Solution

1. The genetic code is a triplet code. That is, it takes a sequence of three nucleotides on the coding strand of DNA to specify one amino acid. The DNA of T4 contains:

$\frac{1.3\times10^{8}}{650} = 2\times10^{5}$ nucloeotide pairs $= 2\times10^{5}$ nucleotides in the coding strand.

$\frac{2\times10^{5}}{3} = \sim 6.7\times10^{4}$ codons.

2. The average MW of an amino acid residue is 110. A protein of MW 55000 contains:

$\frac{55000}{110} = 500$ amino acids.

6.7×10^{4} codons can yield: $\frac{6.7\times10^{4}}{500} = 134$.

Nucleic acid conversion factors

Average MW of a DNA base pair = 650 Da

1 A_{260} unit = ~50 microgram/ml of double strand DNA

1 A_{260} unit = ~40 microgram/ml of single strand RNA

1 A_{260} unit = ~33 microgram/ml of single strand DNA

1000 bp DNA open reading frame = 333 amino acids = 37,000 Da protein

To calculate the concentration of plasmid DNA in solution using absorbance at 260 nm:

(Observed A_{260}) × (dilution factor) × (0.050) = DNA concentration in µg/µl

1.8 Carbohydrates

Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones, or compounds that can be hydrolyzed to them. In the majority of carbohydrates, H and O are present in the same ratio as in water, hence also called as *hydrates of carbon*. Carbohydrates are the most abundant biomolecules on Earth. Carbohydrates are classified into following classes depending upon whether these undergo hydrolysis and if so on the number of products form:

Monosaccharides are simple carbohydrates that consist of a single polyhydroxy aldehyde or ketone unit.

Oligosaccharides are polymers made up of two to ten monosaccharide units joined together by glycosidic linkages. Oligosaccharides can be classified as di-, tri-, tetra- depending upon the number of monosaccharides present. Amongst these the most abundant are the disaccharides, with two monosaccharide units.

Polysaccharides are polymers with hundreds or thousands of monosaccharide units. Polysaccharides are not sweet in taste hence they are also called *non-sugars*.

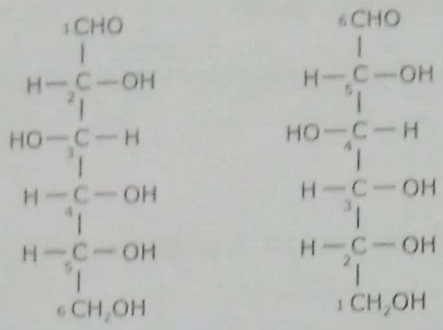
1.8.1 Monosaccharide

Monosaccharides consist of a single polyhydroxy aldehyde or ketone unit. Monosaccharides are the simple sugars and they have a general formula $C_nH_{2n}O_n$. Monosaccharides are colorless, crystalline solids that are freely soluble in water but insoluble in nonpolar solvents. The most abundant monosaccharide in nature is the D-glucose. Monosaccharides can be further sub classified on the basis of:

1. *Number of the carbon atoms*

Monosaccharides can be named by a system that is based on the number of carbons with the suffix-*ose* added. Monosaccharides with four, five, six and seven carbon atoms are called *tetroses, pentoses, hexoses* and *heptoses*, respectively.

System for numbering the carbons : The carbons are numbered sequentially with the aldehyde or ketone group being on the carbon with the lowest possible number.



II. *Types of functional groups*

Monosaccharides can be classified into aldoses and ketoses.

Aldoses are monosaccharides with an aldehyde group.

Ketoses are monosaccharides containing a ketone group.

For example, the monosaccharide *glucose* is an *aldohexose;* that is, it has six-carbon (-hexose) and an aldehyde group (aldo-). Similarly *fructose* is a *ketohexose;* that is, it has six-carbon (-hexose) and a ketone group (keto-). Trioses are simplest monosaccharides. There are two trioses– dihydroxyacetone and glyceraldehyde. Dihydroxyacetone is a *ketose* because it contains a *keto* group, whereas glyceraldehyde is an *aldose* because it contains an *aldehyde* group.

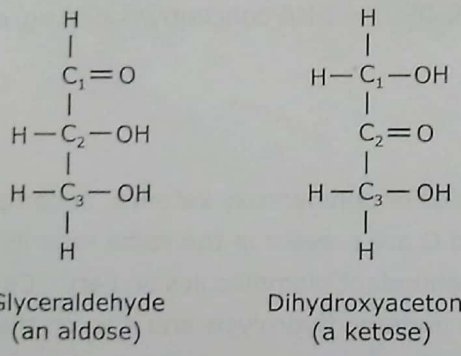


Figure 1.60 Trioses, the simplest monosaccharides.

All the monosaccharides except *dihydroxyacetone* contain one or more chiral carbon atoms. Glyceraldehyde has a central carbon (C-2) which is chiral or asymmetrical. Chiral molecules such as glyceraldehyde can exist in two configurations that are non-superimposable mirror images of each other. These two configurations are called *enantiomers*. An enantiomer is identified by its absolute configuration. Glyceraldehyde has two absolute configurations. When the hydroxyl group attached to the chiral carbon is on the left in a Fischer projection, the configuration is L; when the hydroxyl group is on the right, the configuration is D.

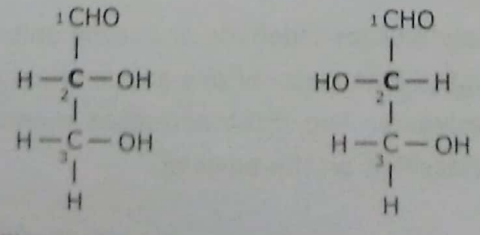


Figure 1.61 The enantiomers of glyceraldehyde. The configuration of groups around the chiral carbon 2 (shown in bold) distinguishes D-glyceraldehyde from L-glyceraldehyde. The two molecules are mirror images and cannot be superimposed on one another.

The absolute configurations of monosaccharide containing more than one chiral centers like hexose are determined by comparing the configuration at the highest-numbered chiral carbon (the chiral carbon farthest from the aldehyde or ketone group) to the configuration at the single chiral carbon of glyceraldehyde.

1.8.2 Epimers

Many common sugars are closely related, differing only by the stereochemistry at a single carbon atom. For example, D-glucose and D-mannose differ only at carbon 2. Sugars that differ only by the stereochemistry at a single carbon (other than anomeric carbon) are called *epimers*. Similarly D-glucose and D-galactose are epimers. D-mannose and D-galactose are not epimers because their configuration differ at more than one carbon.

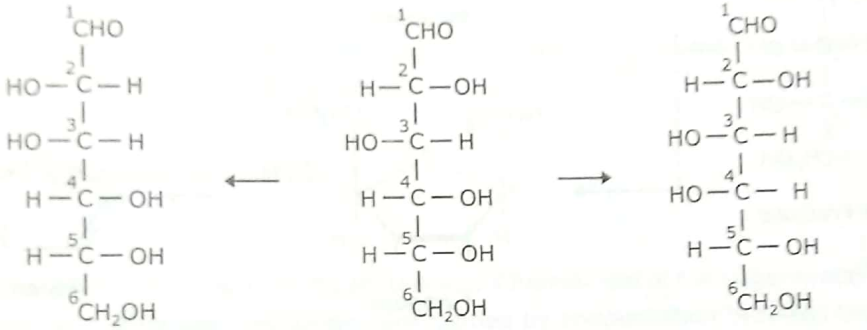


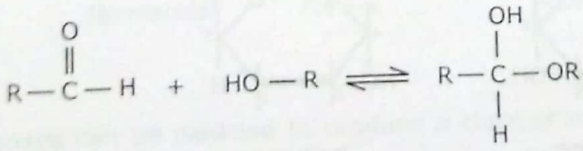
Figure 1.62 D-Glucose and two of its epimers.

1.8.3 Cyclic forms

Monosaccharides having 5 or 6 carbons in the chain gives cyclic structure in aqueous solution via internal hemiacetal or hemiketal formation.

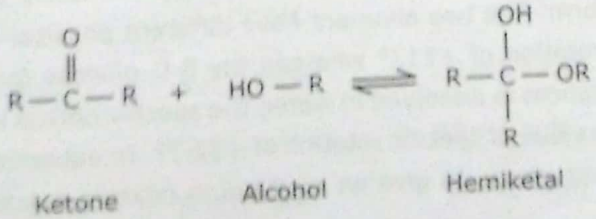
Hemiacetal

In general, an aldehyde can react with an alcohol to form a *hemiacetal*.



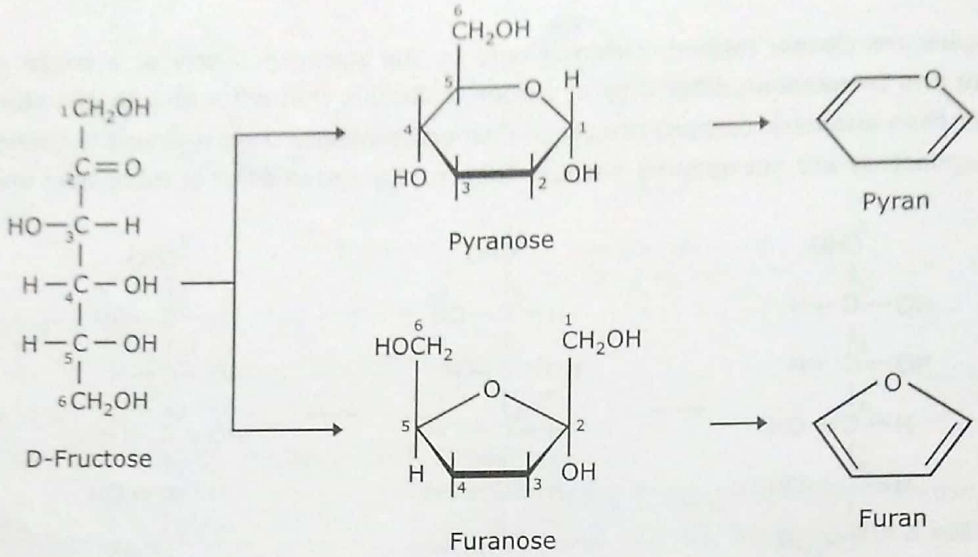
Hemiketal

A ketone can react with an alcohol to form a *hemiketal*.



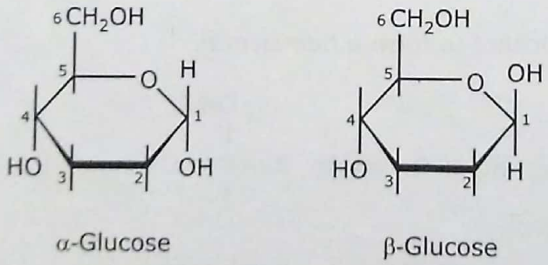
For an aldohexose such as glucose, the C-1 aldehyde group in the open-chain form of glucose reacts with the C-5 hydroxyl group to form an intramolecular *hemiacetal*. The resulting cyclic hemiacetal, a six-membered ring, is called *pyranose* because of its similarity to *pyran*.

Similarly, the C-2 keto group in the open-chain form of a ketohexose, such as fructose, can form an intramolecular *hemiketal* by reacting with either the C-6 hydroxyl group to form a six-membered cyclic hemiketal or the C-5 hydroxyl group to form a five-membered cyclic hemiketal. The five-membered ring is called a *furanose* because of its similarity to *furan*. Thus, fructose forms both pyranose and furanose rings.

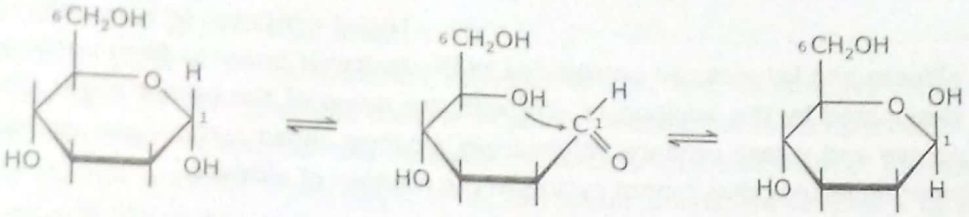


Aldopentoses such as ribose can form furanose or pyranose rings. For the five carbon sugar ribose, the pyranose form arises when the carbonyl group reacts with the terminal hydroxyl group. The carbon 5 is incorporated into the ring. If cyclization occurs between the hydroxyl group on carbon 4 and the carbonyl group then the furanose ring forms. This places the carbon 5 outside the ring.

Cyclic structure exists in two different configurational forms. If the hydroxyl on the anomeric carbon is below the plane of the ring, it is said to be in the α-position; if above the plane of the ring, it is in the β-position. These two diastereoisomers are called **anomers** and the hemiacetal or hemiketal carbon is known as *anomeric carbon*.



In glucose, the C-1 carbon atom is called the *anomeric carbon atom*, and the α and β forms are called *anomers* (as shown in the figure). An equilibrium mixture of glucose contains approximately 37% α-form and 63% β-form and less than 1% of the open chain form. The two anomers have different physical and chemical properties. For example α-D-glucose has a specific rotation of +112° whereas the β-D-glucose form has a specific rotation of +19°. When either of these pure substances is dissolved in water, the specific optical rotation of the solution slowly changes until it reaches an equilibrium value of specific rotation of +52.7°. In aqueous solution the interconversion of α- and β-forms via the open chain structure, to give an equilibrium mixture is known as **mutarotation**.



α-D-Glucose
MP = 146°C
[α] = +112°

D-Glucose

β-D-Glucose
MP = 150°C
[α] = +19°

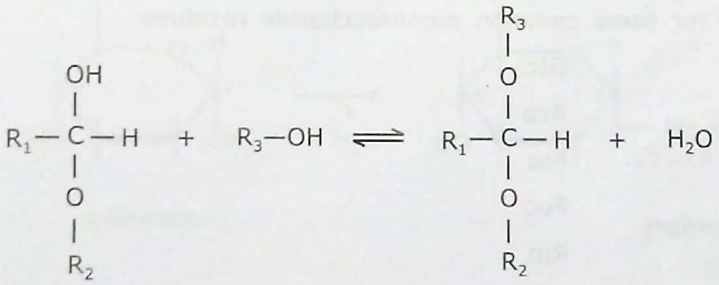
Figure 1.63 The α and β cyclic isomers of D-glucose can interconvert, with the open-chain structure as the intermediate.

The same nomenclature applies to the furanose ring form of fructose, except that α and β refer to the hydroxyl groups attached to C-2, the anomeric carbon atom.

1.8.4 Derivatives of monosaccharide

Glycosides

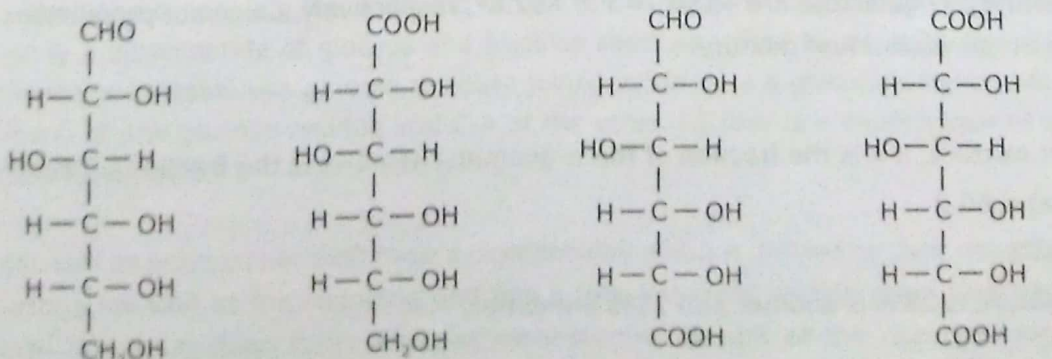
When hemiacetals react with alcohols, it forms acetals and if hemiacetal of the sugar reacts with an alcohol to form an acetal, it is known as a *glycoside*. Glycosides are formed by condensation between the hydroxyl group of the anomeric carbon of a monosaccharide, and a second compound that may or may not be another monosaccharide. If the hemiacetal portion is glucose, the resulting compound is *glucoside*; if galactose, a *galactoside*; and so on. Glycosides are widely distributed in nature. A very common glycoside is *ouabain* which inhibits the action of enzymes that pump Na^+ and K^+ ions across cell membranes. Other glycosides include antibiotics such as streptomycin.



Hemiacetal Alcohol Acetal

Sugar acids

The aldehyde group in aldoses can be oxidized to produce a class of monosaccharides called **aldonic acids** (if glucose, it is gluconic acid). One important aldonic acid is L-ascorbic acid or vitamin C. Aldoses can undergo selective oxidation also. If terminal –OH group oxidizes, it produces **uronic acid** (if glucose, it is glucuronic acid). If both the aldehyde group and the terminal –OH oxidizes then **aldaric acid** (if glucose, it is glucaric acid) is produced.



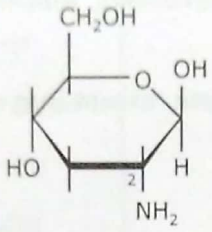
D-Glucose D-Gluconic acid D-Glucuronic acid D-Glucaric acid

Sugar alcohols

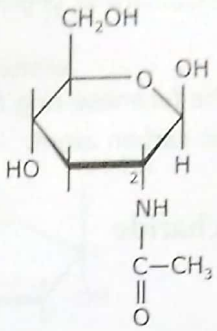
Carbonyl groups in aldoses and ketoses can be reduced to the hydroxyl group to form *sugar alcohols* or alditols. Sugar alcohols are designated by the addition of *-itol* with the name of the parent sugar. The reduction of the carbonyl group of glucose and xylose produce polyhydroxy alcohols called *sorbitol* and *xylitol*, respectively. The sugar alcohols are linear molecules that cannot cyclize in the manner of aldoses.

Amino sugars

In amino sugars, a hydroxyl group is replaced by an *amino* or an *acetylamino* group. Most often found as monomer residues in complex oligosaccharides and polysaccharides.



β-D-Glucosamine



N-Acetyl-β-D-glucosamine

N-acetylglucosamine, a glucosamine derivative, is part of many structural polymers, including those of the bacterial cell wall. Bacterial cell walls also contain a derivative of glucosamine, N-acetylmuramic acid, in which lactic acid is ether-linked to the oxygen at C-3 of N-acetylglucosamine.

Table 1.12 Abbreviations for some common monosaccharide residues

Glucose	Glc
Arabinose	Ara
Fructose	Fru
Fucose	Fuc
Ribose	Rib
Xylose	Xyl
N-Acetylgalactosamine	GalNAc
N-Acetylglucosamine (NAG)	GlcNAc
N-Acetylmuramic acid (NAM)	MurNAc

Problem

An aqueous solution of D-galactose has an $[\alpha]_D^{25}$ of +80.2° after standing for some hours. The specific rotations of pure α-D-galactose and β-D-galactose are +150.7° and +52.8°, respectively. Calculate proportions of α-D-galactose and β-D-galactose in the equilibrium mixture.

Solution

For the equilibrium mixture, if x is the fraction of the α-anomer, then 1–x is the fraction of the β-anomer and

$150.7x + 52.8(1-x) = 80.2$

$x = 0.28;\ 1-x = 0.72$

The equilibrium mixture is 28% α-anomer and 72% β-anomer.

1.8.5 Disaccharides and glycosidic bond

Disaccharides are the simplest and most common oligosaccharides. Oligosaccharides containing three or more residues are relatively rare, occurring almost entirely in plants. A *disaccharide* consists of two monosaccharides joined by an *O*-glycosidic bond. A bond formed between the anomeric carbon atom of a monosaccharide and the oxygen atom of an alcohol is called a *glycosidic bond*. Glycosidic bonds are labeled α or β depending on the anomeric configuration of the carbon involved in the glycosidic bond. For example, in **maltose** two molecules of glucose are linked by an $\alpha 1 \rightarrow 4$ glycosidic bond. The glycosidic bond forms between C-1 (the anomeric carbon) of one glucose residue and the hydroxyl oxygen atom on C-4 of the other. The configuration of the anomeric carbon atom participates in this glycosidic bond formation is α.

Similarly, **Sucrose** is a disaccharide of glucose and fructose residues joined by an $\alpha 1 \leftrightarrow 2\beta$ glycosidic bond between C-1 (the anomeric carbon) of glucose residue and C-2 (the anomeric carbon) of the fructose residue. The anomeric carbons of both monosaccharide units are involved in the glycosidic bond. The configurations of the anomeric carbon atom involved in the glycosidic bond formation are α for glucose and β for fructose. The abbreviated name of sucrose is either Glc ($\alpha 1 \leftrightarrow 2\beta$) Fru or Fru ($2\beta \leftrightarrow \alpha 1$) Glc.

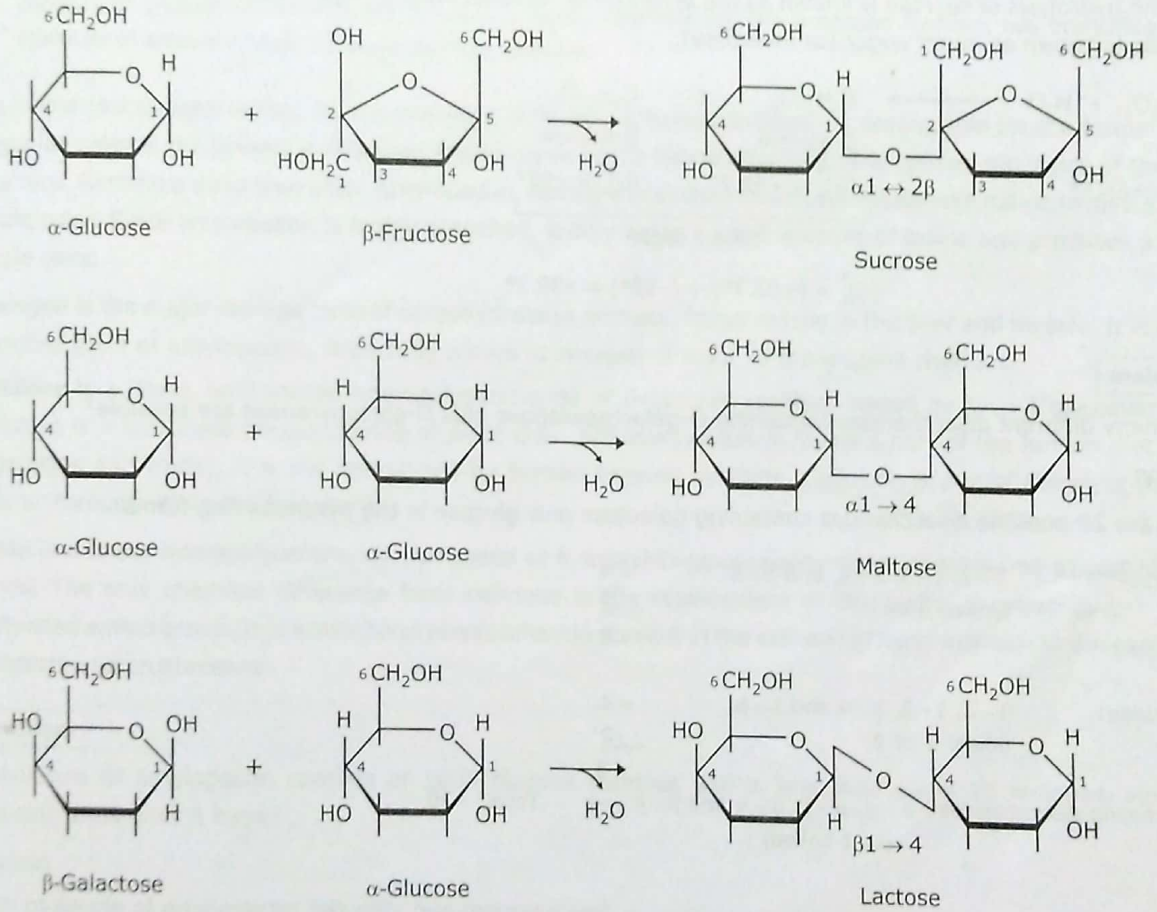


Figure 1.64 The bond connecting the anomeric carbon to the hydroxyl oxygen atom is termed a glycosidic bond. *Sucrose* is a disaccharide of glucose and fructose residues joined by $\alpha 1 \leftrightarrow 2\beta$ glycosidic bond. The disaccharide *maltose* contains two glucose residues joined by an $\alpha 1 \rightarrow 4$ glycosidic bond between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other. *Lactose* is a disaccharide of galactose and glucose residues joined by a $\beta 1 \rightarrow 4$ glycosidic bond.

Oligosaccharides (as well as polysaccharides) have a directionality which is defined by their reducing and nonreducing ends. The monosaccharide unit at the reducing end has a free anomeric carbon atom that has reducing activity because it can form the open-chain form whereas monosaccharide unit at the non-reducing end has no free anomeric carbon due to its participation in the glycosidic bond formation.

Table 1.13 Occurrence and biochemical roles of some representative disaccharides

Disaccharides	*Structure*	*Physiological role*
Sucrose	Glucose ($\alpha 1 \leftrightarrow 2\beta$) Fructose	A product of photosynthesis.
Lactose	Galactose ($\beta 1 \rightarrow 4$) Glucose	A major animal energy source.
Trehalose	Glucose ($\alpha 1 \leftrightarrow 1\alpha$) Glucose	A major circulatory sugar in insects; used for energy.
Maltose	Glucose ($\alpha 1 \rightarrow 4$) Glucose	The dimer derived from the starch and glycogen.
Cellobiose	Glucose ($\beta 1 \rightarrow 4$) Glucose	The dimer of the cellulose polymer.
Gentiobiose	Glucose ($\beta 1 \rightarrow 6$) Glucose	Constituent of plant glycosides and some polysaccharides.

Hydrolysis of sucrose

Sucrose is dextrorotatory, its specific rotation being +66.5°. Hydrolysis of sucrose with hot dilute acid yields D-glucose and D-fructose. D-glucose is also dextrorotatory, $[\alpha]_\lambda^T = +52.7°$, but D-fructose is levorotatory, $[\alpha]_\lambda^T = -92°$. Since D-fructose has a greater specific rotation than D-glucose, the resulting mixture is levorotatory. Because of this, the hydrolysis of sucrose is known as the *inversion of sucrose*, and the equimolecular mixture of glucose and fructose is known as *invert sugar* (or invertose).

$$\underset{\substack{\text{Sucrose}\\ [\alpha]_\lambda^T = +66.5°}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underbrace{\underset{\substack{\text{D-glucose}\\ [\alpha]_\lambda^T = +52.7°}}{C_6H_{12}O_6} + \underset{\substack{\text{D-fructose}\\ [\alpha]_\lambda^T = -92°}}{C_6H_{12}O_6}}_{\text{Invert sugar}}$$

$$[\alpha]_\lambda^T = (+52.7°) + (-92°) = -39.3°$$

Problem

How many different disaccharides containing D-galactopyranose plus D-glucopyranose are possible?

Solution

There are 20 possible disaccharides containing galactose plus glucose in the pyranose ring forms:

Galactosides :	1 – 2, 1 – 3, 1 – 4, and 1 – 6 Linked α or β	= 4 $\therefore \times 2$ = 8
Glucosides :	1 – 2, 1 – 3, 1 – 4, and 1 – 6 linked α or β	= 4 $\therefore \times 2$ = 8
Nonreducing disaccharides :	$\alpha - \alpha$, $\alpha - \beta$, $\beta - \alpha$ and $\beta - \beta$ (1 – 1 linked)	= 4

Total : 20

1.8.6 Polysaccharides

Polysaccharides are ubiquitous in nature. They are also called *glycans*. They can be classified into two separate groups, based on their functions: *Structural* and *storage polysaccharides*. Structural polysaccharides provide mechanical stability to cells, organs, and organisms. Storage polysaccharides serve as carbohydrate stores that release monosaccharides as required. Polysaccharides may be *homopolysaccharides* (contain only a single type of monomeric unit) or *heteropolysaccharides* (contain two or more different kinds of monomeric units).

Homopolysaccharides

Starch is a branched chain of D-glucose units. It is the storage form of glucose in plants. It contains a mixture of *amylose* and *amylopectin*. *Amylopectin* is a branched polymer of α-D-glucose with $\alpha 1 \rightarrow 4$-glycosidic bonds and with

$\alpha 1 \rightarrow 6$ branching points that occur at intervals of approximately 25 to 30 α-D-glucose residues. *Amylose* is a linear unbranched polymer of α-D-glucose units in a repeating sequence of $\alpha 1 \rightarrow 4$-glycosidic bonds.

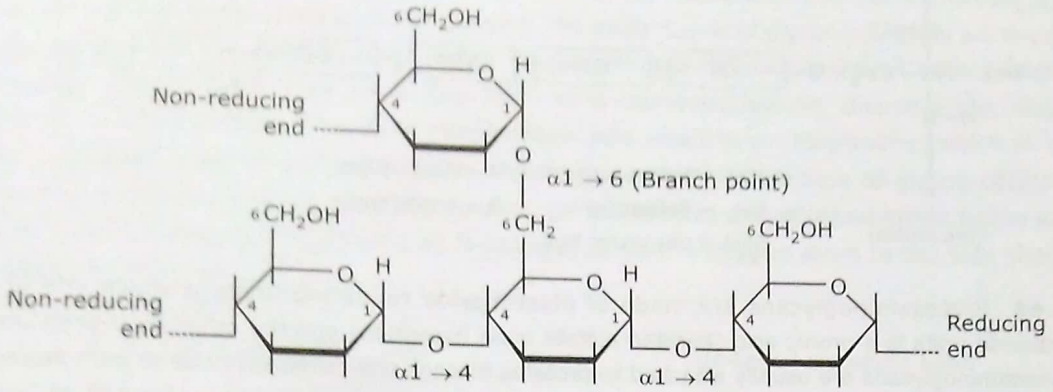


Figure 1.65 The storage polysaccharide in plants is starch. *Amylose,* the unbranched fraction of starch, consists of glucose residues joined by $\alpha 1 \rightarrow 4$ bond. *Amylopectin,* the branched fraction, has branching at intervals of approximately 25 to 30 glucose residues.

The *iodine test* is used to test for the presence of starch. Amylose in starch is responsible for the formation of a deep blue color in the presence of iodine. The amylose forms helical structure. The iodines slip inside of the helical structure, forming a deep blue color. Amylopectin, having a branched structure, reacts with iodine to give a reddish purple color. Since amylopectin is highly branched, it only binds a small amount of iodine and produces a reddish purple color.

Glycogen is the major storage form of carbohydrate in animals, found mostly in the liver and muscle. It is a highly branched form of amylopectin; branching occurs at intervals of eight to ten glucose residues.

Cellulose is a linear, unbranched homopolysaccharide of D-glucose residues joined by $\beta 1 \rightarrow 4$ glycosidic bonds. Cellulose is a structural polysaccharide of plant cells. Although cellulose forms a part of the human diet (e.g. in vegetables and fruits), it is not hydrolyzed by human enzyme systems. *Cellulose is one of the most abundant organic compounds in the biosphere.*

Chitin is a linear homopolysaccharide composed of N-acetyl-D-glucosamine residues joined by $\beta 1 \rightarrow 4$ glycosidic bonds. The only chemical difference from cellulose is the replacement of the hydroxyl group at C-2 with an acetylated amino group. It is a structural polysaccharide present in the cell wall of fungi and also in the exoskeleton of insects and crustaceans.

Problem

A molecule of amylopectin consists of 1000 glucose residues and is branched every 25 residues. How many reducing ends does it have?

Solution

Each molecule of amylopectin has only one reducing end.

Heteropolysaccharides

Glycosaminoglycans are negatively charged, unbranched heteropolysaccharide composed of repeating disaccharide units, [Acidic sugar — amino sugar]$_n$. Amino sugar is always either N-acetylglucosamine or N-acetylgalactosamine and the acidic sugar in most cases is a uronic acid, usually glucuronic acid. The simplest glycosaminoglycan hyaluronan (hyaluronic acid) contains alternating residues of D-glucuronic acid and N-acetylglucosamine. Chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, dermatan sulfate and hyaluronate are the major glycosaminoglycans. These polysaccharides are unique to animals and bacteria and are not found in plants. With the exception of hyaluronic acid, all the GAGs contain sulfate groups, either as O-esters or as N-sulfate. All of the glycosaminoglycans except *hyaluronic acid* are found covalently attached to protein forming **proteoglycan**.

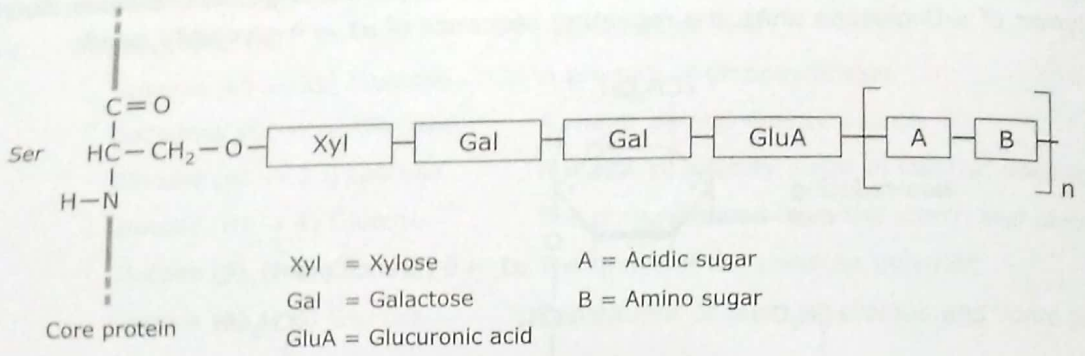


Figure 1.66 Glycosaminoglycans are made of disaccharide repeating units in which one of the two monosaccharide units is a uronic acid (keratan sulfate is an exception) and the other an N-acetylated amino sugar. Glycosaminoglycans are usually attached to proteins through link tetrasaccharide to form *proteoglycans*. In a typical link tetrasaccharide, the xylose residue at the reducing end of the linker is joined by its anomeric carbon to the hydroxyl of the Ser residue.

The polysaccharide chain is mainly assembled on the core protein in the Golgi bodies. A specific *link tetrasaccharide* is first assembled on a serine residue. In most cases, it is not clear how the serine residue is selected. The rest of the GAG chain, consisting mainly of a repeating disaccharide unit, is then synthesized, with one sugar residue being added at a time. The covalent bond forms between Ser residue of the protein and xylose sugar residue of link tetrasaccharide is O-glycosidic bond.

Table 1.14 Characteristics of Glycosaminoglycans (GAGs)

GAG	*Sugar A* (acidic sugar)	*Sugar B* (amino sugar)	*Sulfates*	*Links to protein*
Hyaluronic acid	D-Glucuronic acid	N-acetyl-D-glucosamine	–	–
Chondroitin sulfate	D-Glucuronic acid	N-acetyl-D-galactosamine	+	+
Dermatan sulfate	D-Glucuronic acid *or* L-iduronic acid	N-acetyl-D-galactosamine	+	+
Heparan sulfate	D-Glucuronic acid *or* L-iduronic acid	N-acetyl-D-glucosamine	+	+
Heparin	D-Glucuronic acid *or* L-iduronic acid	N-acetyl-D-glucosamine	+	+
Keratan sulfate	D-Galactose (non acidic sugar)	N-acetyl-D-glucosamine	+	+

A **peptidoglycan** or *murein* (present in eubacterial cell walls) consists of a glycosaminoglycan formed by alternating residues of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). NAG and NAM are joined through $\beta 1 \rightarrow 4$ glycosidic bond. A peptide chain of four alternating D- and L- amino acids called *tetrapeptide* is connected to the carboxyl group of the NAM.

Table 1.15 Important polysaccharides

Name	*Monomer*	*Main linkages*	*Branch linkages*
Murein	NAG + NAM	$\beta 1 \rightarrow 4$	—
Dextran	D-glucose	$\alpha 1 \rightarrow 6$	$\alpha 1 \rightarrow 2$; $\alpha 1 \rightarrow 3$ and $\alpha 1 \rightarrow 4$
Cellulose	D-glucose	$\beta 1 \rightarrow 4$	—
Starch (Amylose)	D-glucose	$\alpha 1 \rightarrow 4$	—
(Amylopectin)	D-glucose	$\alpha 1 \rightarrow 4$	$\alpha 1 \rightarrow 6$
Inulin	D-fructose	$\beta 2 \rightarrow 1$	—
Chitin	N-acetyl-glucosamine	$\beta 1 \rightarrow 4$	—
Glycogen	D-glucose	$\alpha 1 \rightarrow 4$	$\alpha 1 \rightarrow 6$
Callose	D-glucose	$\beta 1 \rightarrow 3$	—

1.8.7 Glycoproteins

Various types of compound consisting of carbohydrates covalently linked with non-carbohydrates constituent are classified under the general name called *glycoconjugates*. The major types of glycoconjugates are the glycoproteins, peptidoglycans, glycolipids and lipopolysaccharides. Carbohydrates covalently linked with proteins are called *glycoproteins*. The carbohydrate may be in the form of a monosaccharide, disaccharide, oligosaccharide, polysaccharide, or their derivatives. The term *glycoprotein* also includes *proteoglycans*, which in the past were considered as a separate class of glycoconjugates. Proteoglycans are a subclass of glycoproteins in which the carbohydrates are glycosaminoglycans. In glycoproteins, carbohydrates are attached either to the amide nitrogen atom in the side chain of asparagine (termed as N-*linkage*) or to the oxygen atom in the side chain of serine or threonine (termed as O-*linkage*).

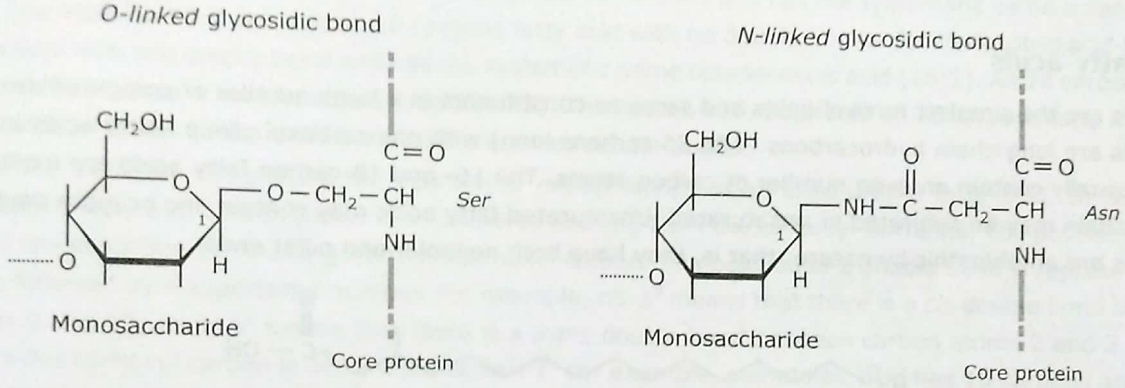


Figure 1.67 Carbohydrates are covalently attached to many different proteins to form *glycoproteins*. Carbohydrates are attached either to the amide nitrogen atom in the side chain of asparagine (termed an N-*linkage*) or to the oxygen atom in the side chain of serine or threonine (termed an O-*linkage*).

1.8.8 Reducing and non–reducing sugar

Sugars capable of reducing ferric or cupric ion are called **reducing sugar**. A reducing sugar is any sugar that either has an aldehyde group or is capable of forming one in solution through isomerisation. This functional group allows the sugar to act as a reducing agent.

All monosaccharides whether aldoses and ketoses, in their hemiacetal and hemiketal form are reducing sugars. All disaccharides formed from head to tail condensation are also reducing sugar i.e. disaccharides except *sucrose, trehalose* are reducing sugars. All reducing sugars undergo mutarotation in aqueous solution.

Sugars like sucrose, trehalose not capable of reducing ferric or cupric ion are called **non-reducing sugar**. In sucrose and trehalose, anomeric carbon becomes involved in a glycosidic bond. So they do not contain free anomeric carbon atoms. Sucrose and trehalose are therefore not a reducing sugar, and have no reducing end. So it cannot be oxidized by cupric or ferric ion. In describing disaccharides or polysaccharides, the end of a chain that has a free anomeric carbon (i.e. is not involved in a glycosidic bond) is commonly called the reducing end of the chain.

1.9 Lipids

Biological lipids are a chemically diverse group of organic compounds which are insoluble or only poorly soluble in water. They are readily soluble in nonpolar solvents such as ether, chloroform, or benzene. The hydrophobic nature of lipids is due to the predominance of hydrocarbon chains ($-CH_2-CH_2-CH_2-$) in their structures. Unlike the proteins, nucleic acids, and polysaccharides, lipids are not polymers.

Functions

Biological lipids have diverse functions. The four general functions of biological lipids have been identified.

- They serve as a storage form of metabolic fuel.
- They serve as a transport form of metabolic fuel.
- They provide the structural components of membranes.
- They have protective functions in bacteria, plants, insects, and vertebrates, serving as a part of the outer coating between the body of the organism and the environment.

Apart from the general functions biological lipids serve as *pigments* (carotene), *hormones* (vitamin D derivatives, sex hormones), *signaling molecules* (eicosanoids, phosphatidylinositol derivatives), *cofactors* (vitamin K), *detergents* (bile salt) and many other specialized functions.

1.9.1 Fatty acids

Fatty acids are the simplest form of lipids and serve as constituents in a large number of complex forms of lipids. Fatty acids are long-chain hydrocarbons (4 to 36 carbons long) with one carboxyl group. Fatty acids in biological systems usually contain an *even* number of carbon atoms. The 16- and 18-carbon fatty acids are most common. The alkyl chain may be saturated or unsaturated. Unsaturated fatty acids may contain one or more double bonds. Fatty acids are amphipathic by nature; that is, they have both nonpolar and polar ends.

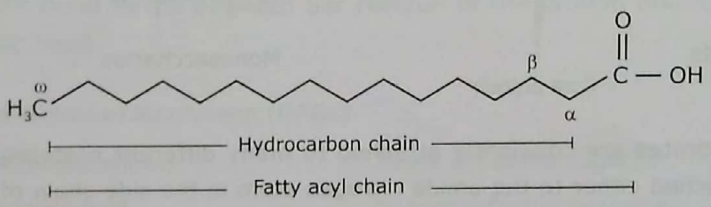


Figure 1.68 Structure of fatty acid.

By an older system, in a fatty acid second carbon is referred to as the α-carbon, third carbon as the β-carbon and the end methyl carbon as the ω-carbon.

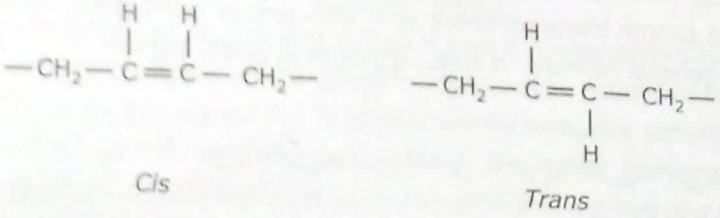
Table 1.16 Predominant naturally occurring fatty acids

Common name	Systematic name	Carbon atoms : Double bonds
Saturated fatty acid		
Lauric acid	Dodec*anoic* acid	12 : 0
Myristic acid	Tetradec*anoic* acid	14 : 0
Palmitic acid	Hexadec*anoic* acid	16 : 0
Stearic acid	Octadec*anoic* acid	18 : 0
Arachidic acid	Eicos*anoic* acid	20 : 0
Unsaturated fatty acid		
Palmitoleic acid	*cis*-Δ^9-Hexadec*enoic* acid	16 : 1
Oleic acid	*cis*-Δ^9-Octadec*enoic* acid	18 : 1
Linoleic acid	all *cis*-$\Delta^{9,12}$-Octadeca*dienoic* acid	18 : 2
Linolenic acid	all *cis*-$\Delta^{9,12,15}$-Octadeca*trienoic* acid	18 : 3
Arachidonic acid	all *cis*-$\Delta^{5,8,11,14}$-Eicosa*tetraenoic* acid	20 : 4

Saturated and unsaturated fatty acids

Saturated fatty acids have no double bonds in the chain. Their general formula is $CH_3—(CH_2)_n—COOH$ where n specifies the number of methylene groups between the methyl and carboxyl carbons. Examples of predominant saturated fatty acids are lauric, myristic, palmitic and others.

Unsaturated fatty acids have one or more double bonds, and called *monounsaturated* or *polyunsaturated* respectively. The double bonds in naturally occurring fatty acids are *generally* in a *cis* as opposed to a *trans* configuration. The double bonds of polyunsaturated fatty acids are almost never conjugated (alternating single and double bonds).



Cis *Trans*

The *systematic name* includes the number of carbons, the number of double bonds, and the positions of the double bonds. For example, stearic acid (a saturated fatty acid) has 18 carbons and has the systematic name octade*canoic acid* (18:0). The notation 18:0 denotes an 18 carbons fatty acid with no double bonds. Similarly, oleic acid is an 18 carbons fatty acid with one double bond and has the systematic name octadec*enoic* acid (18:1). An 18 carbons fatty acid with two double bonds is octadeca*dienoic* acid (18:2). The notation 18:1 denotes an 18 carbons fatty acid with one double bond, whereas 18:2 signifies that there are two double bonds.

Two systems are used for designating the position of double bonds in an unsaturated fatty acid. In *carboxyl-reference system,* fatty acid carbon atoms are numbered starting from the carboxyl terminus. The positions of the double bonds are described by counting from the carboxyl carbon. The position of a double bond is represented by the symbol Δ followed by a superscript number. For example, *cis*-Δ^9 means that there is a *cis* double bond between carbon atoms 9 and 10; *trans*-Δ^2 means that there is a *trans* double bond between carbon atoms 2 and 3. In this nomenclature the carboxyl carbon is designated carbon 1. For example, palmitoleic acid has 16 carbons and has a double bond between carbons 9 and 10. It is designated as 16:1:Δ^9.

In *omega-reference system*, the position of the double bond are indicated relative to the omega carbon (i.e. number 1 is assigned to the omega carbon). For example, ω6 indicates a double bond on the sixth carbon counting from the ω-carbon.

Essential fatty acids

Essential fatty acids are those fatty acids which are not synthesized by animals and must be obtained from diet. Linoleate and linolenate are the two essential fatty acids for humans and other animals. Humans lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain. Hence, humans cannot synthesize linoleate and linolenate. Fatty acids that can be endogenously synthesized are termed as *nonessential* fatty acids. They are nonessential in the sense that they do not have to be obligatorily obtained from diet.

Melting point of fatty acids

The melting point of fatty acids depends on chain length, presence or absence of double bond and number of double bonds (i.e. degree of unsaturation). The longer the chain length, the higher the melting point, and the greater the number of double bonds, the lower the melting point. The presence of double bonds makes unsaturated chain more rigid. As a result, unsaturated chains cannot pack themselves in crystals efficiently and densely as saturated chain, so, they have a lower melting point as compared to saturated fatty acids. Similarly, the unsaturated fatty acids with *cis* configuration have lower melting points than the unsaturated fatty acids with *trans* configuration.

Problem

Why unsaturated fatty acids have low melting points?

Solution

The presence of double bonds makes unsaturated chain more rigid. As a result, unsaturated chains cannot pack themselves in crystals efficiently and densely as saturated chain, so, they have lower melting point as compared to saturated fatty acids.

1.9.2 Triacylglycerol and Wax

Triacylglycerols (also called triglycerides) are triesters of fatty acids and glycerol. They are composed of three fatty acids and a glycerol molecule. Triacylglycerols are of two types – *simple* and *mixed* type. Those containing a single kind of fatty acids are called simple triacylglycerols and with two or more different kinds of fatty acids are called mixed triacylglycerols. The general formula of triacylglycerol is given below:

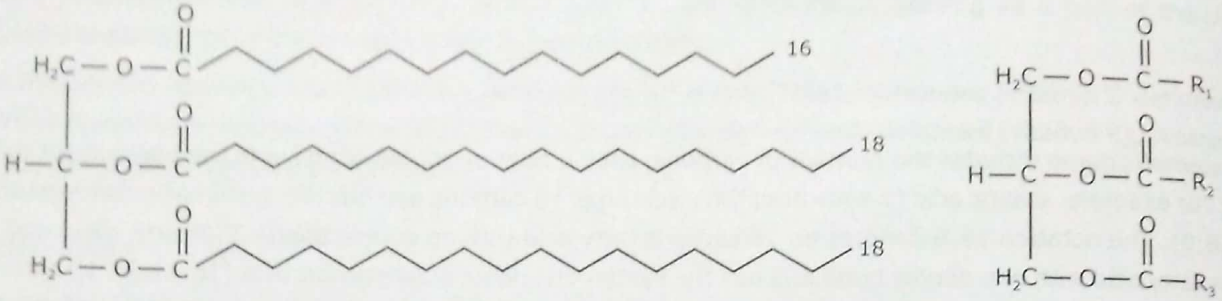


Figure 1.69 General structure of triacylglycerol.

Triacylglycerols are nonpolar, hydrophobic in nature and a major form of stored lipids. Because triacylglycerols have no charge (i.e. the carboxyl group of each fatty acid is joined to glycerol through a covalent bond), they are sometimes referred to as **neutral lipid**. Triacylglycerol molecules contain fatty acids of varying lengths, which may be unsaturated or saturated. Triacylglycerols can be distinguished as fat and oil on the basis of physical state at room temperature. *Fats,* which are solid at room temperature, contain a large proportion of saturated fatty acids. *Oils* are liquid at room temperature because of their relatively high unsaturated fatty acid content.

Hydrolysis of triacylglycerols with alkalis such as NaOH or KOH is called **saponification**. Saponification yields salts of free fatty acids (termed *soap*) and glycerol. The number of milligrams of KOH required to saponify one-gram of fat is known as *saponification number*. The saponification number measures the average molecular weight of fats. Similarly, the number of grams of iodine that can be added to 100g sample of fat or oil is called *iodine number*, which is used to determine the degree of unsaturation (i.e. extent of unsaturation).

Waxes

Natural waxes are typically esters of fatty acids and long chain alcohols. They are formed by esterification of long chain fatty acids (saturated and unsaturated) and high molecular weight monohydroxy alcohols (C14 to C36). Waxes are biosynthesized by many plants or animals. The best known animal wax is beeswax. Triacontanoylpalmitate (an ester of palmitic acid with the alcohol triacontanol) is the major component of beeswax.

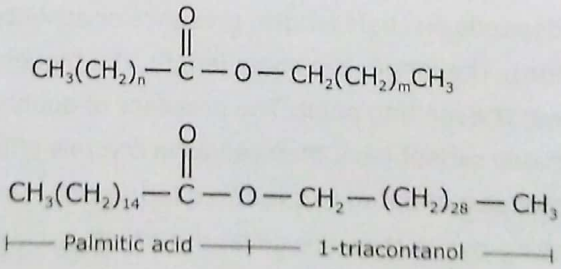


Figure 1.70 The general structure of a wax.

1.9.3 Phospholipids

A *phospholipid* is an amphipathic molecule constructed from four components: fatty acids, a platform to which the fatty acids are attached, a phosphate and an alcohol attached to the phosphate. The platform on which phospholipids are built may be *glycerol* or *sphingosine*.

Phosphoglycerides

Phospholipids derived from glycerol are called *phosphoglycerides* (or glycerophospholipids). A phosphoglyceride consists of *a* glycerol molecule, two fatty acids, a phosphate, and an alcohol (e.g. choline). Phosphoglycerides are the most numerous phospholipid molecules found in cell membranes.

In phosphoglycerides, the hydroxyl groups at C-1 and C-2 of glycerol are esterified to the carboxyl groups of the two fatty acid chains. The C-3 hydroxyl group of the glycerol backbone is esterified to phosphoric acid. When no further additions are made, the resulting compound is **phosphatidic acid**, the simplest phosphoglyceride. Phosphatidic acids are found in small amount in most natural systems. The major phosphoglycerides are derived from phosphatidic acid by the formation of an ester bond between the phosphate group and the hydroxyl group of one of several alcohols. The common alcohol moieties of phosphoglycerides are serine, ethanolamine, choline, glycerol, and the inositol. If the alcohol is choline, the phosphoglyceride molecule is called *phosphatidylcholine* (also referred to as *lecithin*) and if serine then it is called *phosphotidylserine*.

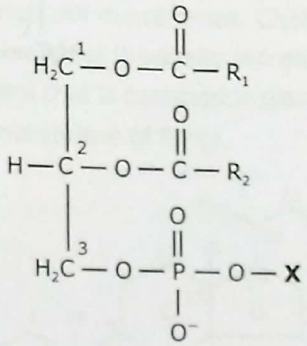


Figure 1.71 The parent compound for glycerophospholipid.

Name of X–OH	*Name of phospholipid*	*Net charge (at pH 7)*
Water (X=H)	Phosphatidic acid	−1
Choline	Phosphatidylcholine (Lecithin)	0
Ethanolamine	Phosphatidylethanolamine (Cephalin)	0
Serine	Phosphatidylserine	−1
Glycerol	Phosphatidylglycerol	−1
Phosphatidylglycerol	Diphosphatidylglycerol (Cardiolipin)	−2
Inositol	Phosphatidylinositol	−1

Ether glycerophospholipids

Ether glycerophospholipids possess an ether linkage instead of an acyl group at the C-1 position of glycerol. One very common example of ether glycerophospholipid found in mammals is *platelet activating factor* or PAF. PAF is a potent signal molecule that causes platelet aggregation and dilation of blood vessels. In ether glycerophospholipids, the ether-linked chain may be saturated or may contain a double bond between C-1 and C-2. Ether glycerophospholipids in which the alkyl moiety is cis-α,β-unsaturated is termed as **plasmalogen**.

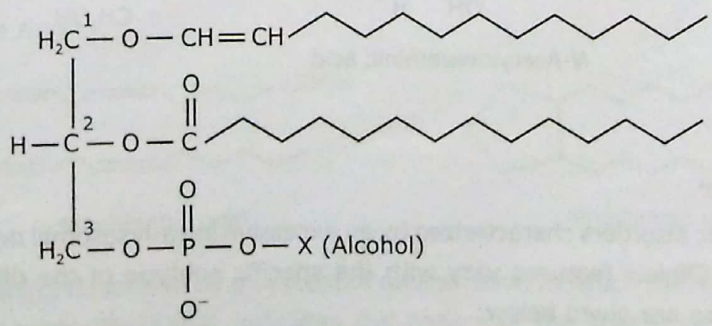
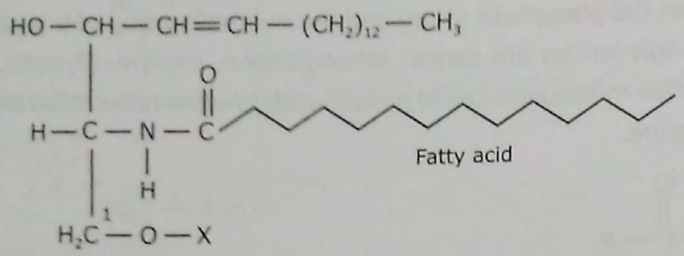


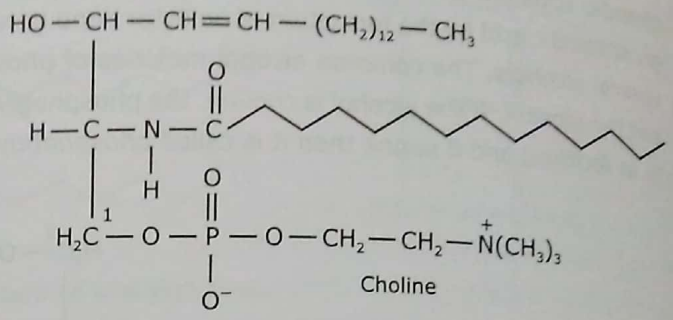
Figure 1.72 Structure of plasmalogen. One hydrocarbon group is linked to the C–1 position of glycerol as a vinyl ether.

Sphingophospholipids

Phospholipids derived from sphingosine are called **sphingophospholipids**. A sphingophospholipid contains a 18-carbon amino alcohol called sphingosine instead of glycerol. A fatty acid is joined to a sphingosine via an amide linkage to form a *ceramide*. Ceramide is the structural parent of all sphingolipids. The addition of phosphorylated alcohol to the C–1 position of sphingosine, leads to a variety of sphingophospholipids. One important example is *sphingomyelin*, in which a phosphocholine group is attached to the C–1 hydroxyl. Sphingomyelins are major constituent in the nervous tissue of higher animals.



If X = H then it is called *ceramide*

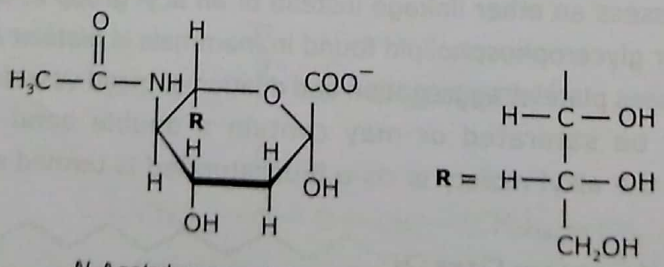


Sphingomyelin

1.9.4 Glycolipids

Lipid containing saccharide groups go under the general name of glycolipids. The glycosphingolipid is the most important type of glycolipid and constitute third major class of membrane lipids. In glycosphingolipid head group contains one or more sugars connected directly to the —OH at C–1 of the ceramide moiety (do not contain phosphate). It can be:

- *Cerebroside* : Have a single monosaccharide linked to ceramide.
- *Globoside* : With oligosaccharide linked to ceramide.
- *Gangliosides* : Globoside that contains complex oligosaccharides as their polar head groups with one or more residues of *N-acetylneuraminic acid*, a sialic acid. Sialic acid gives gangliosides the negative charge at pH 7. More than 60 different gangliosides have been characterized. Gangliosides with one sialic acid residue are in the GM (M for mono-) series, those with two are in the GD (D for di-) series, and so on. The normal function of gangliosides is not known.



N-Acetylneuraminic acid

Sphingolipid storage disease

A group of inherited metabolic disorders characterized by an excessive intra-lysosomal deposition of sphingoglycolipids and sphingophospholipids. Clinical features vary with the specific subtype of the disease. Examples of selected sphingolipid storage diseases are given below:

Table 1.17 Sphingolipid storage diseases

Disease	*Accumulating sphingolipid*	*Enzyme deficiency*
Tay-Sachs	Ganglioside GM2	β-Hexosaminidase A
Gaucher's	Glucocerebroside	β-Glucosidase
Niemann Pick	Sphingomyelin	Sphingomyelinase
Fabry's	Trihexosylceramide	α–Galactosidase A
Krabbe's	Deacylated galactocerebroside	Galactocerebrosidase

1.9.5 Steroid

Steroids are complex derivatives of triterpenes. Each type of steroid is composed of four fused rings called *steroids nucleus*. A *sterol* is a class of steroid characterized by a hydroxyl group at C-3. Cholesterol is an example of the sterol and an essential component in animal cell membranes. Cholesterol acts as a precursor for the biosynthesis of all *steroid hormones* and *bile salts*. Cholesterol is usually stored within cells as a fatty acid ester. Cholesterol is not found in plants and fungi. Types of sterol that is common in plants are stigmasterol, sitosterol and campesterol. Ergosterol is a sterol present in the cell membrane of fungi.

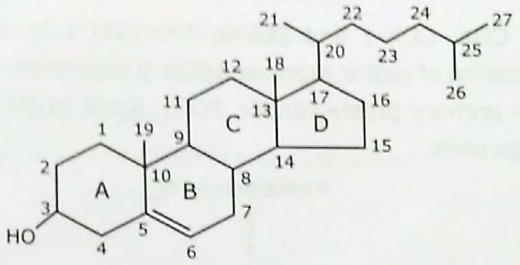


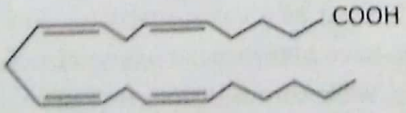
Figure 1.73 Structure of cholesterol.

1.9.6 Eicosanoid

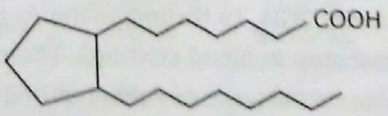
Eicosanoids are a family of very potent biological signaling molecules that act as short range messengers. It includes *prostanoids* and *leukotrienes*. It is known as eicosanoids because of their common origin from 20 carbons polyunsaturated fatty acids, eicosanoic acids, particularly arachidonic acid. Eicosanoids are potent hormones and act locally instead of being transported in the blood to act on cells of other tissues or organs.

Prostanoids and leukotrienes – two major groups of eicosanoids - formed via two distinct pathways, the *cyclooxygenase* and *lipoxygenase* pathways. Prostanoids, which include the *prostaglandins, prostacyclin* and *thromboxanes*, are formed through the *cyclooxygenase* pathway (cyclic pathway) and leukotrienes come from the *lipoxygenase* pathway (linear pathway).

Prostaglandins were first identified in human semen. Almost all mammalian cells except RBC produce prostaglandins. Prostaglandins are all derivatives of the hypothetical C_{20} fatty acid **prostanoic acid** in which carbon atoms 8 to 12 comprise a cyclopentane ring.



Arachidonic acid



Prostanoic acid

Individual prostaglandins are described by a system of abbreviation in which the name prostaglandin is designated PG, followed by a third letter (A–I) that indicates the nature of the substituents on the cyclopentane ring. A numerical subscript indicates the total number of double bonds in the aliphatic chains.

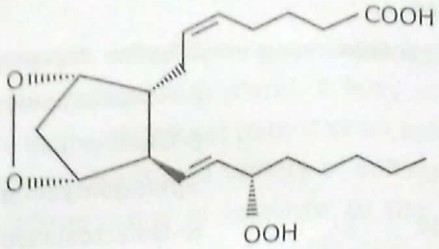
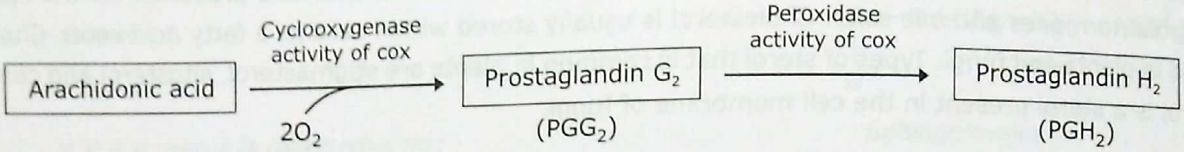


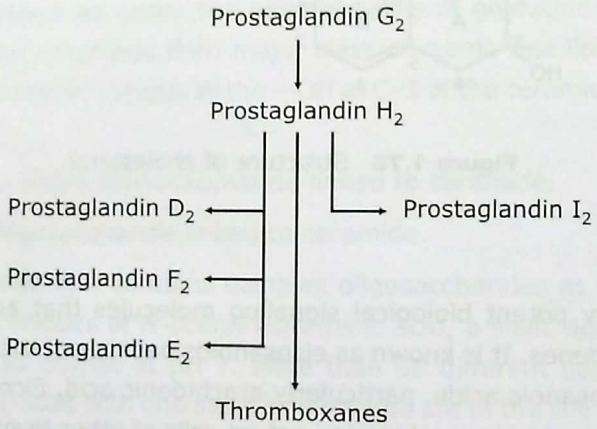
Figure 1.74 Prostaglandin G_2 (PGG_2).

Synthesis of prostaglandins

In humans, the most prevalent prostaglandin precursor is *arachidonic acid.* Prostaglandins are synthesized as shown in the figure from arachidonic acid in a metabolic pathway that begins with plasma membrane phospholipids. The enzyme *cyclooxygenase* (cox), also called *prostaglandin H_2 synthase* catalyzes the introduction of oxygen and the cyclization of the carbon chain of arachidonic acid in the region of the double-bond positions at C-8 and C-11.



Mammals have two isozymes of COX, COX-1 and COX-2. The COX-1 is responsible for the synthesis of the prostaglandins that regulate the secretion of gastric mucin and COX-2 for the prostaglandins that mediate inflammation and pain. The biosynthesis of the primary prostaglandin, PGG_2, leads to the biosynthesis of a large number of chemically related secondary compounds.



Prostaglandins perform diverse functions. They stimulate uterine contraction, lowering blood pressure, vasodilation, and inflammation and pain. **Aspirin** (acetyl salicylate) used as an analgesic (pain-relieving), antipyretic (fever-reducing), and anti-inflammatory agent inhibits the synthesis of prostaglandins. It *irreversibly* inhibits cyclooxygenase by acetylating a *Ser* residue and blocking the enzymes active site. This inhibition of cyclooxygenase blocks the synthesis of prostaglandins, which in turn reduces the inflammatory response. **Ibuprofen** is also used as an analgesic and anti-inflammatory agent. It is a non-steroidal anti-inflammatory drug (NSAID), acts as a reversible competitive inhibitor of cyclooxygenase.

Thromboxanes are derived from prostaglandins in the blood platelets (thrombocytes) by enzyme *thromboxane synthase* which converts PGH_2 to thromboxane A_2 (TxA_2). They act as a vasoconstrictor and stimulator of platelet aggregation (an initial step in blood clotting). Thromboxanes have heterocyclic oxane structures. Thromboxane A (TxA) contains an unstable bicyclic oxygenated ring structure, while thromboxane B (TxB) has a stable oxane ring.

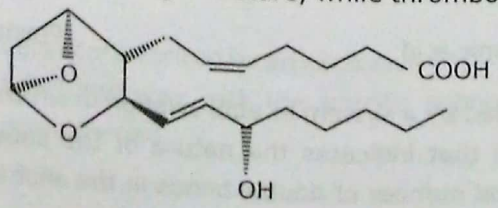


Figure 1.75 Structure of TxA_2.

Prostacyclins, the endogenous antagonist of thromboxane A_2, are generated from prostaglandin H_2 by a prostacyclin synthase that is particularly abundant in endothelial cells. The biosynthesis of prostacyclin starts with COX-1 and is inhibited by aspirin.

Leukotrienes are hydroxy fatty acid derivatives of arachidonic acid and do not contain a ring structure. Leukotrienes are distinguished by containing a conjugated triene double-bond arrangement. They are involved in chemotaxis, inflammation, and allergic reactions.

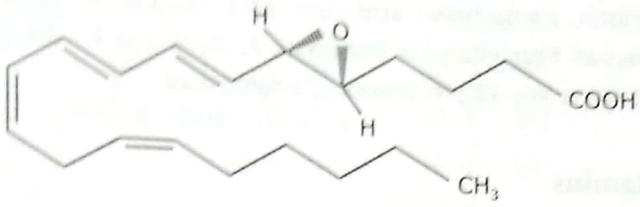


Figure 1.76 Structure of leukotriene A.

Table 1.18 Biological effects of eicosanoids

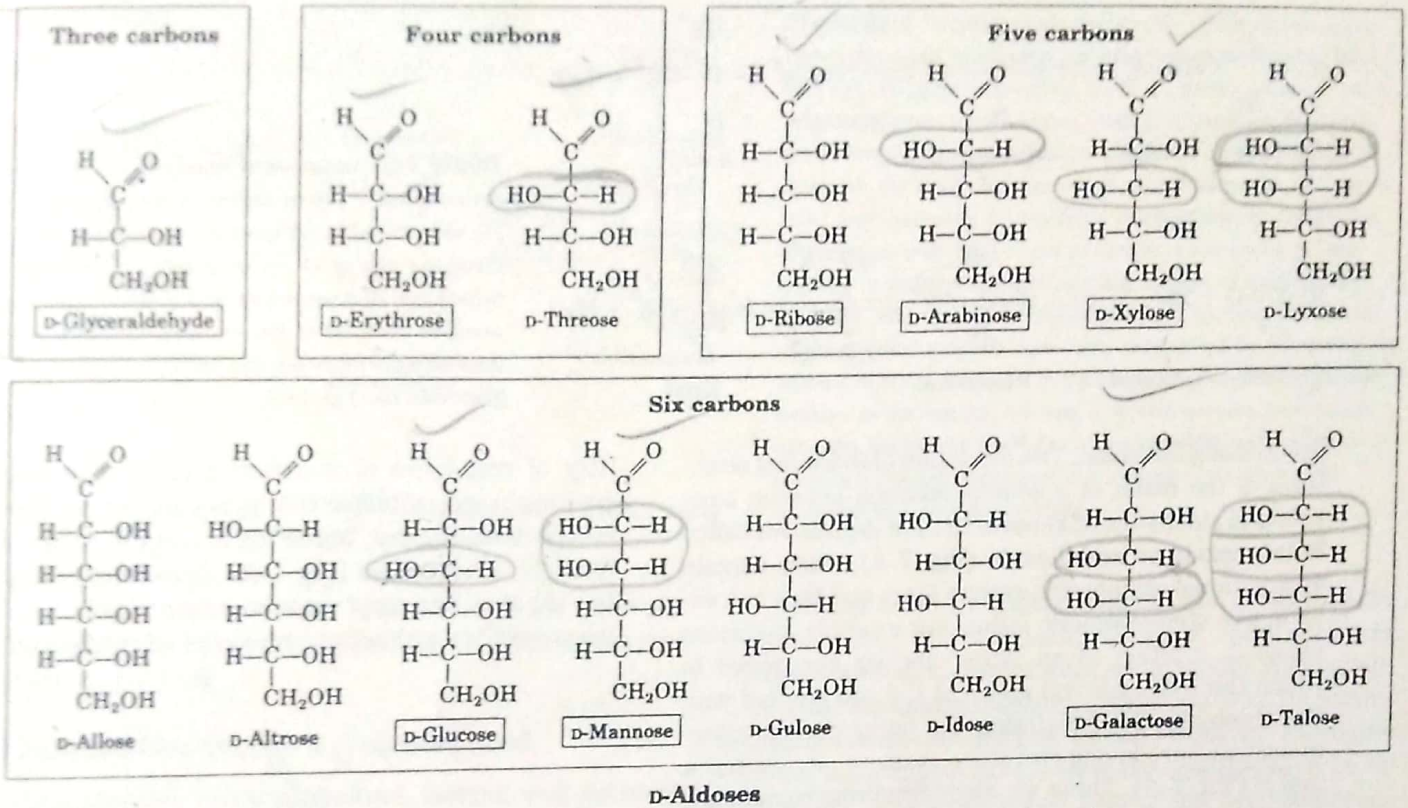
Type	*Major functions*
Prostaglandins	Mediation of inflammatory response
	Regulation of nerve transmission
	Inhibition of gastric secretion
	Sensitization to pain
	Stimulation of smooth muscle contraction
Thromboxanes	Platelet aggregation
	Aorta constriction
Prostacyclins	Thromboxane antagonists
Leukotrienes	Bronchoconstriction
	Leukotaxis

1.9.7 Plasma lipoproteins

Triacylglycerols, phospholipids, cholesterol and cholesterol esters are transported in human plasma in association with proteins as **lipoproteins**. Blood plasma contains a number of soluble *lipoproteins,* which are classified, according to their densities, into four major types. These lipid-protein complexes function as a lipid transport system because isolated lipids are insoluble in blood. There are four basic types of lipoproteins in human blood : *chylomicrons, very low density lipoproteins* (VLDL), *low density lipoproteins* (LDL), and *high density lipoproteins* (HDL). A lipoprotein contains a core of neutral lipids, which includes triacylglyerols and cholesterol esters. This core is coated with a monolayer of phospholipids in which proteins (called *apolipoprotein*) and cholesterol are embedded.

Table 1.19 Some properties of major classes of human plasma lipoproteins

Lipoprotein	*Density (g/mL)*	*Protein*	*Phospho-lipids*	*Free cholesterol*	*Cholesterol esters*	*Triacyl-glycerols*	*Apolipo-protein*
Chylomicrons	<1.006	1.5–2.5	7–9	1–3	3–5	85	A-I, C-I, B-48
VLDL	0.95–1.006	5–10	15–20	5–10	10–15	50	B-100, C-I, C-II
LDL	1.006–1.063	20–25	15–20	7–10	35–40	7–10	B-100
HDL	1.063–1.210	50–55	20–25	3–4	15	3–4	A-I, A-II, C-I



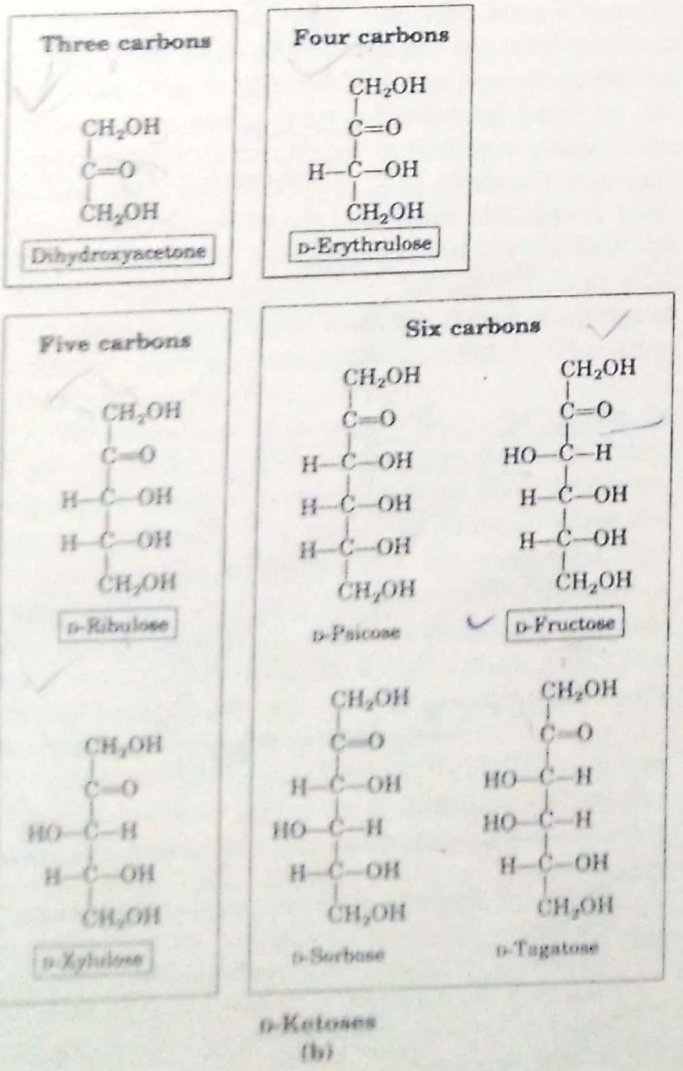


FIGURE 7-3 Aldoses and ketoses. The series of **(a)** D-aldoses and **(b)** D-ketoses having from three to six carbon atoms, shown as projection formulas. The carbon atoms in red are chiral centers. In all these D isomers, the chiral carbon *most distant from the carbonyl carbon* has the same configuration as the chiral carbon in D-glyceraldehyde. The sugars named in boxes are the most common in nature; you will encounter these again in this and later chapters.

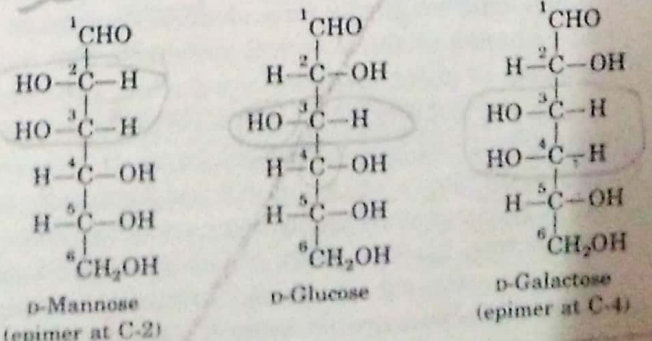


FIGURE 7-4 Epimers. D-Glucose and two of its epimers are shown as projection formulas. Each epimer differs from D-glucose in the configuration at one chiral center (shaded pink).