereals.

The following is the RDA for vitamin B12:

<b>Age</b>	<b>Males and Females</b>	<b>Pregnancy</b>	<b>Lactation</b>
1-3 years	0.9 mcg	N/A	N/A
4-8 years	1.2 mcg	N/A	N/A
9-13 years	1.8 mcg	N/A	N/A
14-19 years	2.4 mcg	2.6 mcg	2.8 mcg
19+ years	2.4 mcg	2.6 mcg	2.8 mcg

Some medications, stomach or intestinal disorders, old age, and a diet free of meat and meat products can increase the risk of vitamin B12 deficiency. Your doctor can perform a blood test to determine if you need to take a supplement.

## **VITAMIN C**

Vitamin C is mostly known for its ability to help with colds, but it has many more important functions in your body. Vitamin C is needed for the growth and repair of tissues in all parts of your body. It helps with the structural part of blood vessels, ligaments, and tendons. It also is an antioxidant, meaning that it protects your body from dangerous products of metabolism called free radicals, which are linked to cancer and other diseases. There are plenty of rich sources of vitamin C so you can reach your needs without a supplement by balancing your diet. Foods that are high in vitamin C are red peppers (higher than oranges), guava, oranges, kiwi, brussels sprouts, and broccoli.

The following is the RDA for vitamin C:

<b>Age</b>	<b>Males</b>	<b>Females</b>	<b>Pregnancy</b>	<b>Lactation</b>
1-3 years	15 mg	15 mg	N/A	N/A
4-8 years	25 mg	25 mg	N/A	N/A
9-13 years	45 mg	45 mg	N/A	N/A



14-18 years	75 mg	65 mg	80 mg	115 mg
19 + years	90 mg	75 mg	85 mg	120 mg

Scurvy is a disease caused by a severe vitamin C deficiency. It's not common any longer, but it can still occur. You can experience a deficiency without getting scurvy. The symptoms are feeling weak, tired, and irritable, dry and splitting hair, bleeding gums, rough, dry, scaly skin, gingivitis, easy bruising, anemia, and a decreased ability to fight infection. Excess intakes can cause stomach upset, diarrhea, and possibly kidney stones.

## VITAMIN D

Vitamin D is known by most as the sunshine vitamin. The ultraviolet rays from the sunlight help your body produce vitamin D when they hit your skin for at least 10 minutes. The sunlight is not the only way to get vitamin D. Foods like seafood, mushrooms, and egg yolks naturally contain this vitamin, and other foods have it added so you can reach your needs with your diet.

Vitamin D is needed to maintain blood levels of calcium and phosphorus. A deficiency in children can cause rickets, a disease that causes soft, weak bones; this results in skeletal deformities (bowed legs), impaired growth, bone pain, and dental problems. In adults, a deficiency can cause osteomalacia and osteoporosis.

The recommendations for vitamin D are listed as an adequate intake in micrograms (mcg) and international units (IU):

Age	Vitamin D
Birth to 13 years	5 mcg (200 IU)
14-18 years	5 mcg (200 IU)
19-50 years	5 mcg (200 IU)
51-70 years	10 mcg (400 IU)
71+ years	15 mcg (600 IU)

## VITAMIN E

Vitamin E naturally occurs in eight different chemical forms, but the only one that meets human requirements is alpha-tocopherol. It's needed to maintain cell integrity and act as a powerful antioxidant. Vitamin E is a fat-soluble vitamin, so foods like nuts, seeds, and vegetable have a high content. While deficiency is rare, it can happen. Symptoms of vitamin E deficiency are peripheral neuropathy, impaired immune system, retinopathy, and skeletal myopathy.

The following is the RDA for vitamin E:

Age	Males and Females	Pregnancy	Lactation
1-3 years	6 mg (9 IU)	N/A	N/A
4-8 years	7 mg (10.5 IU)	N/A	N/A
9-13 years	11 mg (16.5 IU)	15 mg (22.5 IU)	19 mg (28.5 IU)
14 + years	15 mg (22.5 IU)	15 mg (22.5 IU)	19 mg (28.5 IU)

## VITAMIN K

Vitamin K is known by many people taking blood-thinner medications. It is limited by them because of its role in blood clotting. The goal when taking blood thinners is to consume a consistent amount of vitamin K, not to eliminate it from your diet. A deficiency in vitamin K can lead to defective blood clotting and increased bleeding. The foods with the highest amount of vitamin K are kale, spinach, turnips, collards, mustard greens, and brussels sprouts.

There is no data to establish an RDA for vitamin K. Therefore, adequate intakes (AI) have been established:

Age	Males	Females	Pregnancy	Lactation
0-6 months	2.0 mcg	2.0 mcg	N/A	N/A
7-12 months	2.5 mcg	2.5 mcg	N/A	N/A
1-3 years	30 mcg	30 mcg	N/A	N/A
4-8 years	55 mcg	55 mcg	N/A	N/A
9-13 years	60 mcg	60 mcg	N/A	N/A

14-18 years	75 mcg	75 mcg	75 mcg	75 mcg
19+ years	120 mcg	90 mcg	90 mcg	90 mcg

A well-balanced diet is often enough to reach all of your recommended vitamins. Certain medical conditions, dietary restrictions, and medications can require that you take a vitamin K supplement in order to reach your goals. It's best to work with a health-care professional to determine how much you should take. Excess quantities can be as dangerous as not consuming enough. The key is always to give your body the optimal levels for optimal health.

### **Secondary metabolites**

**Secondary metabolites** are compounds that are not required for the growth or reproduction of an organism but are produced to confer a selective advantage to the organism. For example, they may inhibit the growth of organisms with which they compete and, as such, they often inhibit biologically important processes.

### NATURAL PRODUCTS CHEMISTRY

#### **Definition :**

“That branch of chemistry which deals with the isolation, identification, structure elucidation, and study of the chemical characteristics of chemical substances produced by living organisms”

#### Problems with Synthetic Drugs

- Potency
- Cost
- Side effects
- Requires close supervision of clinician
- Resistance
- Unavailability (Sometimes)
- Stability....

#### Source of Natural Products

- **Plants**

- Microorganisms
  - Bacteria, fungus
  - Marine organisms
  - Animal products

Plant research is at the forefront to find an effective, safe pharmacological treatment of diseases.

WHO estimates 65-80% of world population use traditional medicine & 80 % of these involves plant extracts.

## **Metabolites**

### **Primary metabolites**

(needful for the cell survival and present in all living system, plants and animals)

- Carbohydrates
- Lipids
- Proteins
- Organic acids
- Vitamins
- Chlorophylls

### **Secondary metabolites**

(synthesized from primary metabolites; they are not needful for the cell survival, but contribute to the survival of the whole organism)

- Glycosides
- Phenolic compounds
- Terpenoids
- Alkaloids

## **ALKALOIDS**

**Alkaloids** are naturally-occurring organic compounds containing nitrogen moiety, and are usually heterocyclic in nature. They are nitrogen based organic compounds, with nitrogen enclosed in an heterocyclic ring.

The alkyl amines are referred to as proalkaloids.

### **Characteristics of alkaloids**

- (1) They are basic in nature due to the presence of nitrogen in their ring.
- (2) They have complex structures.
- (3) They have bitter principles.
- (4) They are mostly obtained from plant materials.
- (5) They have high pharmacological and physiological activities.

Examples of alkaloids are:

- (1) Quinine — an antimalarial drug isolated from a plant called *Cinchonia officinalis*

Quinine is an antipyretic alkaloid. Its molecular formula is  $C_{20}H_{24}N_2O_2$ .

Functional groups present in quinine are: methoxyl –  $OCH_3$ , hydroxyl –  $OH$ , tertiary amine group, etc. Other examples of alkaloids are: morphine, cocaine, heroine, etc. Most are highly narcotic in nature.

### **Cocaine -**

- Cocaine is obtained from coca leaves,
- Cocaine is the first local anaesthetic ever discovered by man,
- Cocaine is highly narcotic and stimulates the central nervous system i.e. CNS depressant,
- Cocaine can lead to psychiatric problem when taken in high dose or when addicted to it.

**Caffeine** is an alkaloid obtained from coffee, tea. It is also a strong stimulant which can increase alertness, thereby causing insomnia when the body gets addicted.

### **Alkaloid as Natural Products**

- Largest class of secondary metabolites, >6500 compounds known
- Contains N, most compounds are basic (alkaline)
- Often highly toxic

- Found in certain higher plants
- Little is known regarding why alkaloids are produced
- Biosynthesis from amino acids

Many of these substances have marked physiological effects, a fact discovered by many ancient people long before organic chemistry developed.

Like, alkaloid quinine, a chief constituent of bark of Cinchona, has been used as effective antimalarial since 1639.

Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants and animals and are part of the group of natural products (also called secondary metabolites).

### **Effects of alkaloids on humans**

- High biological activity
- Produce varying degrees of physiological and psychological responses - largely by interfering with neurotransmitters
  - others interfere with membrane transport, protein synthesis or other processes
- In large doses - highly toxic - fatal
- In small doses, many have therapeutic value
  - muscle relaxants, tranquilizers, pain killers, mind altering drugs, chemotherapy

### **Tests for Alkaloids**

- Most alkaloids are precipitated from neutral or slightly acidic solution by
- Mayer's reagent (potassium mercuric iodide solution) Cream coloured precipitate.
- Dragendorff's reagent (solution of potassium bismuth iodide) orange coloured precipitate.
- Wagner's reagent (iodine in potassium iodide) red-brown precipitate
- Hager's reagent (picric acid) precipitate

### **Physical & Chemical Properties of Alkaloids**

MW: 100 – 900

Most bases which do not contain Oxygen are liquid at room temperature (nicotine), while those that do are solids.

In rare cases they are coloured.

Most solid bases rotate the plane of polarized light, have high melting points.

Normally are not soluble in water (occasionally slightly soluble).

Soluble in non polar or slightly polar organic solvent

The basicity of alkaloids depends on the availability of the lone pair of electrons on the N atoms: Electron donating groups enhance basicity, while e- withdrawing groups decrease it.

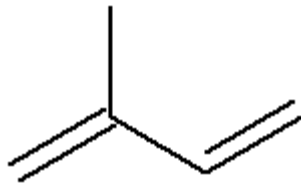
Because some alkaloids have a carbonyl group on the amide, they can also be neutral (colchicine & piperine).

## Terpenoids

The **terpenoids**, sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene, and the isoprene polymers called terpenes. Most are multicyclic structures with oxygen-containing functional groups.

You are not expected to memorize all the details of the synthetic mechanisms for terpenoids. However, you should know the overall general synthetic pathway illustrated under “Terpenoid Biosynthesis.” As you read through the details of these mechanisms, realize that they may be complex, but they are based on experimental evidence. You should also note the important role of enzymes in many natural systems transformations. Finally, you will recognize that essentially, individual steps are often reactions you have already encountered in previous sections.

The terpenoids (aka isoprenoids) are a large (estimated 60% of known natural products ) and diverse group of lipids derived from five-carbon isoprene units assembled in thousands of combinations. Technically a terpenoid contains oxygen, while a [terpene](#) is a hydrocarbon. Often the two terms are used to refer collectively to both groups.



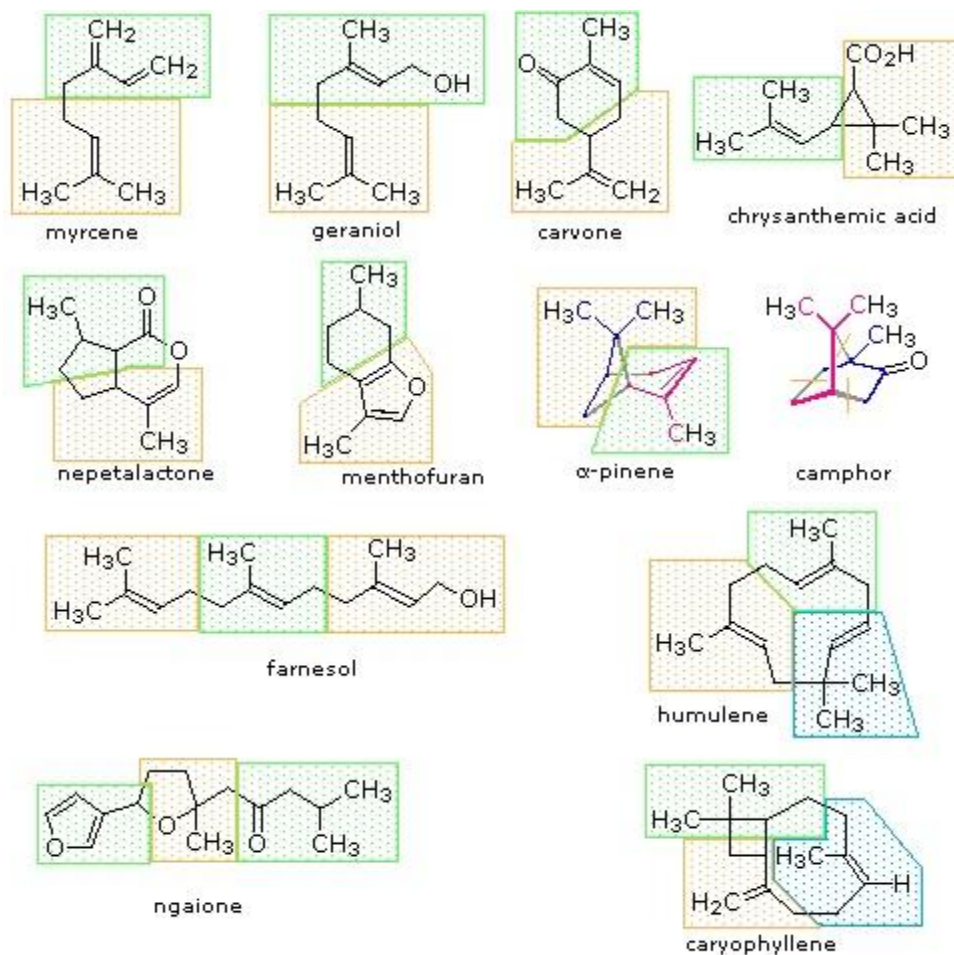
isoprene

### Isoprene Rule

Compounds classified as terpenes constitute what is arguably the largest and most diverse class of natural products. A majority of these compounds are found only in plants, but some of the larger and more complex terpenes (e.g. squalene & lanosterol) occur in animals. Terpenes incorporating most of the common functional groups are known, so this does not provide a useful means of classification. Instead, the number and structural organization of carbons is a definitive characteristic. Terpenes may be considered to be made up of isoprene (more accurately isopentane) units, an empirical feature known as the [isoprene rule](#). Because of this, terpenes usually have  $5n$  carbon atoms ( $n$  is an integer), and are subdivided as follows:

Isoprene itself, a  $C_5H_8$  gaseous hydrocarbon, is emitted by the leaves of various plants as a natural byproduct of plant metabolism. Next to methane it is the most common volatile organic compound found in the atmosphere. Examples of  $C_{10}$  and higher terpenes, representing the four most common classes are shown in the following diagrams. Most terpenes may be structurally dissected into isopentane segments. How this is done can be seen in the diagram directly below.





**Figure 27.5.1:** Monoterpenes and sesquiterpenes

The isopentane units in most of these terpenes are easy to discern, and are defined by the shaded areas. In the case of the monoterpene camphor, the units overlap to such a degree it is easier to distinguish them by coloring the carbon chains. This is also done for alpha-pinene. In the case of the triterpene lanosterol we see an interesting deviation from the isoprene rule. This thirty carbon compound is clearly a terpene, and four of the six isopentane units can be identified. However, the ten carbons in center of the molecule cannot be dissected in this manner. Evidence exists that the two methyl groups circled in magenta and light blue have moved from their original isoprenoid locations (marked by small circles of the same color) to their present location. This rearrangement is described in the [biosynthesis](#) section. Similar alkyl group rearrangements account for other terpenes that do not strictly follow the isoprene rule.

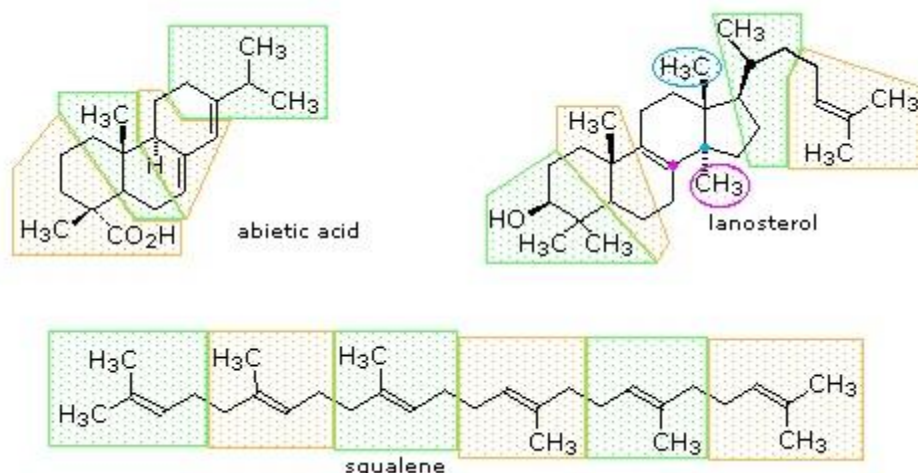
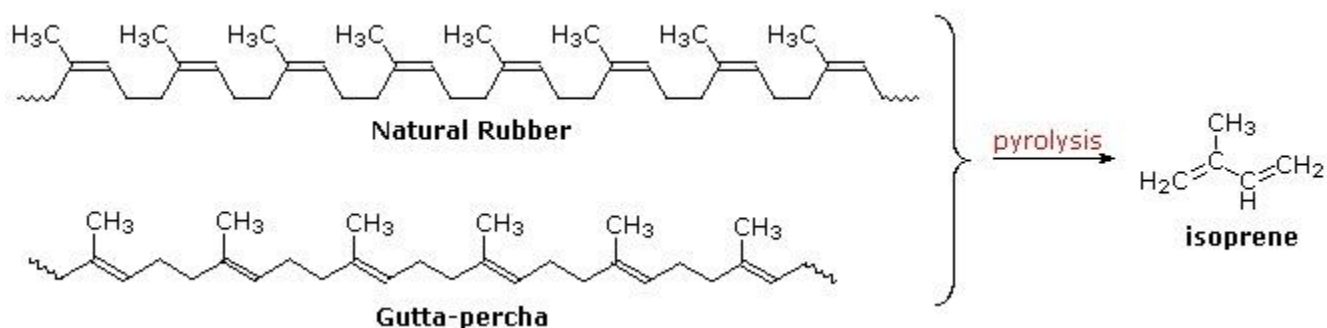


Figure 27.5.2: Triterpenes

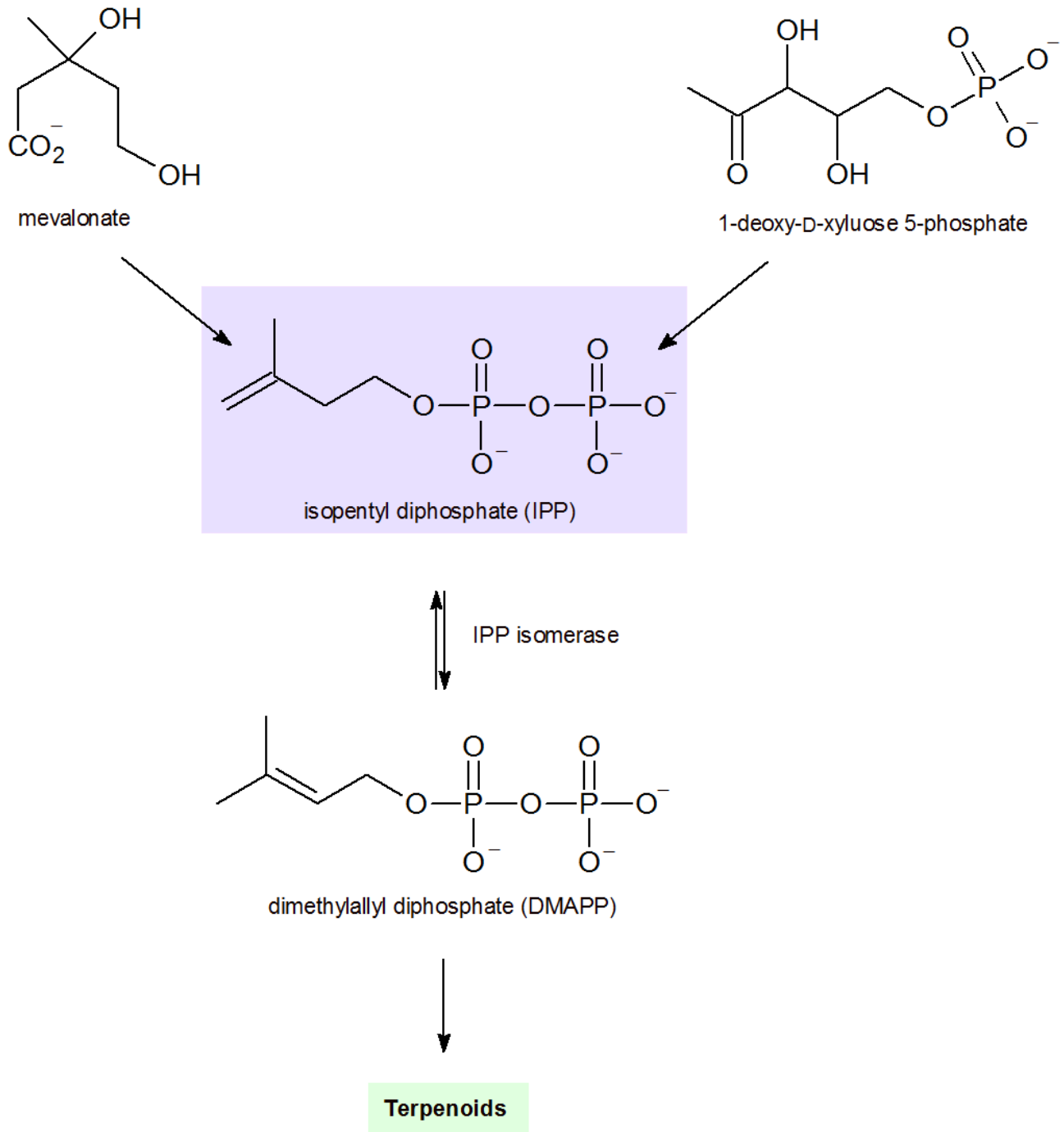
Polymeric isoprenoid hydrocarbons have also been identified. Rubber is undoubtedly the best known and most widely used compound of this kind. It occurs as a colloidal suspension called latex in a number of plants, ranging from the dandelion to the rubber tree (*Hevea brasiliensis*). Rubber is a polyene, and exhibits all the expected reactions of the C=C function. Bromine, hydrogen chloride and hydrogen all add with a stoichiometry of one molar equivalent per isoprene unit. Ozonolysis of rubber generates a mixture of levulinic acid (CH<sub>3</sub>COCH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H) and the corresponding aldehyde. Pyrolysis of rubber produces the diene isoprene along with other products.



The double bonds in rubber all have a Z-configuration, which causes this macromolecule to adopt a kinked or coiled conformation. This is reflected in the physical properties of rubber. Despite its high molecular weight (about one million), crude latex rubber is a soft, sticky, elastic substance. Chemical modification of this material is normal for commercial applications. Gutta-percha (structure above) is a naturally occurring E-isomer of rubber. Here the hydrocarbon chains adopt a uniform zig-zag or rod like conformation, which produces a more rigid and tough substance. Uses of gutta-percha include electrical insulation and the covering of golf balls.

## Terpenoid Biosynthesis

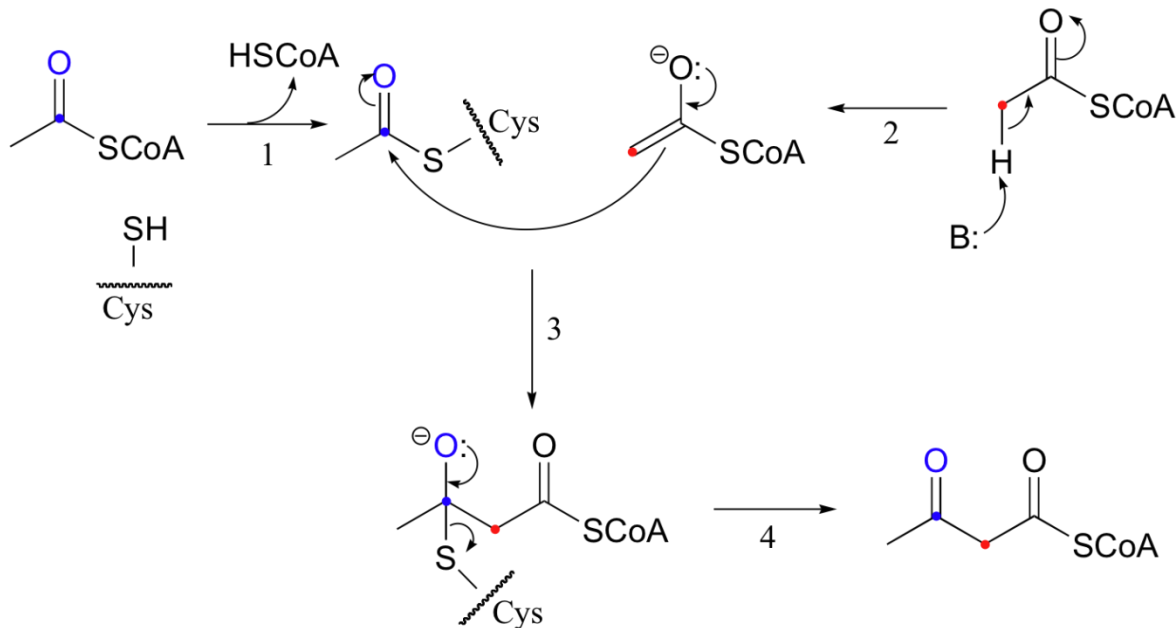
While we can identify isoprene units within a terpenoid structure and use that in its classification, the building block for terpenoid synthesis in nature is isopentenyl diphosphate (formerly called isopentenyl pyrophosphate and abbreviated IPP). There are two major routes to the synthesis of IPP; namely (1) the mevalonate pathway and (2) the 1-deoxyxylulose pathway.



## Mevalonate Pathway

### Step 1 - Claisen Condensation

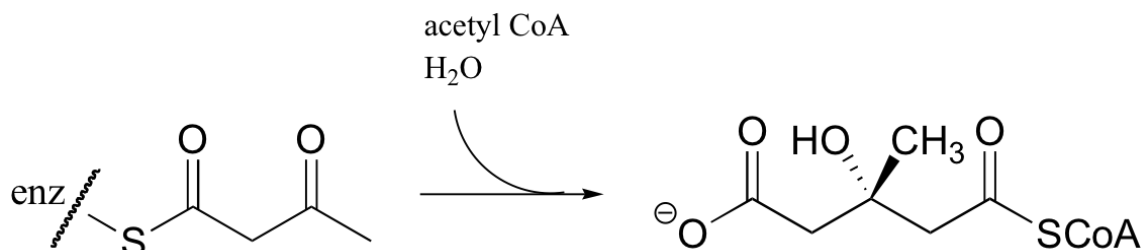
An early step in the biosynthesis of cholesterol and other 'isoprenoid' compounds is a [Claisen condensation](#) between two acetyl CoA molecules. An initial trans-thioesterase process transfers the acetyl group of the first acetyl CoA to an enzymatic cysteine (Reaction 1). In the Claisen condensation phase of the reaction, the alpha-carbon of a second acetyl CoA is deprotonated, forming an enolate (Reaction 2).



The enolate carbon attacks the electrophilic thioester carbon, forming a tetrahedral intermediate (Reaction 3) which quickly collapses to expel the cysteine thiol (Reaction 4) and produce acetoacetyl CoA.

### Step 2 - Aldol Condensation

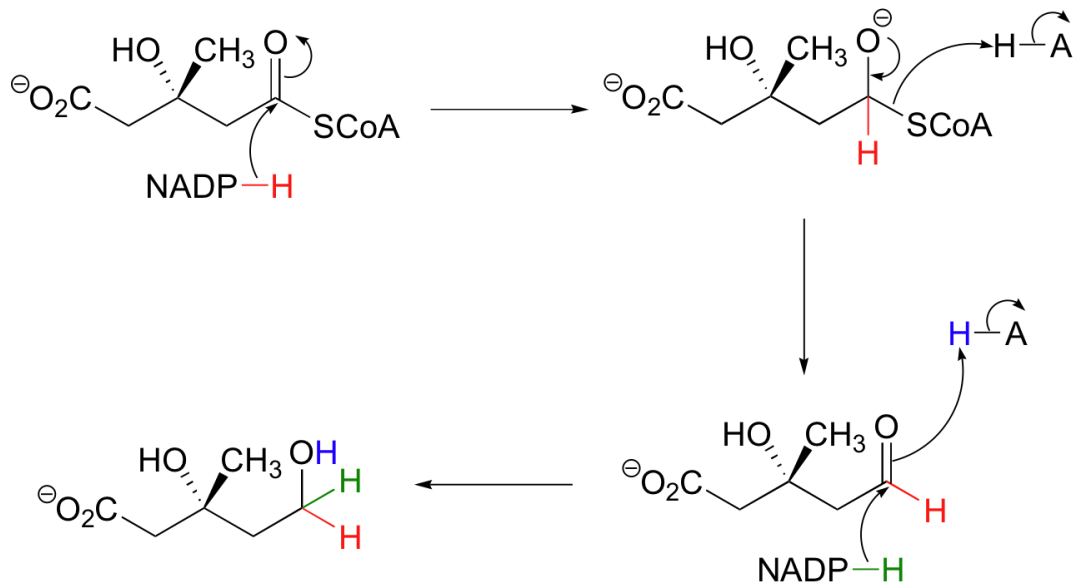
Acetyl CoA then reacts with the acetoacetyl CoA in an aldol-like addition. Subsequent hydrolysis produces (3*S*)-3-hydroxy-3-methylglutaryl CoA (HMG-CoA).



## Generating HMG-CoA

### Step 3 - Reduction of the Thioester

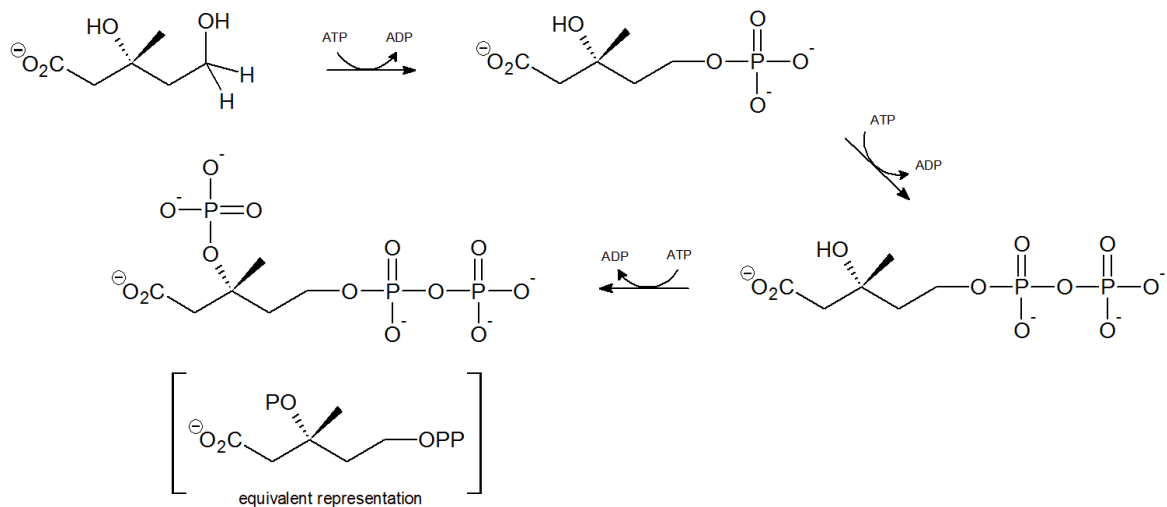
The thioester is reduced first to an aldehyde, then to a primary alcohol by two equivalents of NADPH producing (*R*)-mevalonate. The enzyme catalyzing this reaction is the target of the statin family of cholesterol-lowering drugs.



## Generating (*R*)-Mevalonate

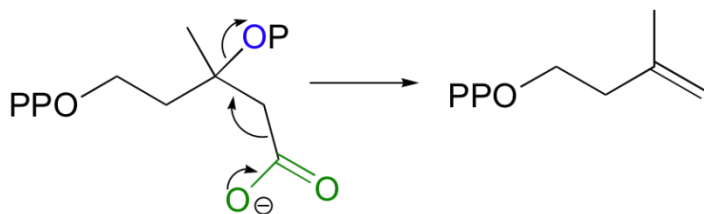
### Step 4 - Mevalonate Phosphorylation

Two phosphorylations by [adenosine triphosphate \(ATP\)](#) occur at the terminal hydroxyl/phosphorus group through nucleophilic substitution, followed by a third ATP phosphorylation of the tertiary hydroxyl group.



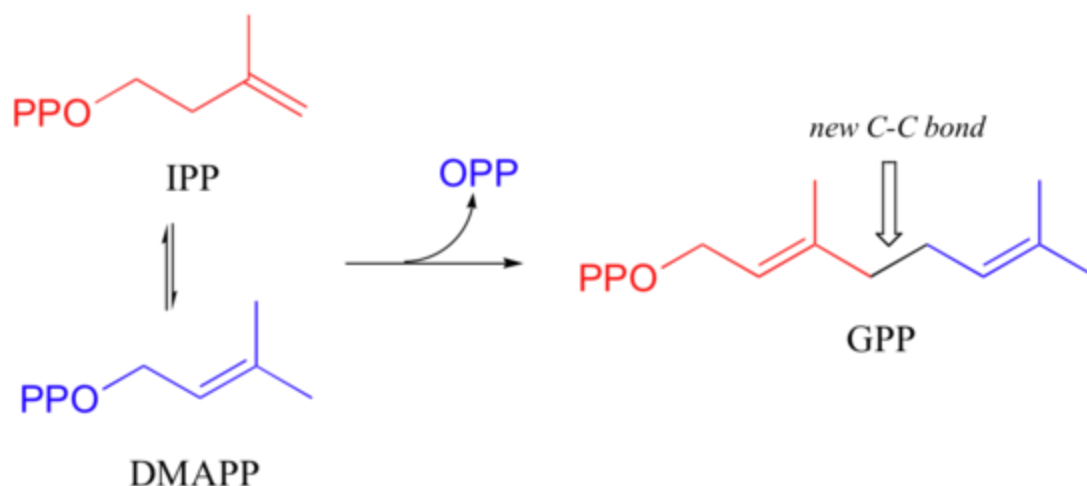
### Step 5 - Decarboxylation

Finally isopentenyl diphosphate (IPP), the 'building block' for all isoprenoid compounds, is formed from a decarboxylation-elimination reaction.

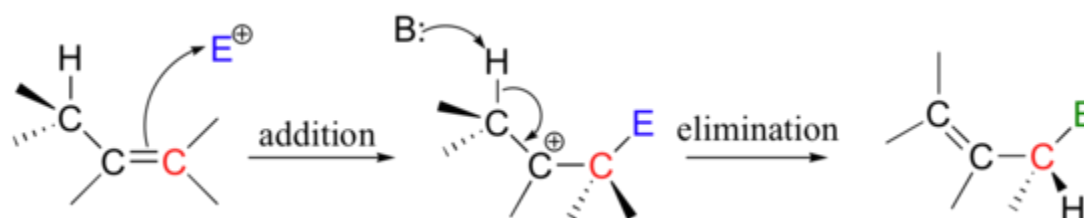


### Conversion of IPP to Terpenoids

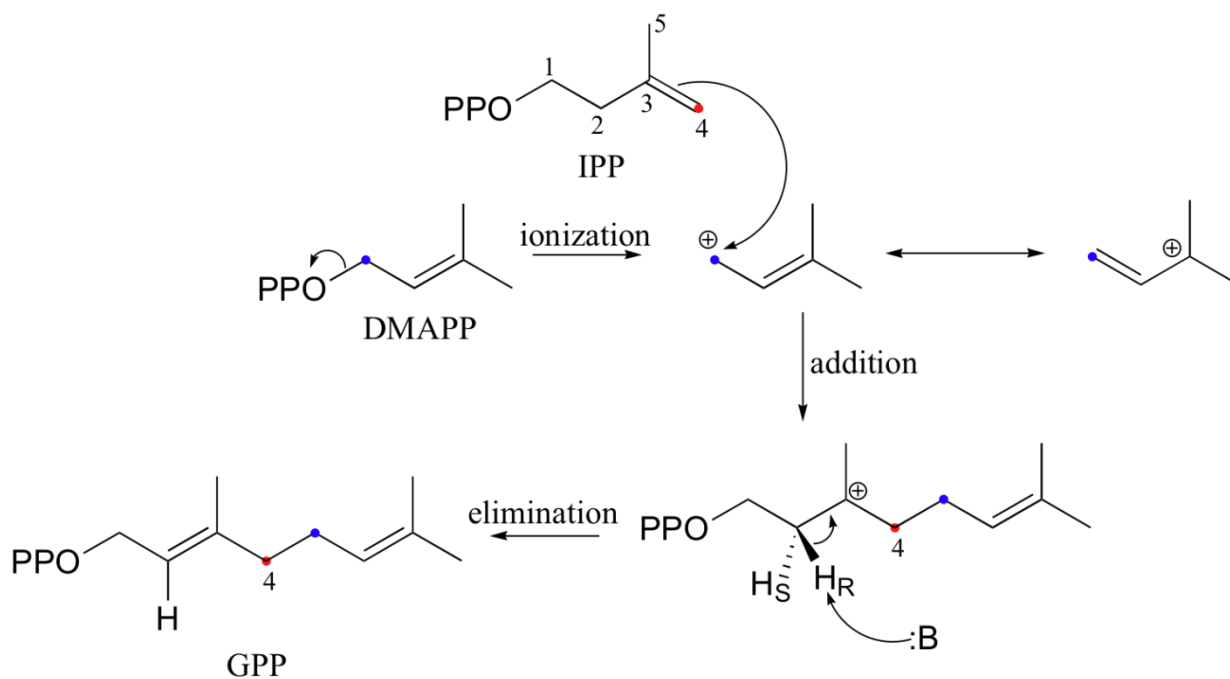
The electrophilic double bond isomerization catalyzed by IPP isomerase is a highly reversible reaction, with an equilibrium IPP:DMAPP ratio of about 6:1. In the next step of isoprenoid biosynthesis, the two five-carbon isomers condense to form a 10-carbon isoprenoid product called geranyl diphosphate (GPP).



This is a nice example of an electrophilic addition/elimination mechanism:

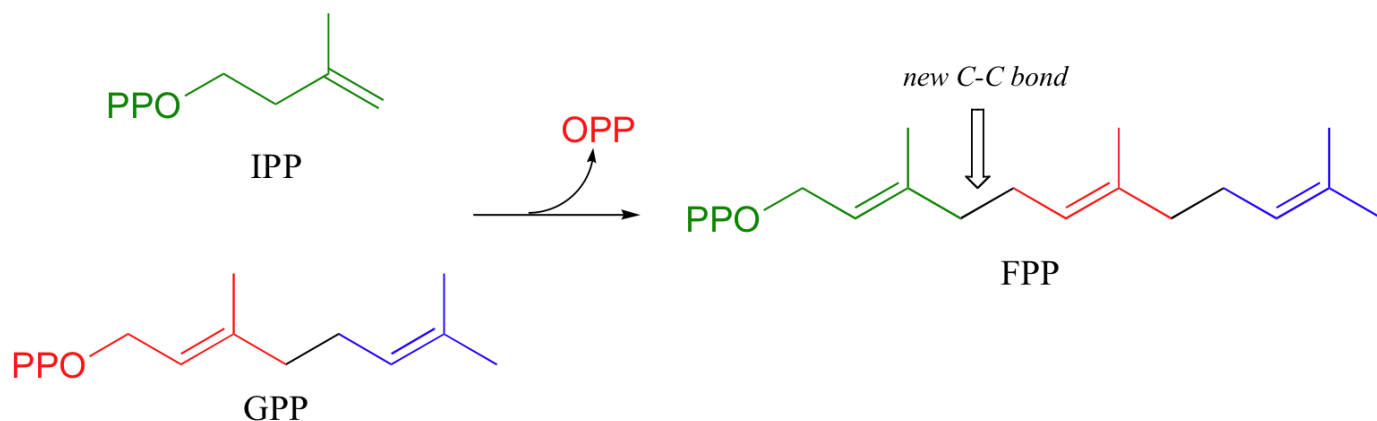


The first step is ionization of the electrophile - in other words, the leaving group departs and a carbocation intermediate is formed. In this case, the pyrophosphate group on DMAPP is the leaving group, and the electrophilic species is the resulting allylic carbocation.

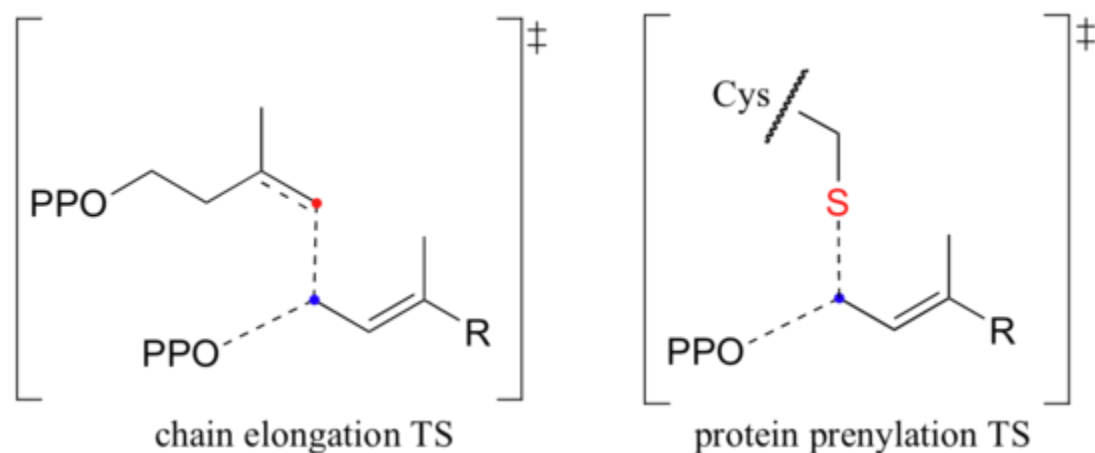


In the condensation (addition) step, the C<sub>3</sub>-C<sub>4</sub> double bond in IPP attacks the positively-charged C<sub>1</sub> of DMAPP, resulting in a new carbon-carbon bond and a second carbocation intermediate, this time at a tertiary carbon. In the elimination phase, proton abstraction leads to re-establishment of a double bond in the GPP product. Notice that the enzyme specifically takes the *pro-R* proton in this step.

To continue the chain elongation process, another IPP molecule can then condense, in a very similar reaction, with C<sub>1</sub> of geranyl diphosphate to form a 15-carbon product called farnesyl diphosphate (FPP).



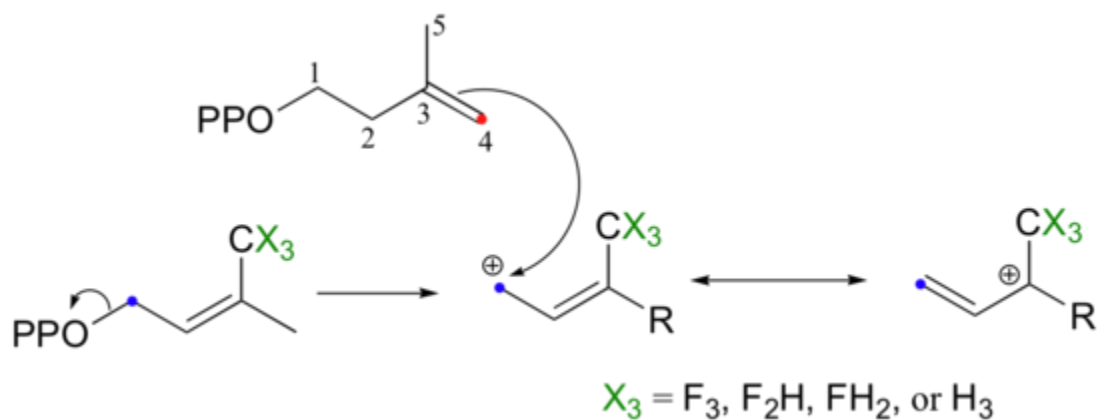
How do we know that these are indeed S<sub>N</sub>1-like mechanisms with carbocation intermediates, rather than concerted S<sub>N</sub>2-like mechanisms? First of all, recall that the question of whether a substitution is dissociative (S<sub>N</sub>1-like) or associative (S<sub>N</sub>2-like) is not always clear-cut - it could be somewhere in between, like the [protein prenyltransferase reaction](#). The protein prenyltransferase reaction and the isoprenoid chain elongation reactions are very similar: the electrophile is the same, but in the former the nucleophile is a thiolate, while in the latter the nucleophile is a pi bond.





This difference in the identity of the nucleophilic species would lead one to predict that the chain elongation reaction has more  $S_N1$ -like character than the protein prenylation reaction. A thiolate is a very powerful nucleophile, and thus is able to *push* the pyrophosphate leaving group off, implying some degree of  $S_N2$  character. The electrons in a pi bond, in contrast, are only weakly nucleophilic, and thus need to be *pulled* in by a powerful electrophile - *ie.* a carbocation.

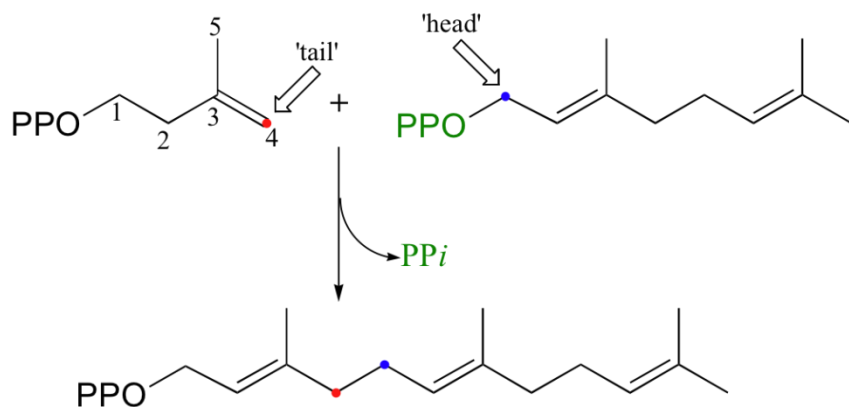
So it makes perfect sense that the chain elongation reaction should more  $S_N1$ -like than  $S_N2$ -like. Is this in fact the case? We know how to answer this question experimentally - just run the reaction with fluorinated DMAPP or GPP substrates and observe how much the fluorines slow things down.



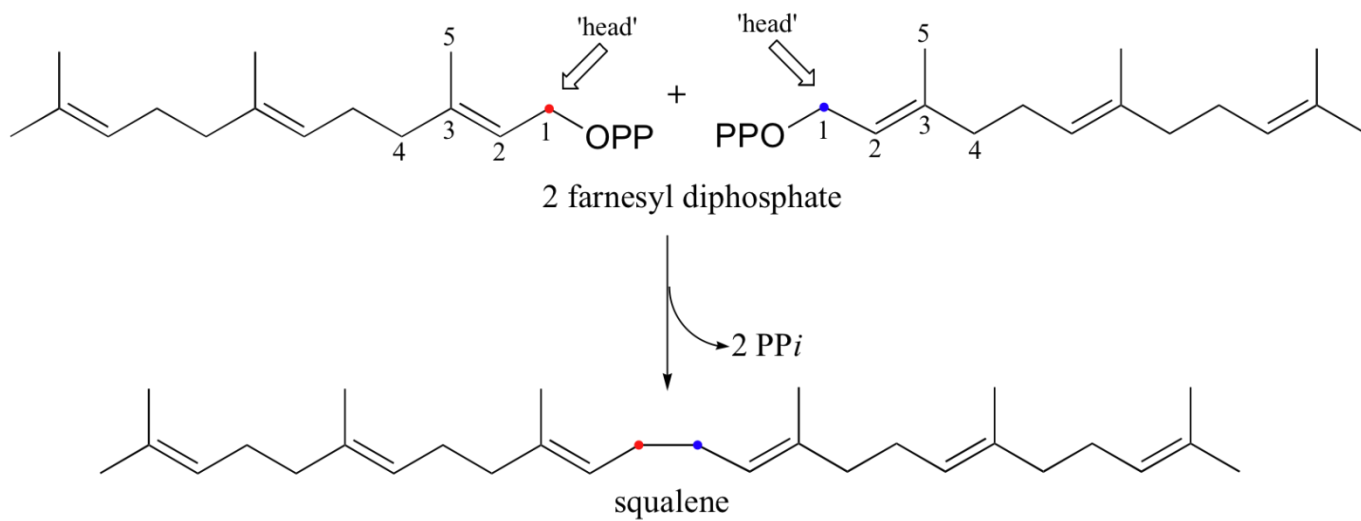
If the reaction is  $S_N1$ -like, the electron-withdrawing fluorines should destabilize the allylic carbocation intermediate and thus slow the reaction down considerably. If the mechanism is  $S_N2$ -like, the fluorine substitutions should not have a noticeable effect, because a carbocation intermediate would not be formed. When this experiment was performed with FPP synthase, the results were dramatic: the presence of a single fluorine slowed down the rate of the reaction by a factor of about 60, while two and three fluorines resulted in a reaction that was 500,000 and 3 million times slower, respectively (*J. Am. Chem. Soc.* **1981**, *103*, 3926.) These results strongly suggest indicate the formation of a carbocation intermediate in an  $S_N1$ -like displacement.

In this section, we will briefly examine the reaction catalyzed by an enzyme called squalene synthase, an important enzymatic transformation that involves some very interesting and unusual electrophilic additions, rearrangements, and reactive intermediates. This particular enzyme is also of interest because it represents a potential new target for cholesterol-lowering drugs.

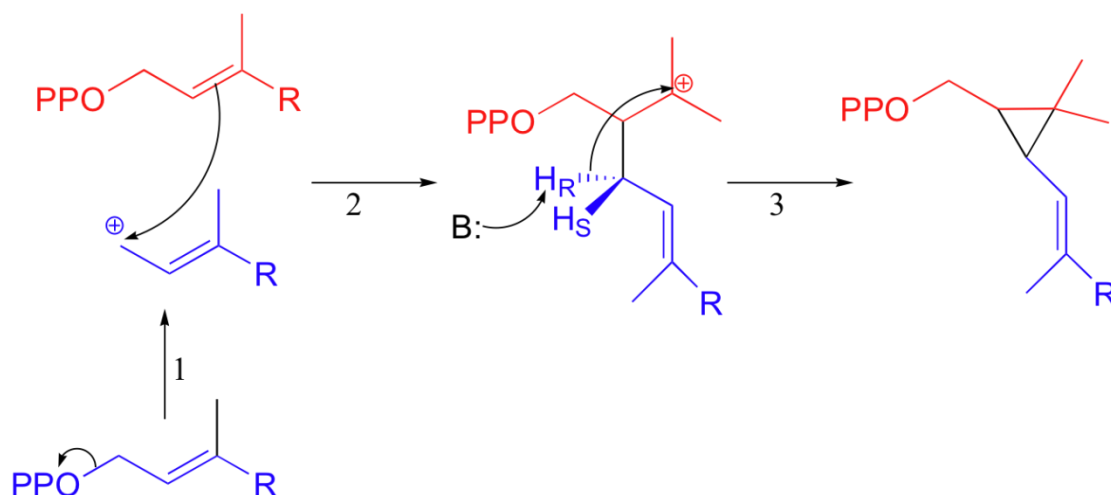
Cholesterol, as we discussed earlier in this chapter, is derived from a 30-carbon isoprenoid molecule called squalene. Squalene, in turn, is derived from the condensation of two molecules of farnesyl diphosphate (FPP), a 15-carbon isoprenoid. You may recall that FPP is the product of the  $C_4$  to  $C_1$ , or 'head to tail' electrophilic condensation of isoprenoid chains:



The condensation of two molecules of FPP to form squalene, however, is something different: this is a 'head to head' condensation, where  $C_1$  of the first molecule forms a bond to  $C_1$  of the second. The chemistry involved is quite a bit more complicated.

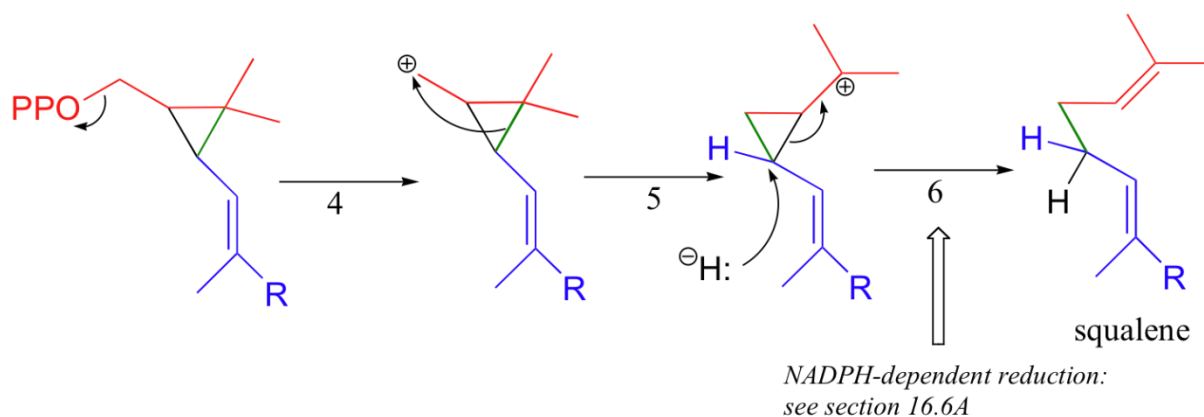


The first two steps are familiar: first, the pyrophosphate on one FPP molecule leaves (step 1), resulting in an allylic carbocation that is attacked by the  $C_2$ - $C_3$   $\pi$  bond of the second molecule (step 2).



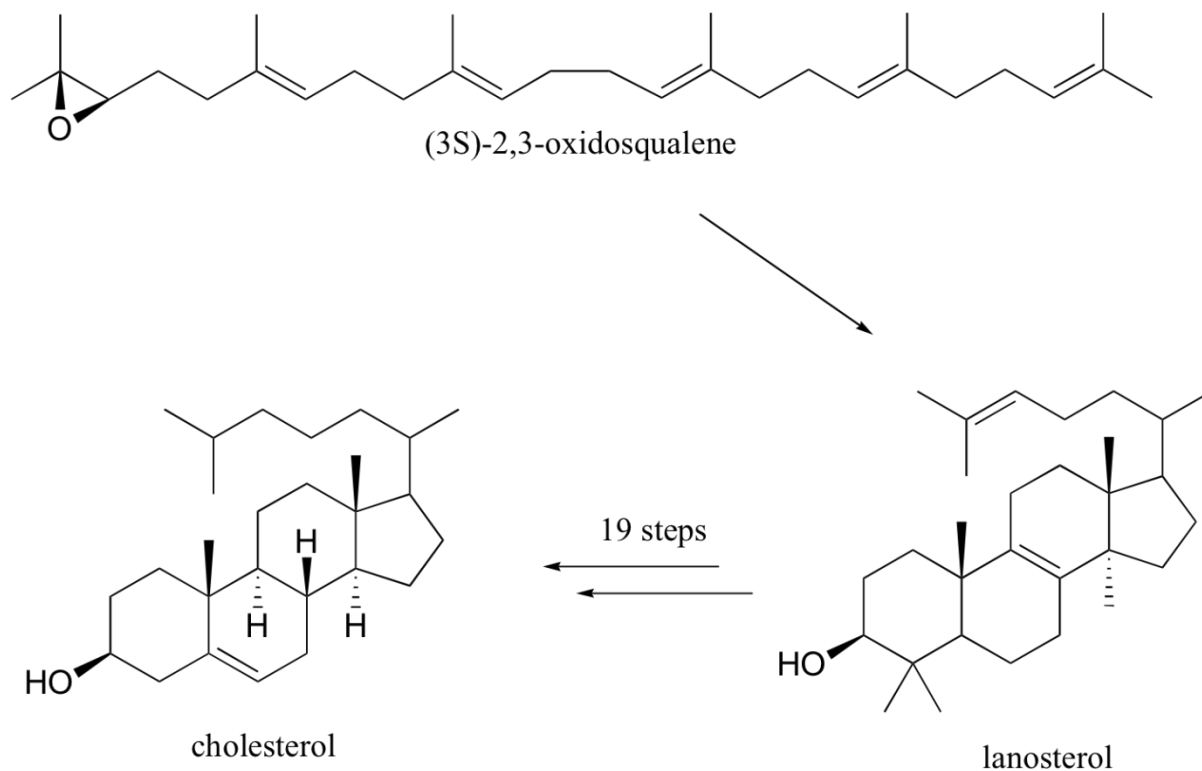
This results in a new carbon-carbon bond between the two FPP molecules, but with incorrect  $C_1$  to  $C_2$  connectivity (remember, the overall reaction is a  $C_1$  to  $C_1$  condensation). In step 3, a proton is abstracted and the electrons from the broken C-H bond bridge across a 2-carbon gap to form a cyclopropyl intermediate.

In the second stage of squalene synthesis, the second pyrophosphate group leaves, generating a cyclopropylcarbinyl cation (step 4). Because this is a primary carbocation, you probably are wondering about how stable it could be (and thus how likely an intermediate). As it turns out, such carbocations are remarkably stable, due to favorable interactions between the empty orbital and orbitals on the three-membered ring (the level of bonding theory needed to really understand this idea is beyond the scope of this text, but you may learn about it if you take a class in advanced organic chemistry). What occurs next is an alkyl shift leading to a tertiary carbocation (step 5).



Discussion of the final step (step 6) will need to be put off - this is a reduction with a hydride nucleophile derived from a coenzyme called NADPH. Although this may seem like an extremely

convoluted (and perhaps unlikely!) mechanism, there is much experimental evidence to back it up.



## Polyphenols

**Polyphenols** are one of the most important and certainly the most numerous among the groups of phytochemicals present in the plant kingdom. Currently, over 8000 phenolic structures have been identified, of which more than 4000 belonging to the class of **flavonoids**, and several hundred occur in edible plants. However, it is thought that the total content of polyphenols in plants is underestimated as many of the phenolic compounds present in fruits, vegetables and derivatives have not yet been identified, escaping the methods and techniques of analysis used, and the composition in polyphenols for most fruits and some varieties of cereals is not yet known.

They are present in many **edible plants**, both for men and animals, and it is thought to be their presence, along with that of other molecules such as carotenoids, vitamin C or vitamin E, the responsible for the health effects of fruits and vegetables. In the human diet, they are the **most abundant natural antioxidants**, and the main sources are fruits, vegetables, whole grains, but also other types of foods and beverages derived from them, such as red wine, rich in

resveratrol, the extra virgin olive oil, rich in hydroxytyrosol, chocolate or tea, in particularly green tea, rich in epigallocatechin gallate (EGCG).

## CONTENTS

- Chemical structure of polyphenols
- Classification
- Variability of polyphenol content of plants and plant products
- References

### Chemical structure of polyphenols

The term polyphenols refers to a **wide variety of molecules** that can be divided into many subclasses, subdivisions that can be made on the basis of their origin, biological function, or chemical structure. Chemically, they are compounds with structural phenolic features, which can be associated with different organic acids and carbohydrates.

In plants, the most part of them are linked to sugars, and therefore they are in the form of glycosides. Carbohydrates and organic acids can be bound in different positions on polyphenol skeletons.

Among polyphenols, there are simple molecules, such as phenolic acids, or complex structures such as proanthocyanidins, that are highly polymerized molecules.

### Classification

They can be classified into **different classes**, according to the number of phenolic rings in their structure, the structural elements that bind these rings each others, and the substituents linked to the rings. Therefore, two main groups can then be identified: the flavonoids group and the non-flavonoid group. **Flavonoids** share a structure formed by two aromatic rings, indicated as A and B, linked together by three carbon atoms forming an oxygenated heterocycle, the C ring; they can be further subdivided into six main subclasses, as a function of the type of heterocycle (the C ring) that is involved:

- flavones
- flavonols
- flavanones
- flavanonols
- flavanols or flavan-3-ols or catechins

- anthocyanins
- isoflavones
- neoflavonoids
- chalcones

**Non-flavonoids** can be subdivided into:

- simple phenols
- phenolic acids
- benzoic aldehydes
- hydrolyzable tannins
- acetophenones and phenylacetic acids
- hydroxycinnamic acids
- coumarins
- benzophenones

### **Variability of polyphenol content of plants and plant products**

Although several classes of phenolic molecules, such as quercetin (a flavonol, see figure), are present in most plant foods (tea, wine, cereals, legumes, fruits, fruit juices, etc.), other classes are found only in a particular type of food (e.g. flavanones in citrus, isoflavones in soya, phloridzin in apples, etc.). However, it is common that different types of polyphenols are in the **same product**; for example, apples contain flavanols, chlorogenic acid, hydroxycinnamic acids, glycosides of phloretin, glycosides of quercetin and anthocyanins.

The polyphenol composition may also be influenced by other parameters such as environmental factors, the degree of ripeness at harvest time, household or industrial processing, storage, and plant variety. From currently available data, it seems that the **fruits** with the highest content of polyphenols are strawberries, lychees and grapes, and the **vegetables** are artichokes, parsley and brussels sprouts. Melons and avocados have the lowest concentrations.

### **Volatile oils**

Volatile oils are mixture of hydrocarbon terpenes, sesquiterpenes and polyterpenes and their oxygenated derivatives obtained from various parts of the plant. Volatile oils evaporate on exposure to air at ordinary temperature and are the odorous constituents. As volatile oil oils are responsible for the essence or odour of the plant they are also known as essential oils.

Volatile oils, when fresh, are colourless liquids. A few are crystalline or amorphous solid. On long standing, they become darker in colour, especially when exposed to air and direct sunlight. Volatile oil should, therefore, be stored in tightly closed amber-coloured bottles in a cool, dry place. Volatile oils are slightly soluble in water but are readily soluble in ether, alcohol and most of organic solvents. Smear on paper, they give a translucent stain which temporary only, disappearing as the oil volatilizes.

### **Extraction of volatile oil:**

#### **(a) Expression Method:**

The plant material is crushed and the juice is screened to remove the large particles. The screened juice is centrifuged in a high-speed centrifugal machine when nearly half of the essential oil is extracted. The other half of the oil is generally not extracted and such residue is used for the isolation of inferior quality of oil by distillation. Citrus, lemon and grass oils are extracted by this method.

#### **(b) Steam Distillation:**

This is the most widely used method; the plant material is macerated and then steam distilled, when the essential oils go into distillate from which they are extracted by the use of pure organic volatile solvents, like light petroleum. However, the method should be used with a great care, since some essential oils are decomposed during distillation and some (ester) are hydrolyzed to none or less fragrant compounds.

#### **(c) Extraction by Mean of Volatile Solvent:**

As described above some essential oil are sensitive to heat and hence decomposed during distillation, in such cases the plant material is directly treated with light petrol at 50°C, and the solvent is removed by distillation under reduce pressure.

#### **(d) Adsorption in Purified Fats (Effleurage):**

The fat taken in glass plates is warmed to about 50°C, then its surface is covered with the petals (part of the flower) and it is allowed to be kept as such for several days until the fat is saturated with the essential oils for which the old petals may be replaced by the fresh ones. The petals are then removed and fat is digested with ethyl alcohol when the essential oils present in the fat are dissolved in ethyl alcohol and if some fat is also dissolved during digestion it is removed by cooling to about 20°C. The extract having ethyl alcohol and essential oils is distilled under reduced pressure to remove the solvent