1. Carbohydrates

Carbohydrates (hydrates of carbon) are naturally occurring organic compounds having general formula $C_x(H_2O)_y$, which are constantly produced and assimilated in nature and take part in many important biochemical reactions. However, number of compounds having general formula as that of carbohydrate has been synthesized. There are many compounds, which are carbohydrate by chemical behaviour but do not confirm to the general formula. For example, 2–deoxyribose, $C_5H_{10}O_4$ is a carbohydrate by chemical reaction but do not confirm to the general formula. All the carbohydrates are optically active. So, carbohydrates are now defined as the polyhydroxy carbonyl compounds α compounds, which give polyhydroxy carbonyl compounds on hydrolysis. Carbohydrates are polyhydroxy aldehydes or ketones that have an OH bonded to each of the carbon other than the C=O group.

1. Classification of Carbohydrates

Chemically carbohydrates are polyhydroxy aldehydes or ketones or substances that yield these on hydrolysis.

(A) On The Basis of Functional Groups:

Carbohydrates with an ***aldehydic*** group are known as ***aldoses*** and those with ***ketonic*** group are called ***ketoses***. The number of carbon atoms in a carbohydrate (monomer unit) is indicated by prefix tri, tetra, penta etc. The name of the carbohydrate containing aldehydic group end in "– ose" while suffix for ketonic group is "– ulose".

For example, 4–carbon containing aldehydic carbohydrate is tetrose. [$OHCH_2(CHOH)_2CHO$].

It contains two chiral carbon and the name of optical isomers are D–erythrose, D–threose, L–erythrose, L–threose.

4–carbon containing ketonic carbohydrate is called keto tetrose [$OHCH_2.CO.CHOH.CH_2OH$].

It contains 1–chiral carbon and name of optical isomers are D– & L–erythrulose.

(b) On the basis of number of monosaccharide units (saccharide is latin name for sugar) carbohydrate yields on hydrolysis.

Carbohydrates are divided into two main classes, sugar and polysaccharides.

Sugars are crystalline substance with a sweet taste and soluble in water. Polysaccharides are more complex than sugar, their molecular weight being far greater, are non–crystalline substances which are not sweet and are insoluble or less soluble in water.

Examples of sugar

(i) Monosaccharides:

These are sugars, which cannot be hydrolysed into smaller molecules. Their general formula is $C_nH_{2n}O_n$ (there are exceptions like deoxyribose) where n is 2–10, and the most important are the pentoses and hexoses. Glucose, Fructose, Ribose are examples of monosaccharides.

(ii) Oligosaccharides:

These yield 2–10 monosaccharide units on hydrolysis. For example, disaccharides (two monosaccharide units, which may, or may not, be the same); trisaccharides (three monosaccharide units) etc.

(iii) Polysaccharides:

Polyaccharides are carbohydrates, which yield a large number of monosaccharide units on hydrolysis. The most widely known polysaccharide have the general formula $(C_6H_{10}O_5)_n$ e.g. starch, cellulose, etc,; a group of polysaccharides which are not so widely spread in nature is pentosans, $(C_5H_8O_4)_n$.

2. Stereochemistry of Carbohydrates

2.1 Fischer Formula For Compounds Containing One Chiral Carbon

All the saccharides are optically active, hence their three–dimensional depiction become important to distinguish between enantiomers. This could be best done with the help Fischer formula. Let us understand the process for writing the correct stereochemical formula on the plane of paper. Following are the three–dimensional structures of two enantiomers of aldotriose (glyceraldehyde).

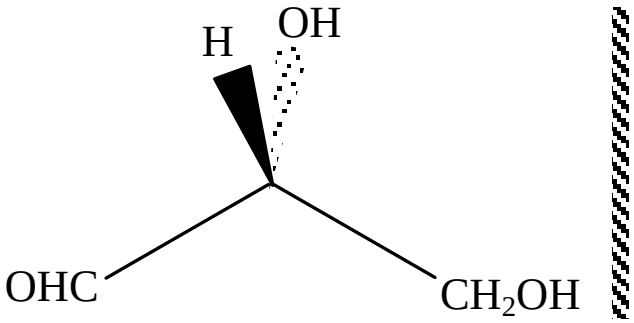


Fig. 2.1

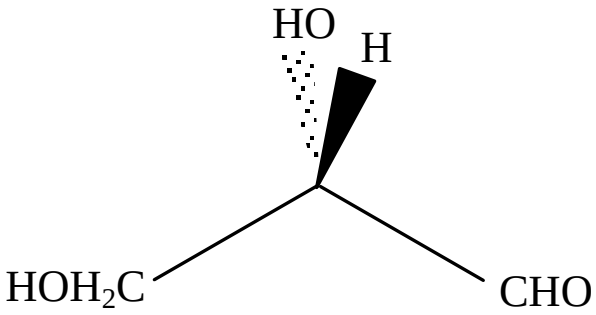


Fig. 2.2

Solid wedge represents groups coming out of plane of paper

Broken wedge represents groups going behind the plane of paper

Solid line represents groups on the plane of paper

For writing correct Fischer formula for compounds, imagine chiral centre being fixed some where in your torso. Stretch your one leg in front of you and pull the other one behind and stretch your arms so that lines joining your palms are perpendicular to line joining your two foot.

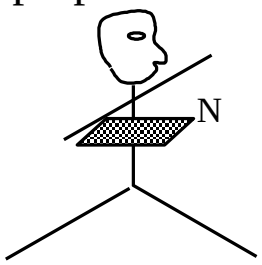


Fig. 2.3

Four groups around tetrahedral carbon will take position of your hands and feet. Atom in place of your front leg should go at top of vertical line in Fisher formula. While towards the rear feet should go to bottom position of vertical line and atom towards left and right hand should go to left and right side of horizontal line. Now writing the Fischer formula for fig. 2.1 and 2.2 you will get fig. (2.5) and fig (2.6) respectively.

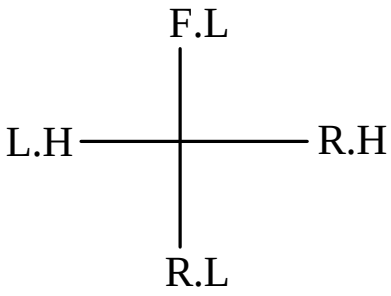


Fig. 2.4

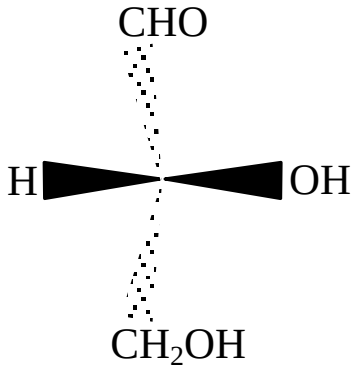


Fig. 2.5

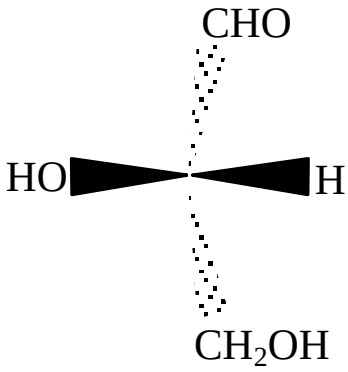


Fig. 2.6

On the Fischer formula cross section of vertical and horizontal line represent chiral centre – horizontal line show group coming out of plane and vertical line going down the paper.

Place Fisher formula of two compounds side by side and perform all possible manipulation which are allowed and if two formulas become the same they represent the formula for same compound. But, if the formulas are mirror images, they represent enantiomers.

The Fischer formula for this compound shown in fig 2.1 and fig 2.2 is fig 2.7 and 2.8 respectively.

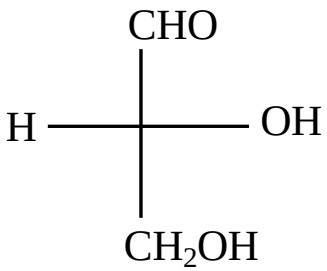


Fig. 2.7

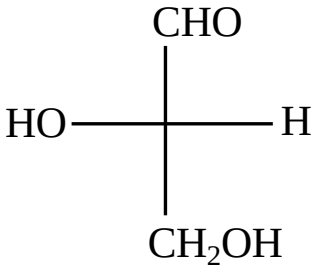


Fig. 2.8

Note:

(i) In the Fischer formula, two bonds above the plane of paper are shown by horizontal line and two bonds below the plane shown by vertical line.

(ii) The point of intersection of horizontal and vertical line represents chiral carbon.

(iii) If Fischer formula for a compound is rotated by 90°, the rotated formula represents mirror image of original compound, hence Fischer formula should not be rotated by 90°. However 180° rotation is permissible.

(iv) Keeping position of one group in Fischer formula fixed, we can rotate other three in clockwise or anticlockwise direction. This manipulation has no effect and manipulated Fischer formula is still representing original molecule.

(v) Two interchanges on Fischer formula do not affect the formula while one interchange will give formula for mirror image.

2.2 Fischer Formula of Compound Containing Many Chiral Carbon

For a chiral carbon containing four asymmetric carbon as shown in figure.

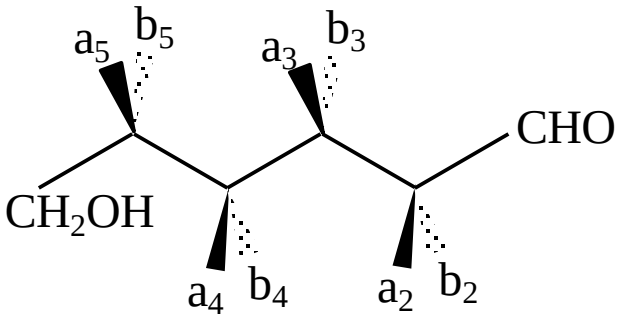


Fig. 2.9

Fischer formula for the above compound is

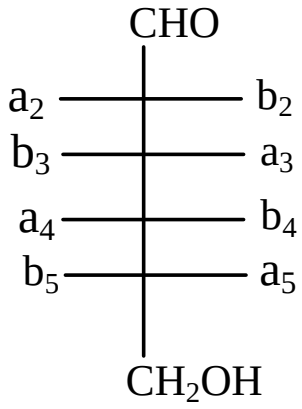


Fig. 2.10

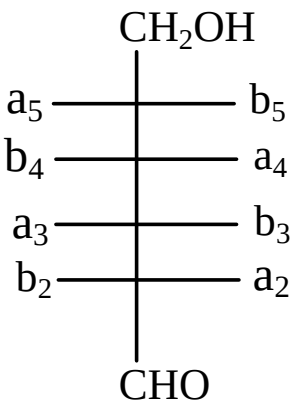


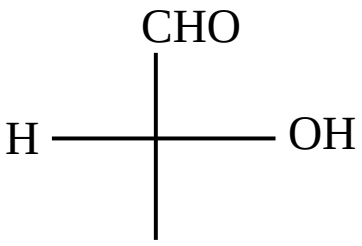
Fig. 2.11

Note that the Fischer formula is written in such a way that numbering of the carbon atom start at the top. It can also be written by keeping CHO at bottom position. Fig 2.10 when rotated by 180° give fig. 2.11.

3. Relative Configurations (D/L Notation)

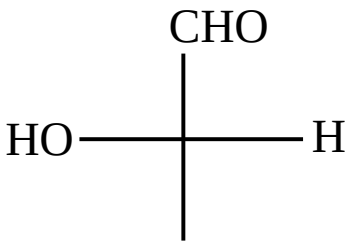
It was difficult to establish absolute configurations of chiral molecules. Until very recently, there was no authentic method of determination of absolute configurations. For example, it was known for certainty that glyceraldehyde show optical isomerism, one isomer is dextrorotatry while other is leavorotatry and the two possible structure of enantiomers are shown in fig I and II.

But there is no method, which can establish weather fig I is dexorotatory (+) or fig. II represents dextrorotatory isomer.



D–glyceraldehyde

Fig. 3.1



L–glyceraldehyde

Fig. 3.2

This problem was resolved by arbitrarily taking one structure to be D–isomer and its structure was fixed as fig 9.1. In this structure, the OH group on the ***highest number stereo centre carbon is*** on right hand side in Fischer formula and the formula is written in such a way that highest priority carbon remains at the top in Fischer representation. The mirror image of D–isomer is called L–isomer.

The D–glyceraldehyde is chosen to be dexorotatory isomer of glyceraldehyde and in this way configuration of stereoisomers is arbitrarily fixed. All the optically active compounds, which can be derived from D–glyceraldehyde, which do not involve reaction at highest number stereo centre carbon is given configuration D.

Now, with sophisticated scientific techniques it is possible to determine absolute configuration of many optically active compounds. But D/L notation is still used extensively in carbohydrate and amino acid chemistry.

The compound $C_6H_{12}O_6$ as shown in figure (11) is a aldohexose. The Fischer formula for the compound and IUPAC numbering of the compound is also shown below. Note that the highest number stereo centre carbon is 5 and CHO is given number 1. For predicting relative configuration, Fischer formula is written in such a way that CHO group remains at top of Fischer formula. Now the OH on C_5 is towards right, hence the compound is D–isomer.

Compound of the formula $HOCH_2.CHOH.CHOH.CHOH.CHOH.CHO$ contains four asymmetric carbon and total number of optical isomers is $2^4 = 16$. 8 of the 16 isomers will be D–isomer and remaining 8 will be L–isomer.

The compound shown in figure is D–iodose and it can be obtained from D–glyceraldehyde provided the synthesis do not involve reaction at highest number stereo centre carbon.

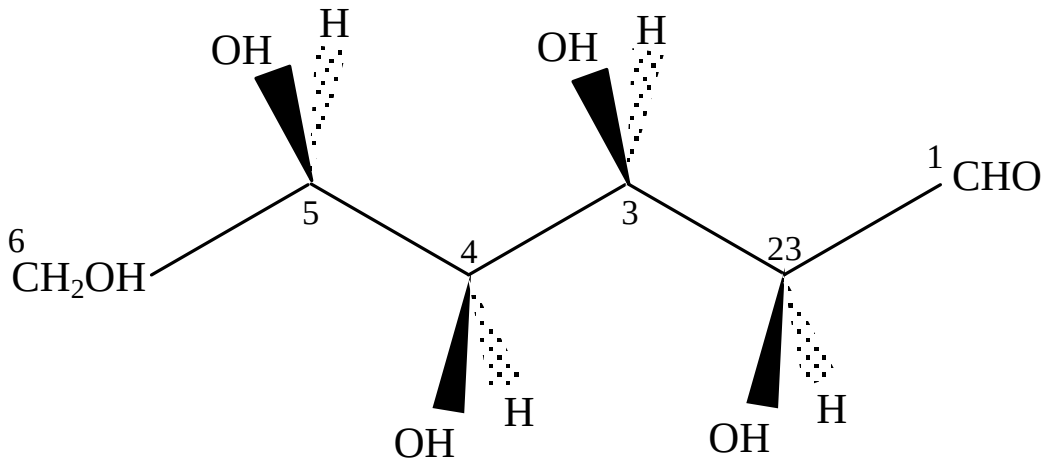


Fig. 3.3

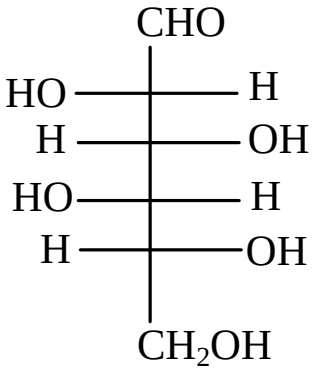


Fig. 3.4

4. Haworth Projection

Many of monosaccharides form cyclic structures. The actual structure is almost planar and can be represented by Haworth projection, which is a way of depicting three–dimensional cyclic structure. Let us understand the Haworth projection with the help of cyclic structure of D–glucose.

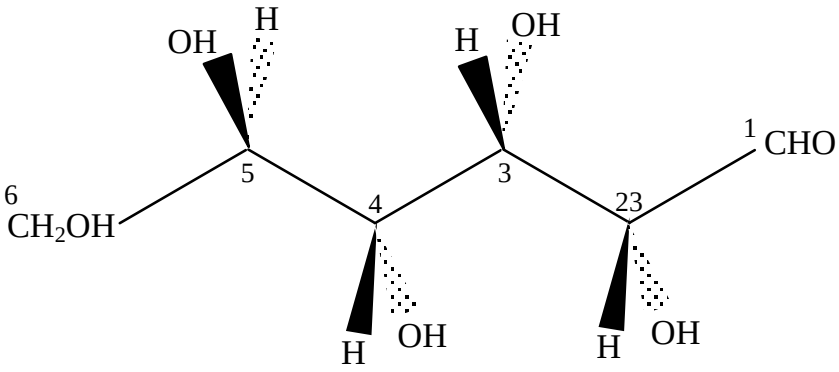


Fig. 4.1

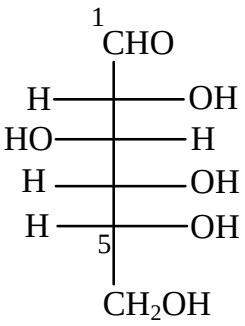


Fig. 4.2

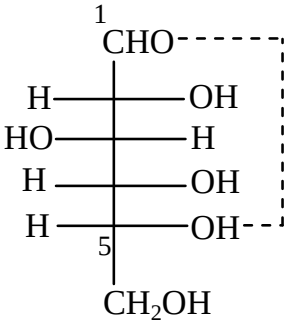
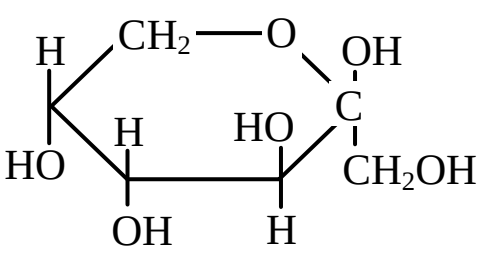
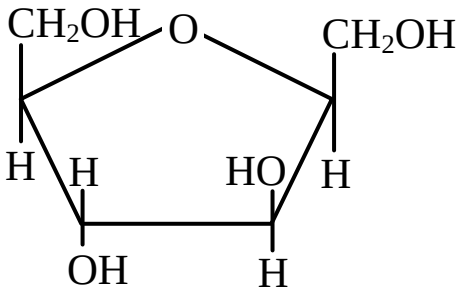


Fig. 4.3

in free state D–Fructose exist as a six membered ring or as pyranose ring. However in combined state as a component of di–saccharides it exist in the furanose forms (five membered ring).



Pysanose



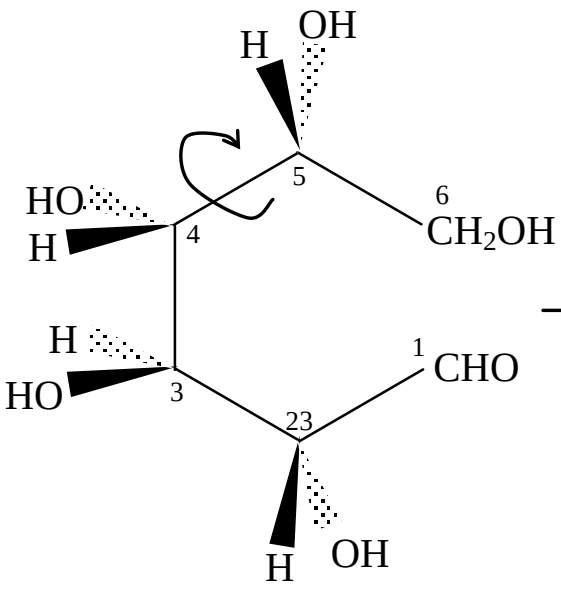


Fig. 4.4

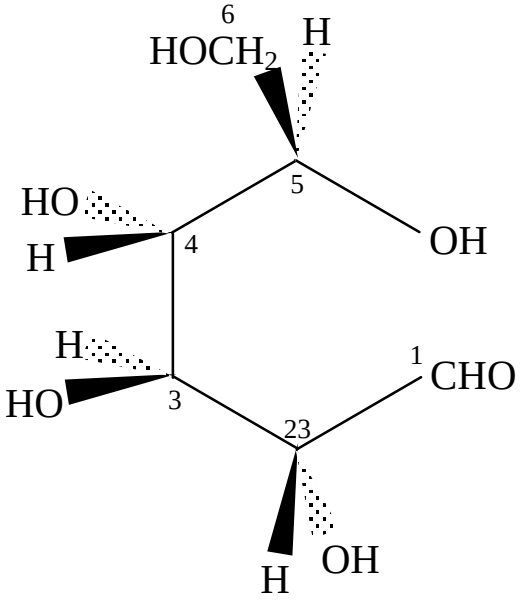


Fig. 4.5

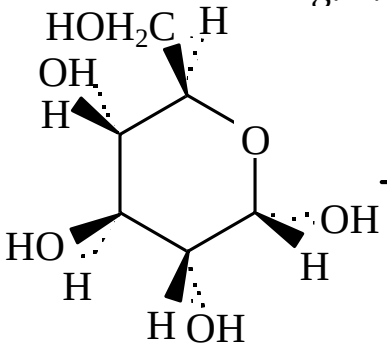


Fig. 4.6

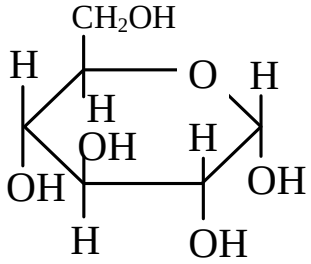


Fig. 4.7

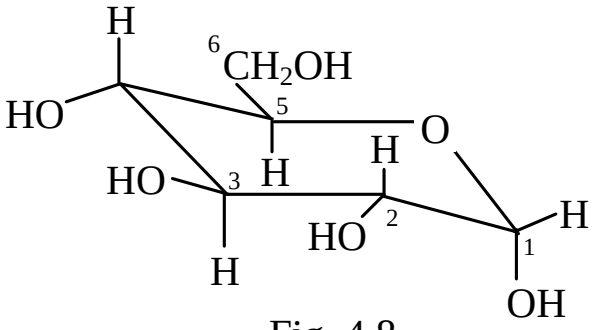


Fig. 4.8

For glucose to form ring structure, the OH at C–5 must interact with CHO and involve in a reaction characteristic of aldehyde, (Fig. 4.1) Hemiacetal and acetal formation. In cyclization, reaction proceeds to Hemiacetal state. For OH at C5 to interact with CHO, the glucose molecule must coil as shown in (Fig. 4.4) Now C–5 is close to CHO. Notice that position of OH at 3, 5–positions is opposite to that of open chain zigzag structure. Now, for –OH at C–5 to involve in acetal formation it must come near CHO, for

this C4–C5 must be rotated by 120° to attain configuration shown in (Fig. 4.4). Notice that $-CH_2OH$ group is now above the plane of paper (Fig. 4.5).

Haworth formula for cyclic glucose is shown in fig. 4.7. Compare it with actual structure of cyclic glucose. (α–D–glucopyranoside). Here pyranoside stands for 6 membered ring containing O, α stands for configuration of C–1OH. If it is towards left hand side in Fischer formula (or if it is same side of highest stereo centre OH) the configuration is α–D.

5. Reactions of Aldoses

5.1 Mild Oxidation

Mild oxidation of aldoses gives aldonic acid. Br_2 water or alkaline solution of iodine oxidises only the aldehydic group to give aldonic acids.

5.2 Strong Oxidation

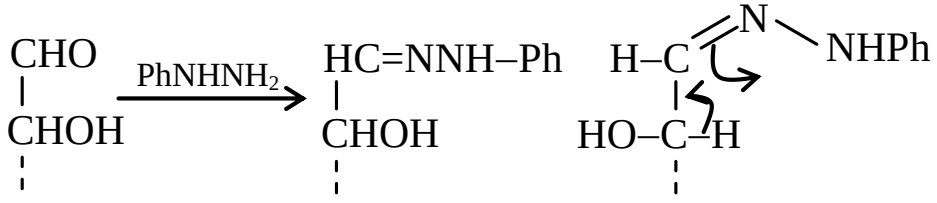
Strong oxidation of aldoses oxidise both –CHO group and terminal $-CH_2OH$ group into –COOH to give aldaric acid.

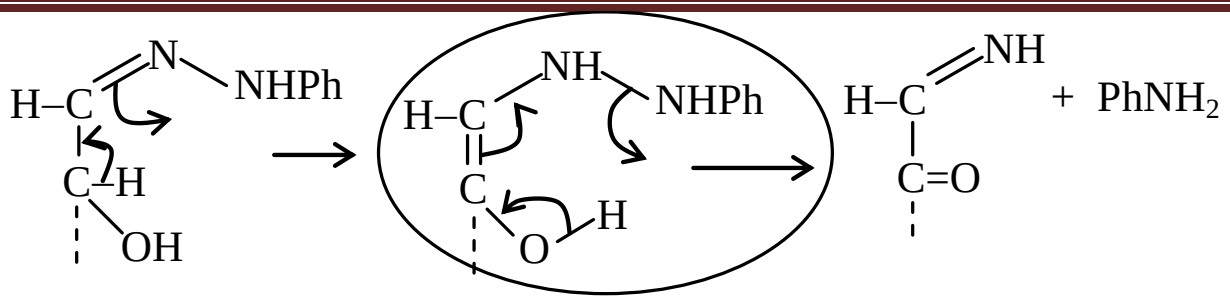
5.3 Reduction of Sugar

Sugar can be reduced into corresponding alcohols by variety of reducing agents like high pressure catalytic hydrogenation (Ni/H_2), $NaBH_4$, (iii) Na/Hg (iv) electrolytic reduction in acidic medium.

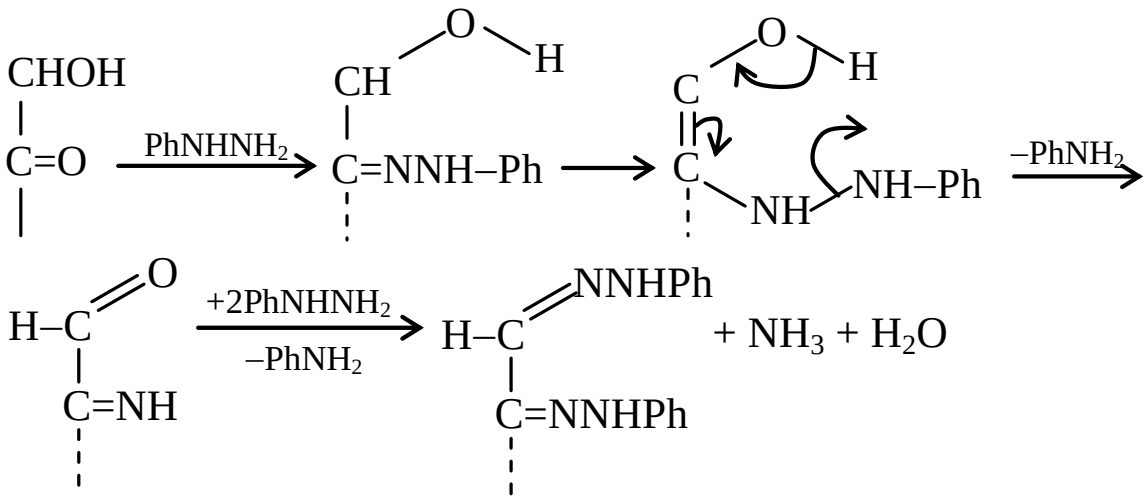
5.4 Reaction of Aldose And Ketose With Phenyl Hydrazine

Aldose and ketose both react with phenyl hydrazine (excess) to form osazones, which contain two phenylhydrazone group and also give aniline and ammonia. The overall reaction may be represented as.





Ketoses (for example, Fructose) also react with phenyl hydrazine in similar way



Only C–1 and C–2 in glucose and fructose are involved in osazone formation and the reaction do not run through out the chain. This is because osazone formed is stabilised by chelation.

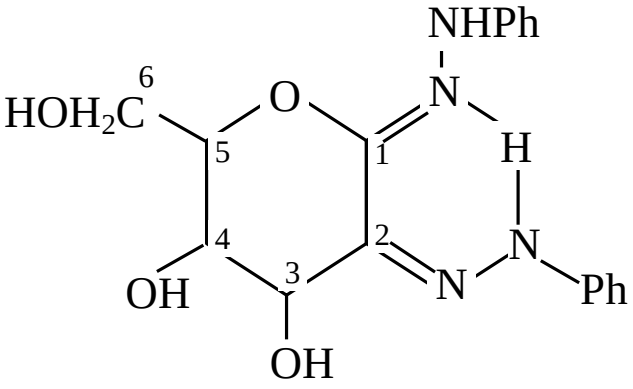
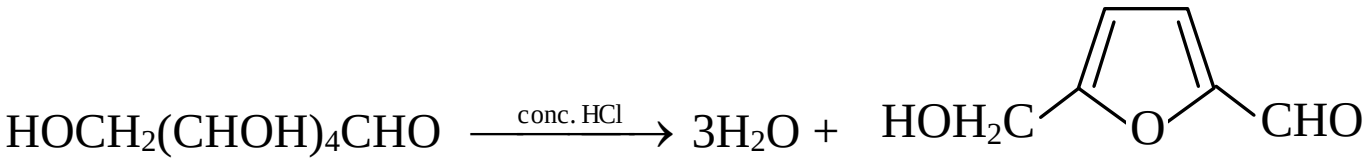


Fig. 5.1

Glucose and fructose both form same osazone. This means configuration of glucose and fructose is similar at all the carbons except C–1 and C–2.

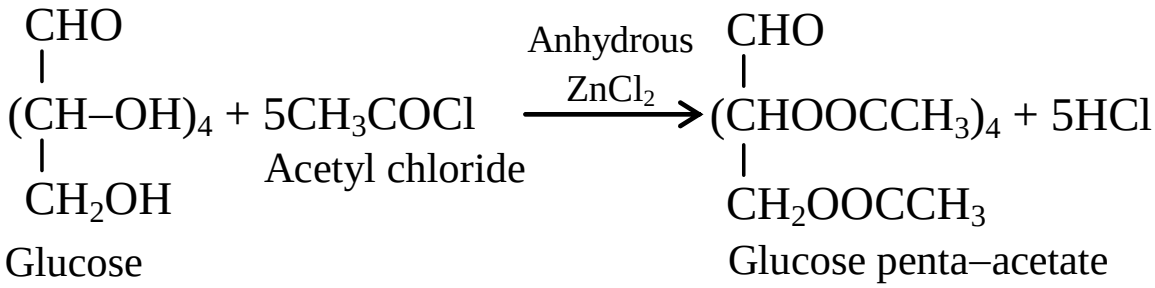
5.5 Reaction With Concentrated HCl

This reaction is only given by aldohexoses and aldopentoses. When aldohexoses are treated with conc. HCl (or H_2SO_4), they first give hydroxymethyl furfural, which then decomposes into other products.



5.6 Reaction With Acid Chlorides And Acid Anhydrides

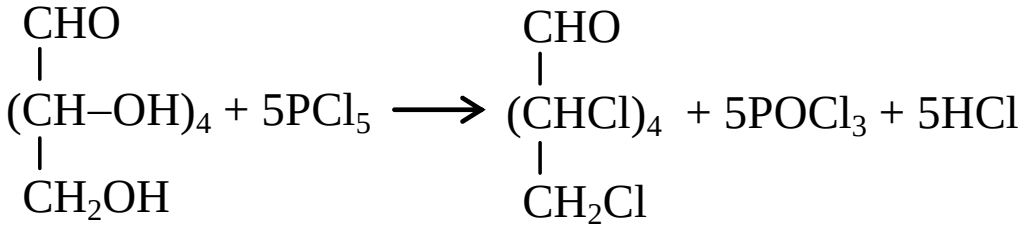
When monosaccharides are acylated with acetyl chloride in presence of anhydrous $ZnCl_2$, all the hydroxyl groups are acylated. For example, glucose gives a penta–acetate.



Glucose — Glucose penta–acetate

5.7 Reaction With PCl_5

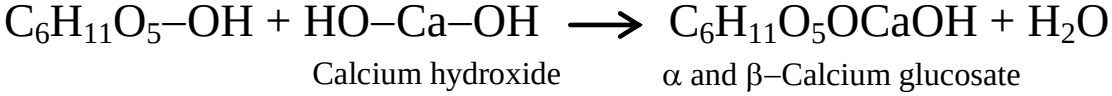
When treated with PCl_5, all the hydroxyl groups are chlorinated.



Glucose — Penta–chloroglucose (Glucose penta–chloride)

5.8 Reaction With Metallic Hydroxides

Glucose reacts as a weak acid and on reaction with calcium hydroxide to form calcium gluocosate.



Calcium hydroxide — α and β–Calcium glucosate

5.9 Formation of Glycosides

Glucose reacts with methyl alcohol in presence of dry HCl to form α and β–methyl glycoside of glucose. The reaction takes place only on OH of hemi–acetylic carbon. Other hydroxyl groups are unreactive.

To methylate all the OH groups, methylating agent used is dimethyl sulphate.

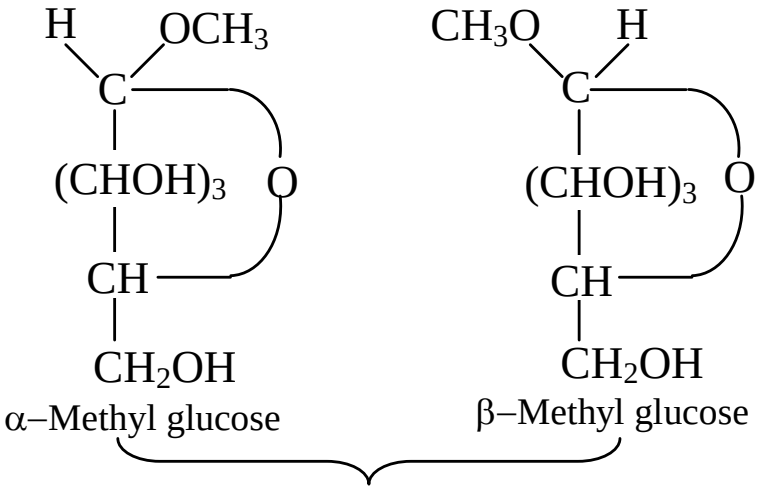


Fig. 5.2

5.10. Reaction of Carbonyl Group (Aldehydic Group)

Glucose is reduced by sodium–amalgam in presence of acid into corresponding hydroxyl compound, **sorbitol**.

$$\underset{\text{Glucose}}{CH_2OH(CHOH)_4CHO} + 2H \xrightarrow[H_2O]{Na–Hg} \underset{\text{Sorbitol}}{CH_2OH(CHOH)_4CH_2OH}$$

5.11 Reaction With HCN

HCN reacts with glucose to form an addition product, glucose cyanohydrin.

$$\underset{\text{Glucose}}{CH_2OH(CHOH)_4CHO} + HCN \longrightarrow \underset{\text{Glucose cyanohydrin}}{CH_2OH(CHOH)_4CH\begin{matrix}CN\\OH\end{matrix}}$$

5.12 Reaction With HIO_4

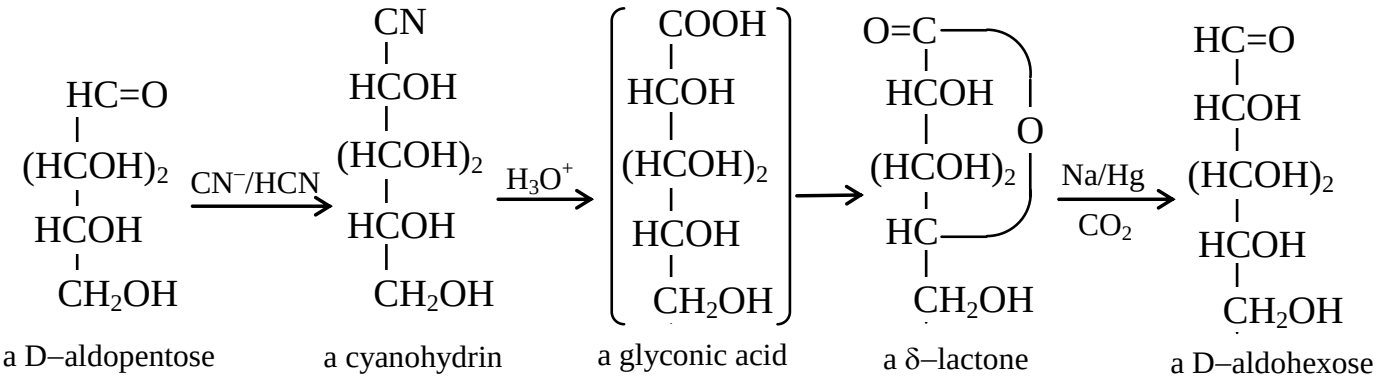
Glucose consumes five moles of HIO_4 to give five moles of formic acid and one mole of formaldehyde. Fructose consumes five moles of HIO_4 to

give three moles of formic acid, two moles of formaldehyde and one mole of CO_2.

6. Method of Accending Sugar Series

Kiliani Fischer Synthesis

The aldoses can be stepped up with the help of following sequence of reactions. Aldose is treated with NaCN /HCN (cyanohydrin formation) and hydrolysed producing aldonic acid. Theoretically, one isomer of an aldose produces two diastereomeric aldonic acid, however one of this is obtained in greater yield in practice. The aldonic acid is separated at this stage and separated acids when heated forms γ–lactone, which when reduced which sodium–amalgam gives higher aldose.



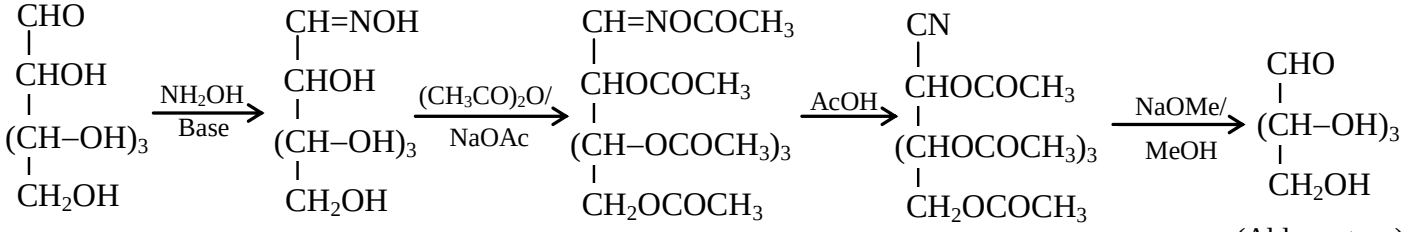
a D–aldopentose | a cyanohydrin | a glyconic acid | a δ–lactone | a D–aldohexose

7. Method of Decending Sugar Series

There are various methods of converting a sugar into its next lower sugar. All of these methods start with the aldose and hence, in order to convert a ketose into the lower aldose, it is first necessary to transform it into an aldose.

7.1 Wohl's Degradation Method

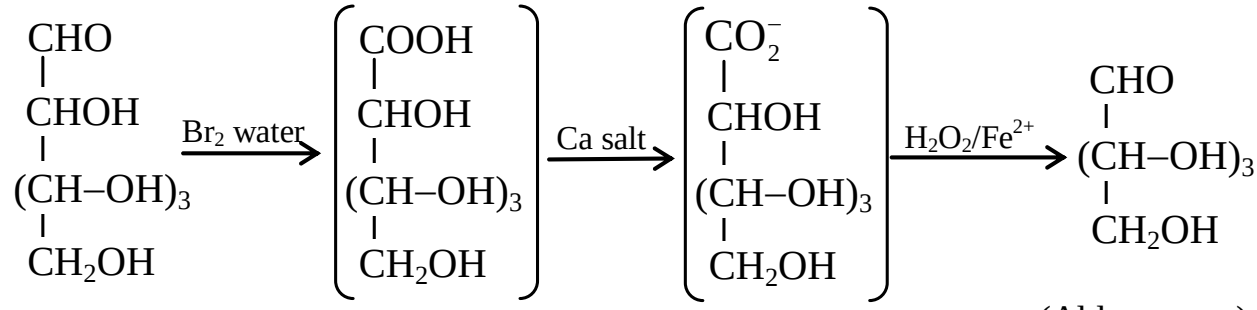
Conversion of aldohexose into aldopentose



(Aldohexose) (Aldopentose)

7.2 Ruff's Degradation Method

In this method, aldose is oxidised by bromine water to corresponding aldaric acid (only CHO is oxidised into COOH) and calcium salt of this acid when treated with Fenton's reagent (H_2O_2/Fe^{2+}) the aldose is converted into corresponding lower aldose.

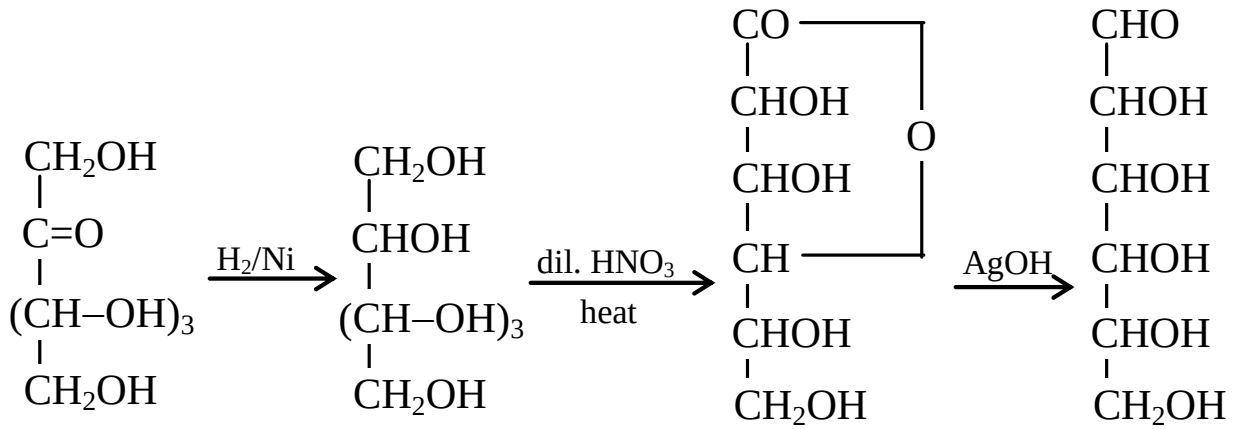


(Aldohexose) (Aldopentose)

This method involves oxidative decarboxylation, where α–CHOH in oxidised to –CH=O without any configurational changes of the other chiral carbons.

8. Conversion of A Ketose to An Aldose & Vice-Versa

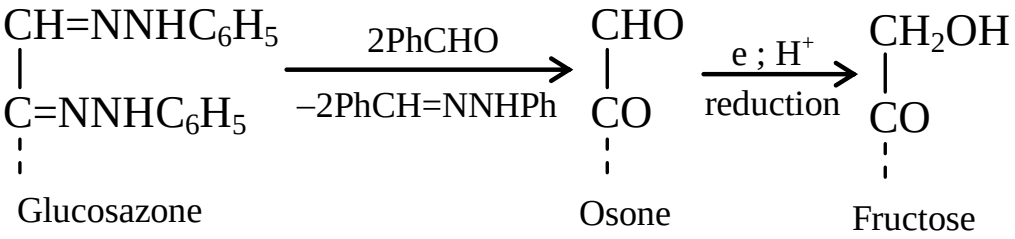
The ketoses are reduced to corresponding polyhydric alcohol, which is then oxidized to a monocarboxylic acid. On warming the acid it is converted into γ–lactone, which on reduction with Na/Hg in faintly acidic medium gives aldose.



(Ketohexose) γ–lactone Aldohexose

Theoretically, two polyhydric alcohol can be formed by reduction of ketose due to the formation of the new asymmetric carbon atom. In practice, however, one isomer is obtained in great yield.

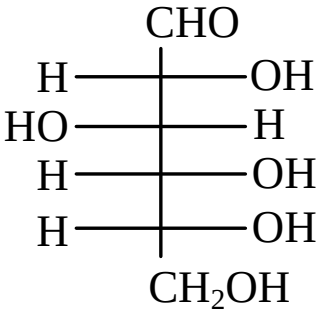
The aldose is converted into its osazone, which is then treated with PhCHO to form osone. On reduction with zinc and acetic acid, the osone is converted into the ketose.



The phenylhydrazinyl group is transferred from the osazone to PhCHO giving PhCH=NNHPh and a dicarbonyl compound called the osone. The more reactive aldehyde group of the osone is reduced and not the less reactive keto group and it gives 2–ketohexose.

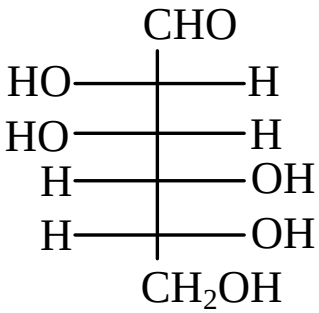
9. Epimerisation

The diastereomers with more than one stereocentre that differs in configuration about only on one carbon atom are called epimers. For example, D–glucose and D–mannose are epimers. Their configuration is different only on C_2.



Glucose

Fig. 8.1

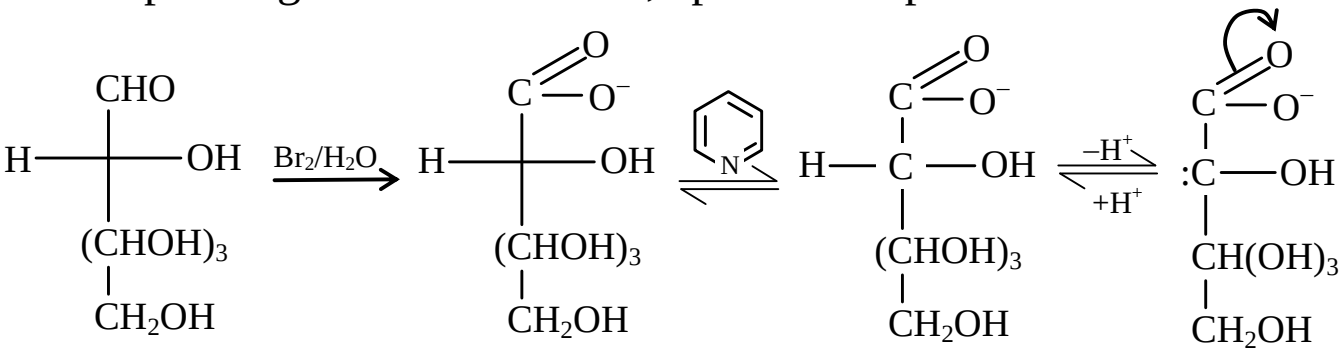


Mannose

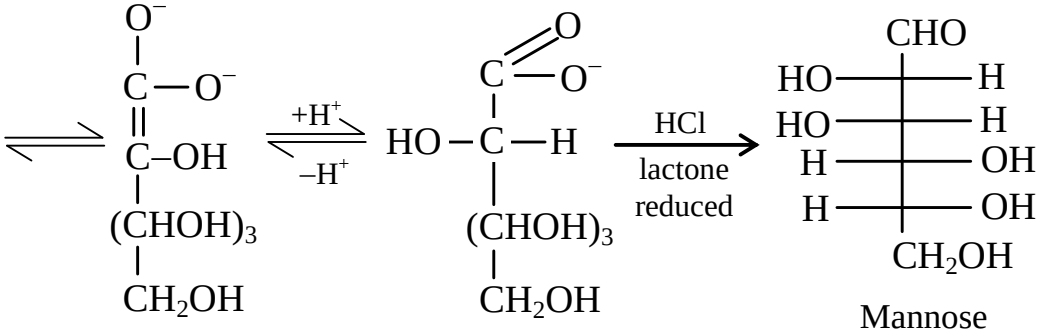
Fig. 8.2

These epimers differ only on configuration of C–2 carbon. Epimerisation of sugar can be done by suitable reagents.

The aldonic acid was heated with pyridine (or quinoline) and gets converted into equilibrium mixture of the original acid and its epimer in the corresponding acids are reduced, epimers are produced.

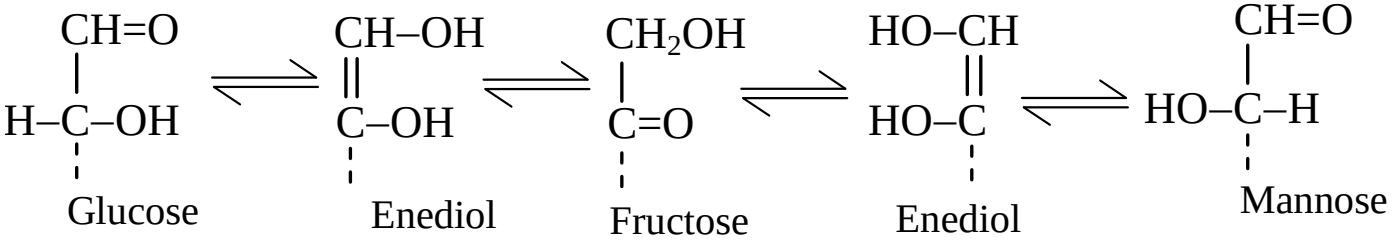


Glucose



Mannose

Glucose can also be epimerised by treating with hot concentrated alkali. Some glucose is also converted into fructose.



Glucose Enediol Fructose Enediol Mannose

This rearrangement is known as Lobry de Bruyn–van Ekenstein rearrangement.

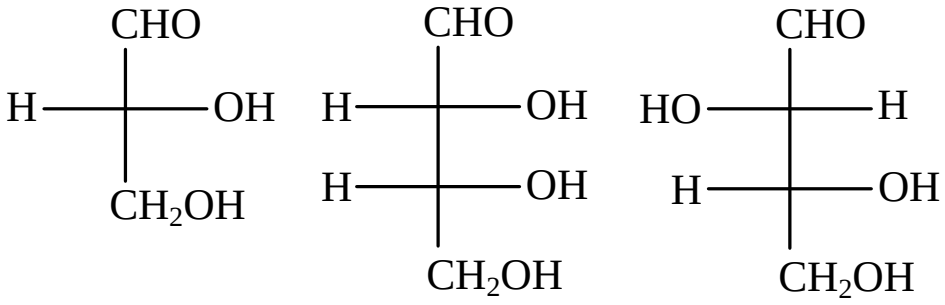
10. Determination of Configuration of Glucose

The relative configuration of D–glucose can be determined with the help of stepping up reaction (Kiliani synthesis) and strong oxidation by HNO_3, which convert aldoses into corresponding aldaric acid.

Glucose can be obtained by systematically stepping up D–glyceraldehyde; hence configuration of OH on highest stereo centre carbon in glucose must

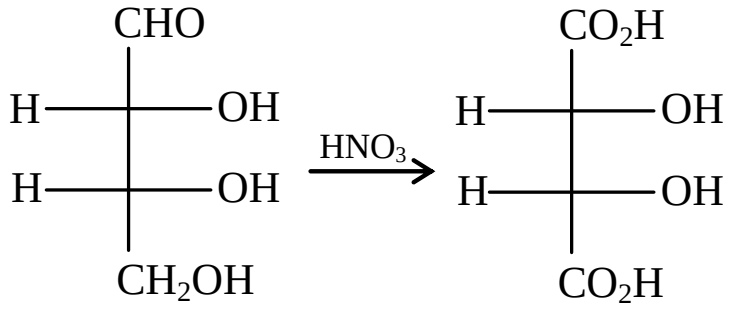
be similar to D–Glyceraldehyde. D–Glucose can be obtained from D–arabinose (pentoses), D–Arabinose can be stepped up from D–erythrose (tetrose) and D–erthyrose can be stepped up from D–Glyceraldehyde (triose).

If D–glyceraldehyde is stepped up, it gives two optical isomers (epimers). The Fischer formula for the possible aldetetrose should be II and III.



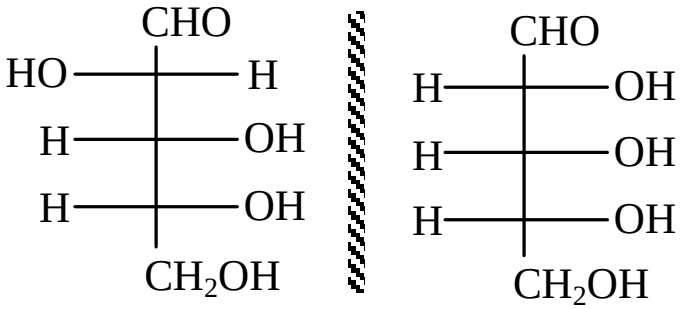
(I) (II) (III)

The one, which on oxidation with HNO_3 gives optically inactive acid, must be D–erythose. Hence, D–erythose must be having configuration (II). (In fact in this way we have also determined configuration of OH on last two optically active stereo centre carbon of glucose).



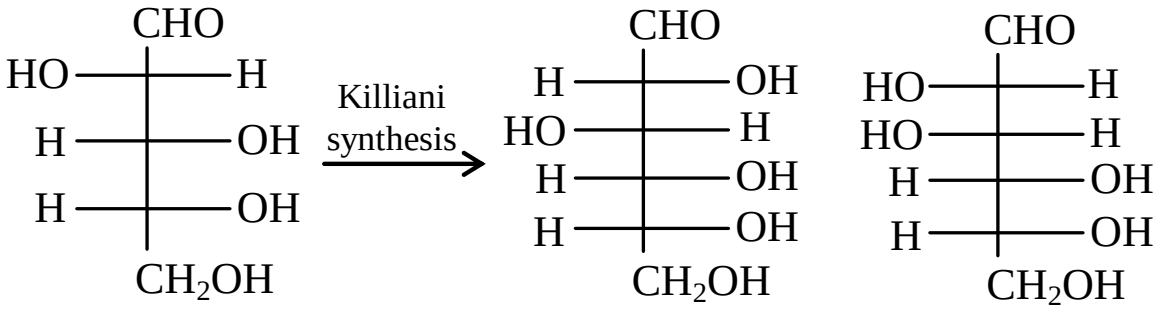
(II) (III)

D–erthyrose gives two optical isomers, (IV) and (V). One isomer, which yields optically active acid upon oxidation, is D–Arabinose. (V on oxidation gives optically inactive acid) so, the configuration of D–arabinose must be (IV).



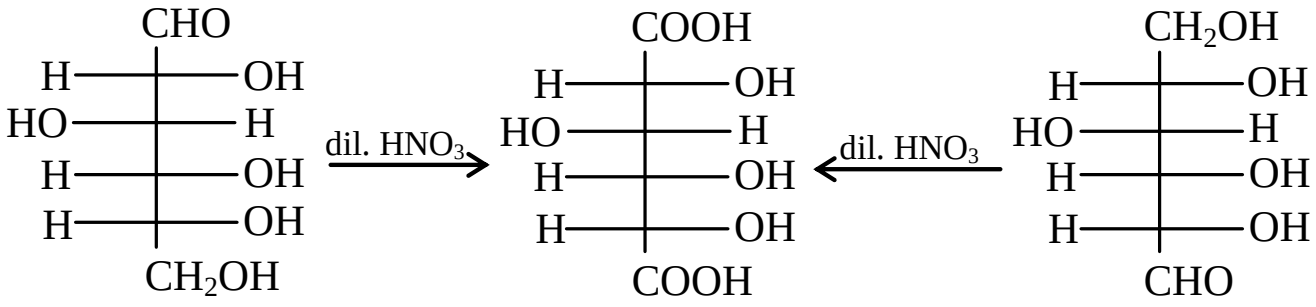
(IV) (V)

D–arabinose upon oxidation gives D–Glucose and D–mannose. They must have structures (VI) or (VII). But which one is D–Glucose and which one is D–mannose?



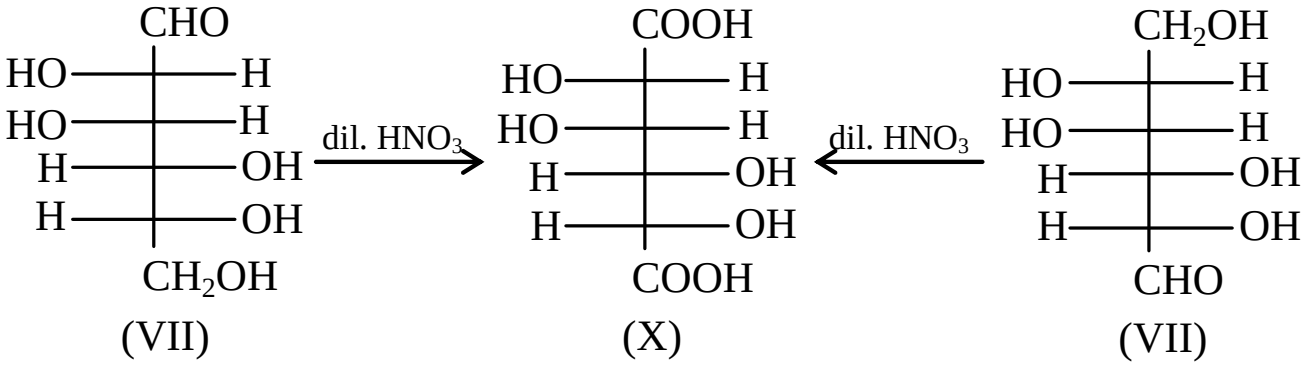
D–Arabinose (VI) (VII)

D–Glucose upon oxidation with HNO_3 gives an optically active acid (VIII), which can also be obtained from another hexose (IX).



(VI) (VIII) (IX)

The acid (X) obtained from D–mannose cannot be obtained from any other source. Hence mannose must be having configuration shown in (VII) and Glucose must be (VI).

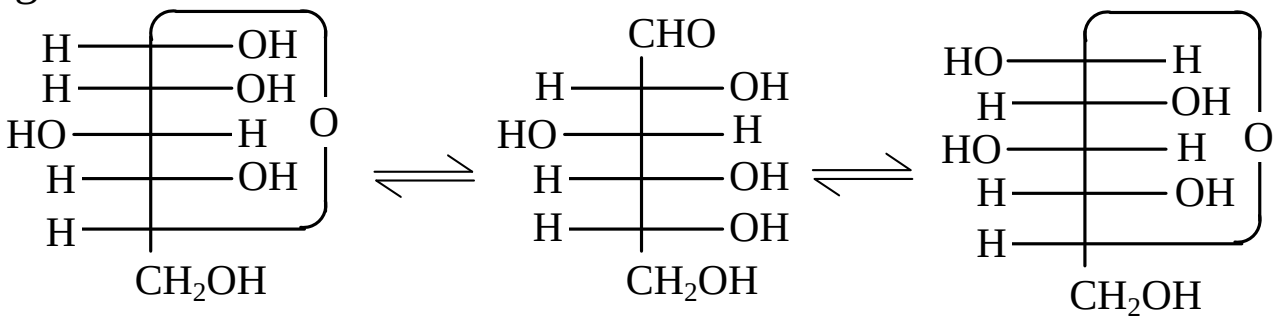


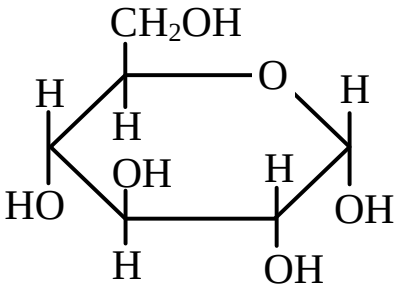
11. Cyclic Structures of Monosaccharides

Many five membered and six membered monosaccharides occur in cyclic form. Cyclic structures of monosaccharides are established by many experiments. The cyclic structure is due to intramolecular hemiacetal formation between aldo /keto group and OH of any one carbon. The ring formed are generally six membered (pyranose) or five membered (furanose).

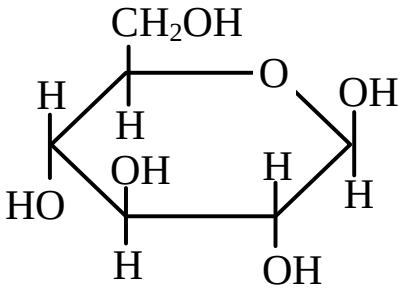
Each cyclization results in creation of a new asymmetric centre apart from the existing ones.

The isomers resulting from cyclizations are called anomers. For example, when D–glucose (open structure) cyclise, it gives α–D–glucose and β–D–glucose.





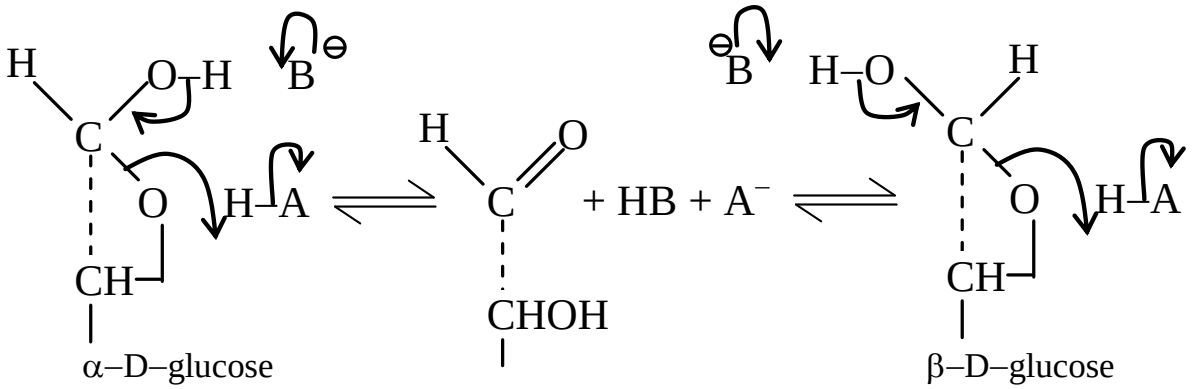
Haworth formula
α–D–Glucose



Haworth formula
β–D–Glucose

When ordinary α–D–glucose is dissolved in water, it has specific rotation of +111° which on standing gradually change until it reaches a constant value of +52.7°. This phenomenon is known as **mutarotation**. Mutarotation is observed because of equilibrium between open chain structure and cyclic structures of monosaccharides in solutions. The specific rotation of β–D–glucose is +19° and equilibrium mixture contains 36.2% & 63.8% α–D–glucose and β–D–glucose respectively.

The following mechanism of mutarotation is proposed which involves a concerted attack by base and acid (water) to produce open chain structure which then recloses to another anomeric form.

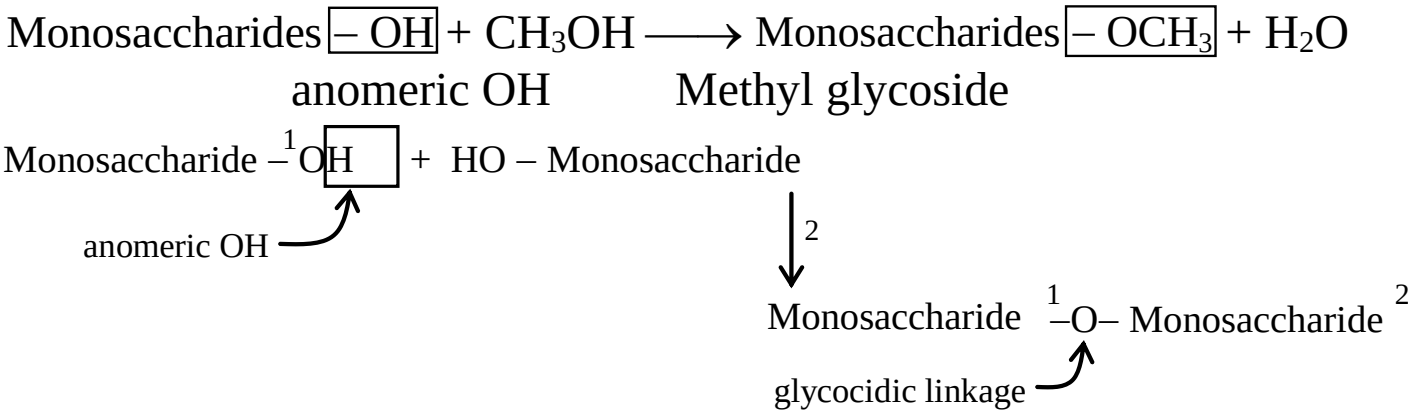


12. Disaccharides

All the disaccharides are crystalline solids, soluble in water and fall into two classes, the reducing sugars and the non–reducing sugars.

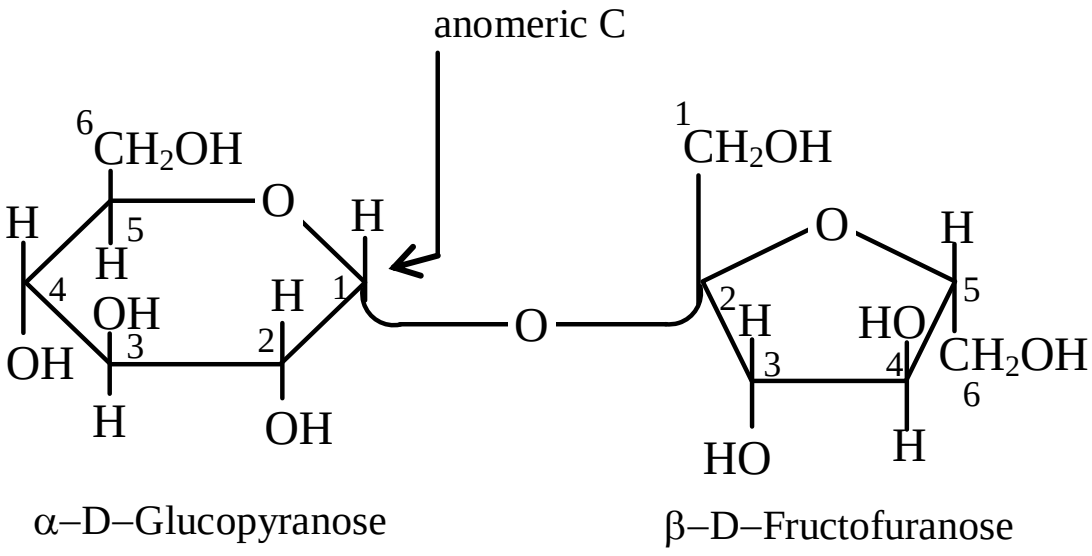
Just like methanol forms methyl glycosides with the monosaccharides (the OH on anomeric carbon reacts with methanol to form methyl ether), other

hydroxy compounds undergo similar union with monosaccharides. Two monosaccharides joined by glycosidic linkage form a disaccharide.



12.1 Sucrose (Cane–Sugar) $C_{12}H_{22}O_{11}$

Sucrose is a white crystalline solid, melting point 180°C and soluble in water. When heated above its melting point, it forms a brown substance known as caramel. Concentrated sulphuric acid chars sucrose, the product being almost pure carbon. Sucrose is dextrorotatory, its specific rotation being +66.5°. On hydrolysis with dilute acids, sucrose yields an equimolecular mixture of D(+)–glucose and D(–)–fructose.

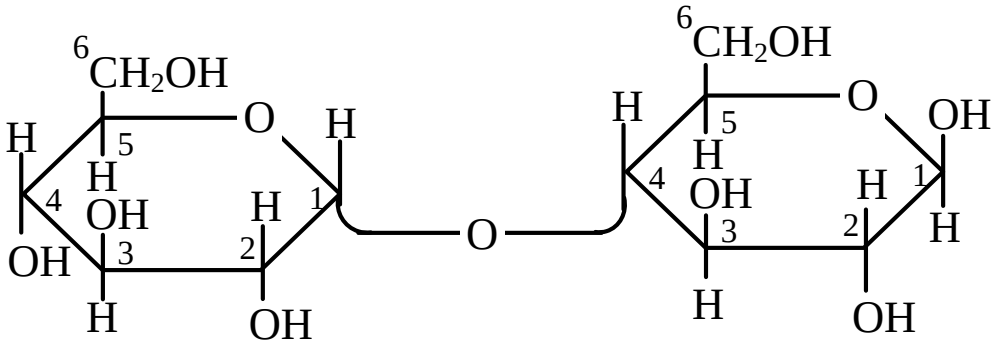


Structure of Sucrose
(α–D–glucopyranosyl–β–D–fructofuranoside
or β–D–fructofuranosyl–α–D–glucopyranoside)

Sucrose is not a reducing sugar, i.e. it will not reduce Fehling's solution, it does not form an oxime or an osazone and does not undergo mutarotation. This indicates that neither the aldehyde group of glucose nor the ketonic group of fructose is free in sucrose. Thus, a tentative structure of sucrose is one in which two molecules, glucose and fructose are linked by the aldehyde group of the former and ketonic group of the latter. This has been amply confirmed by further work and sucrose has been shown to be α–D–glucopyranosyl–β–D–fructofuranoside.

12.2 Maltose (Malt Sugar) $C_{12}H_{22}O_{11}$

Maltose is another disaccharide, which is made up of two α–D–glucose units joined together by glycosidic linkage in such a way that one anomeric OH (C–1) is joined with OH (C–4) in other unit.



α–D–glucopyranose α–D–glucopyranose

Structure of Maltose (α–D– glucopyranose α–D– glucopyranose

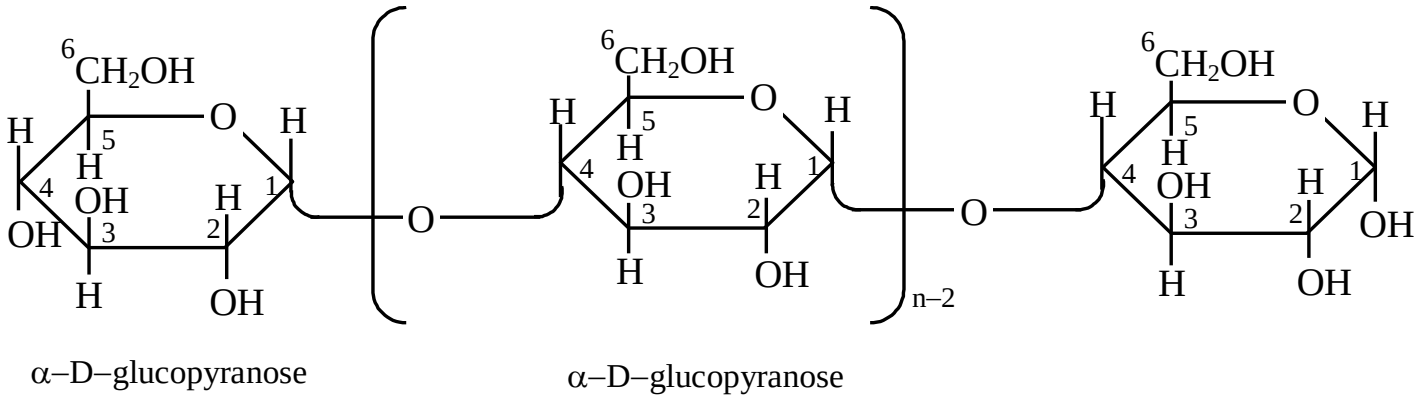
Another example of disaccharide is cellobiose, which is β–D–glucopyranose i.e. it is made up of two β–D–glucose units.
Lactose is another disaccharide, which is made up of D–glucose and D–galactose.

13. Polysaccharides

The polysaccharides are high polymers of the monomeric sugars and have molecular weights that may range from a few thousand to several millions.

13.1 Starch $(C_6H_{10}O_5)_n$:

Occurs in all green plants, the commercial sources of starch are maize wheat, barley, rice, potatoes and sorghum. Starch consists of two fractions, one being known as α–amylose, which gives blue colour with iodine. This blue colour is believed to be due to the formation of an inclusion complex. An aqueous solution of α–amylose slowly forms a precipitate, since α–amylose has a strong tendency to 'revert' to the insoluble state in solution. Amylopectin is insoluble in water and is stable towards both hydrolysis to maltose by the enzyme diastase and to D(+)–glucose by dilute acids (amylopectin gives about 50 percent of maltose). Thus, both contain D–glucopyranose units joined by the α–glycosidic linkage.



Structure of Starch (α–D– glucoamylose)

α–amylase consists of an unbranched chain, with a molecular weight varying between 10,000 (n ≈ 60) and 10,00,000 (n ≈ 6,000). The value of n depends on the source and treatment of α–amylase. Amylopectin differs from α–amylase in that it contains branched chains, the branching occurring through 1, 6–linkages (and other linkages) and the length of the unbranched sections being about 24–30 glucose units. The molecular weights recorded for amylopectin vary between 50,000 and 1,00,00,000.

13.2 Glycogen $(C_6H_{10}O_5)_n$

Glycogen is found in nearly all animals cells, occurring mainly in liver. It is the reserve carbohydrate of animals and so is often known as 'animal starch'. It has also been isolated from plant sources.

Glycogen is a white powder, soluble in water, the solution giving a purplish–red colour with iodine. On hydrolysis with dilute acid, glycogen gives D(+)–glucose. The molecular weight of glycogen has been given as 10,00,000 to 50,000,00 and glycogen contains highly branched chains. Glycogen has a structure similar to amylopectin, except that it has more cross–linking.

13.3 Pectins

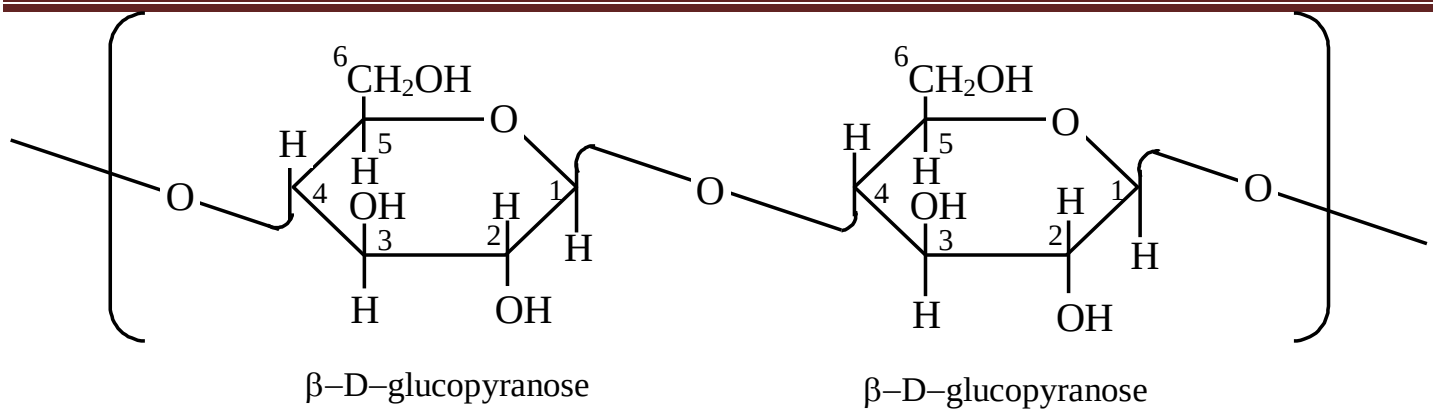
Pectins are found in plant and fruit juices. Their characteristic property is the ability of their solutions to gelate, i.e., form jellies. They have a high molecular weight and are polygalacturonic acids (linked 1, 4) with the carboxyl groups partially esterified with methanol.

13.4 Cellulose $(C_6H_{10}O_5)_n$

Cellulose is the main constituent of the cell–wall of plants and also occurs in certain animal tissues. It is the most widely distributed organic compound on earth, its main source is cotton (almost pure cellulose) and wood (which also contains lignin, which is not a polysaccharide).

Cellulose is a white solid, insoluble in water but soluble in ammoniacal copper hydroxide solution (Schweitzer's reagent). Careful hydrolysis of cellulose gives cellobiose. It is also possible to isolate cellotriose (trisaccharides) and cellotetrose (tetrasaccharide). All of these saccharides, on further hydrolysis, yield only D(+)–glucose, which exists in the β–form in cellulose. Thus, cellulose differs from starch in having β–1, 4–glycosidic linkages and not α.

The molecular weight of cellulose varies between 20,000 and 5,00,000 and the compound consists of an unbranched chain having more glucose units.



Structure of Cellulose

14. Tests For Carbohydrates

(i) When heated in a dry test tube, it melts, turns brown and finally black, giving a characteristic smell of burning sugar.

(ii) When warmed with a little concentrated H_2SO_4, it leaves a charred residue of carbon.

(iii) **Molisch's test:** A drop or two of alcoholic solution of α–naphthol is added to 2 ml of glucose solution and 1 ml of concentrated H_2SO_4 is added carefully along the sides of the test tube. The formation of a violet ring, at the junction of two liquids confirms the presence of a carbohydrate.

Amino Acids And Proteins

The name protein is taken from the Greek word “proteios”, which means “first”. This name is well chosen. Of all chemical compounds, proteins must almost certainly be ranked first, for they are the substance of life.
Proteins make up a large part of the animal body, they hold it together and they run it. They are found in all living cells. They are the principal material of skin, muscle, tendons, nerves and blood of enzymes, antibodies and many hormones.
(Only the nucleic acids, which control heredity, can challenge the position of proteins and the nucleic acids are important because they direct the synthesis of proteins).
Chemically, proteins are high polymers. They are polyamides and the monomers from which they are derived are the α–amino carboxylic acids. A single protein molecule contains hundreds or even thousands of amino acid units, these units can be of twenty–odd different kinds. The number of different combinations, that is, the number of different protein molecules that are possible, is almost infinite. It is likely that tens of thousands of different proteins are required to make up and run an animal body and this set of proteins is not identical with the set required by an animal of a different kind.
In this lesson we shall look first at the chemistry of the amino acids and then at the proteins that they make up. Our chief purpose will be to see the ways in which the structures of these enormously complicated molecules are being worked out and how (in the last analysis) all this work rests on the basic principles of organic structural theory on the concepts of bond angle and bond length, group size and shape, hydrogen bonding, resonance, acidity and basicity, optical activity, configuration and conformation.

15.1 Structure of Amino Acids

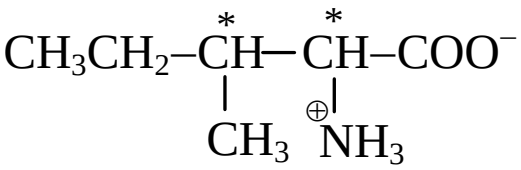
Certain of these (marked *) are the essential amino acids, which must be fed to young animals if proper growth is to take place, these particular amino acids evidently cannot be synthesized by the animal so, it has to be taken from the other materials in its diet.

We see that of all alpha–amino carboxylic acids, in two cases (proline and hydroxyproline) the amino group forms part of a pyrolidine ring. This common feature gives the amino acids a common set of chemical properties, one of which is the ability to form the long polyamide chains that make up proteins. It is on these common chemical properties that we shall concentrate.

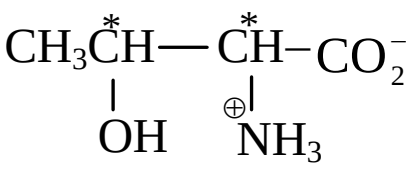
In other respects, the structure of these compounds varies rather widely. In addition to the carboxyl group and the amino group alpha to it, some amino acids contain a second carboxyl group (e.g., aspartic acid or glutamic acid), or a potential carboxyl group in the form of a carboxamide (e.g., asparagine or glutamine), these are called acidic amino acids. Some contain a second basic group, which may be an amino group (e.g., lysine), a guanidine group (arginine), or the imidazole ring (histidine), these are called basic amino acids. Some of the amino acids contain benzene or heterocyclic ring systems, phenolic or alcoholic hydroxyl groups, halogen or sulphur atoms. Each of these ring systems of functional groups undergoes its own typical set of reactions.

Amino acids are categorized as either neutral, acidic or basic, depending on the nature of their R groups. Aspartic acid and glutamic acids, each with a second CO_2H in their side chain are acidic amino acids while lysine, arginine and histidine, each with a basic site in their side chain are basic amino acids. All other are neutral amino acids, which are further classified as polar and non–polar depending on whether their side chains

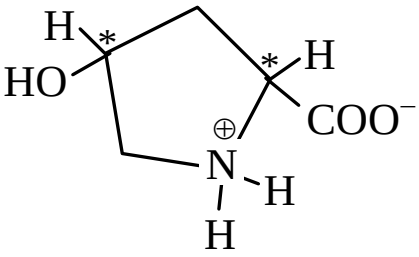
have polar substituents (for example, asparagine with an NH_2CO group) or are completely hydrocarbon in nature (for example alanine, valine etc).
All amino acids are chiral molecules with atleast one chiral carbon except glycine, $H_3N^{\oplus}CH_2COO^-$. Isoleucine, threonine and 4–hydroxy proline are the amino acids with 2 chiral carbons.



Isoleucine

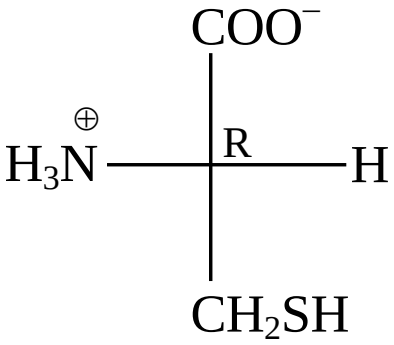


Threonine

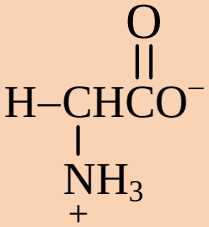


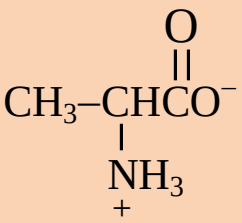
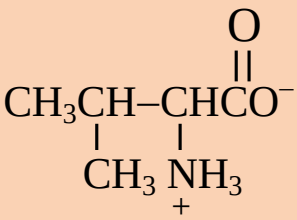
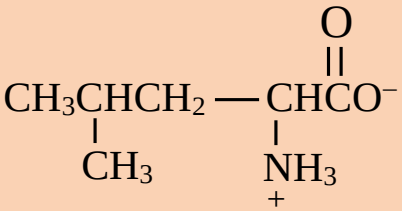
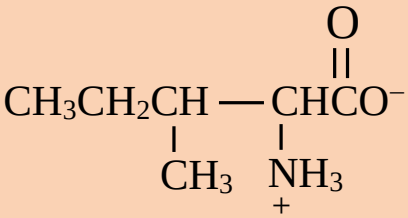
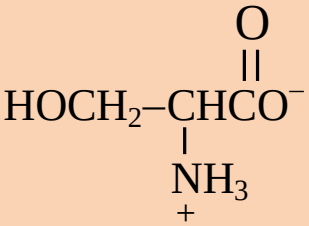
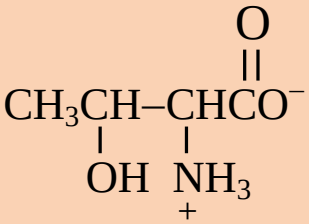
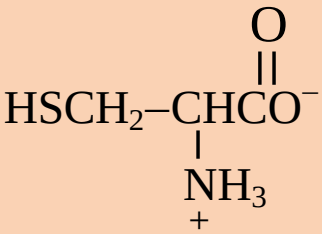
4–hydroxy proline

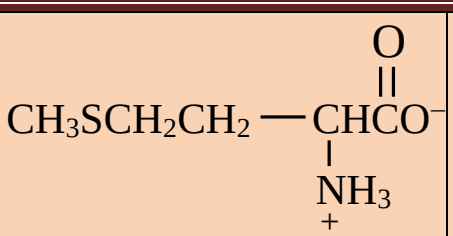
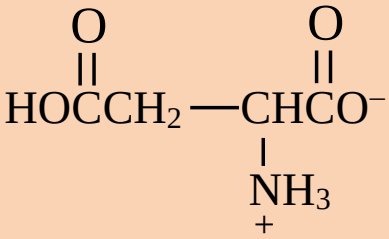
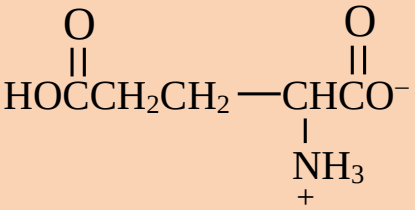
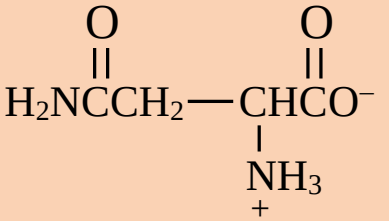
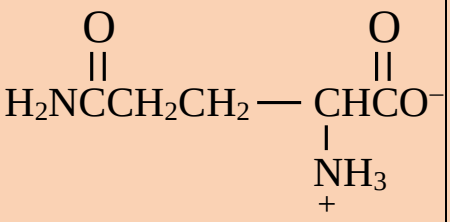
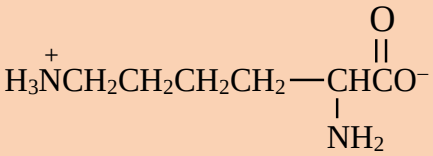
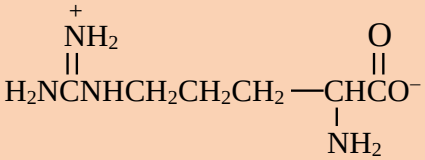
All amino acids have S and L configurations while L–cysteine and L–cystine has R and L configuration because in cysteine CH_2SH group has priority over the COO^- because of higher atomic weight of sulphur than oxygen.

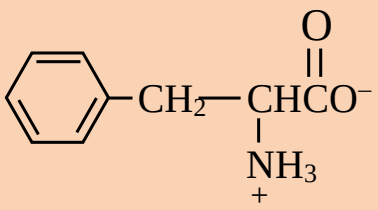
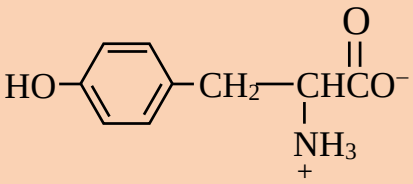
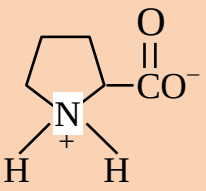
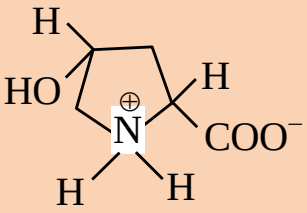
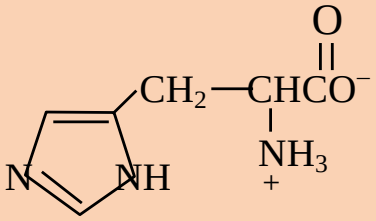
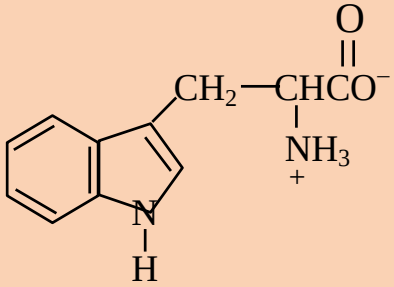


All amino acids are primary amines except proline and 4–hydroxy proline, which are 2° amines.

	Formula	Name	Abbreviations
Aliphatic amino acids		glycine	Gly

		alanine	Ala
		valine*	Val
		leucine*	Leu
		isoleucine*	Ileu
Hydroxy–containing amino acids		serine	Ser
		threonine*	Thr
Sulphur–containing amino acids		cysteine	CySH

		methionine*	Met
Acidic amino acids		aspartic acid	Asp
		glutamic acid	Glu
Amides of acidic amino acids		asparagine	Asp(NH_2)
		glutamine	Glu(NH_2)
Basic amino acids		lysine*	Lys
		arginine*	Arg

Benzene–containing amino acids		phenyl alanine*	Phe
		tyrosine	Tyr
Heterocyclic amino acids		proline	Pro
		4-hydroxy proline	Hypro
		histidine*	His
		tryptophane*	Try

15.2 Amino Acids As Dipolar Ions

Although the amino acids are commonly shown as containing an amino group and a carboxyl group, $H_2NCHRCOOH$, certain properties, (both physical and chemical) are not consistent with this structure

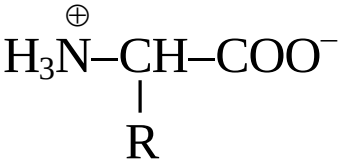
(a) In contrast to amines and carboxylic acids, the amino acids are non–volatile crystalline solids, which melt with decomposition at fairly high temperatures.

(b) They are insoluble in non–polar solvents like petroleum ether, benzene or ether and are appreciably soluble in water.

(c) Their aqueous solutions behave like solutions of substances of high dipole moment.

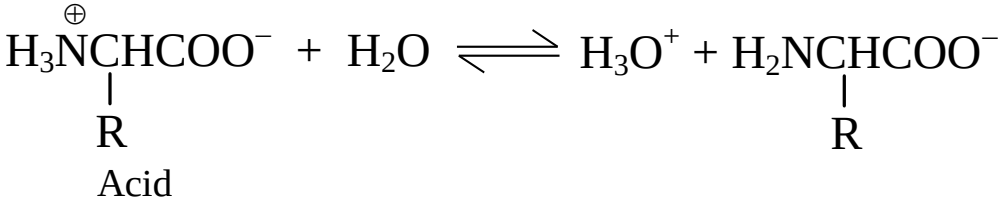
(d) Acidity and basicity constants are ridiculously low for –COOH and $–NH_2$ groups. Glycine, for example, has $K_a = 1.6 \times 10^{-10}$ and $K_b = 2.5 \times 10^{-12}$, whereas most carboxylic acids have K_a values of about 10^{-5} and most aliphatic amines have K_b values of about 10^{-4}.

All these properties are quite consistent with a dipolar ion structure for the amino acids (I).



Amino acids: dipolar ions (Zwitter ion)

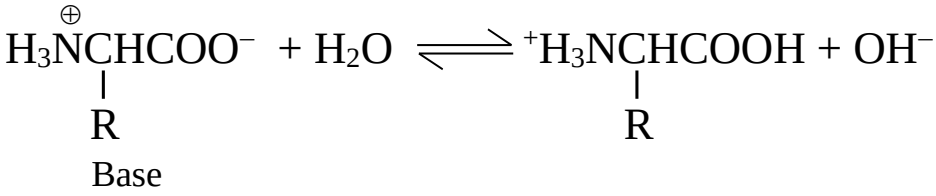
The physical properties–melting point, solubility, high dipole moment–are just what would be expected of such a salt. The acid–base properties also become understandable when it is realized that the measured K_a actually refers to the acidity of an ammonium ion, RNH_3^+,



Acid

$$K_a = \frac{[H_3O^+][H_2NCHRCOO^-]}{[^+H_3NCHRCOO^-]}$$

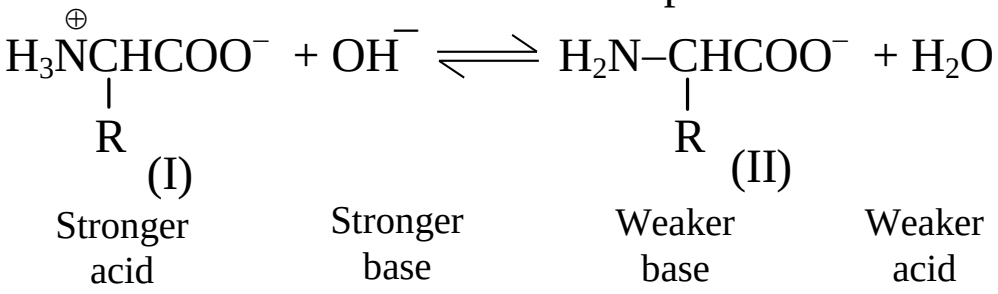
and K_b actually refers to the basicity of a carboxylate ion, $RCOO^-$



$$K_b = \frac{[{}^{+}H_3NCHRCOOH][OH^-]}{[{}^{+}H_3NCHRCOO^-]}$$

In aqueous solution, the acidity and basicity of an acid and its conjugate base (CH_3COOH and CH_3COO^-, or $CH_3NH_3^+$ and CH_3NH_2, for example) are related by the expression $K_a \times K_b = 10^{-14}$. From this it can be calculated that a K_a of 1.6×10^{-10} for the $-NH_3^+$ of glycine means $K_b = 6.3 \times 10^{-5}$ for $-NH_2$: a quite reasonable value for an aliphatic amine. In the same way, a K_b of 2.5×10^{-12} for the $-COO^-$ of glycine means $K_a = 4 \times 10^{-3}$ for $-COOH$: a quite reasonable value for a carboxylic acid containing the strongly electron–withdrawing (acid–strengthening) $-NH_3^+$ group.

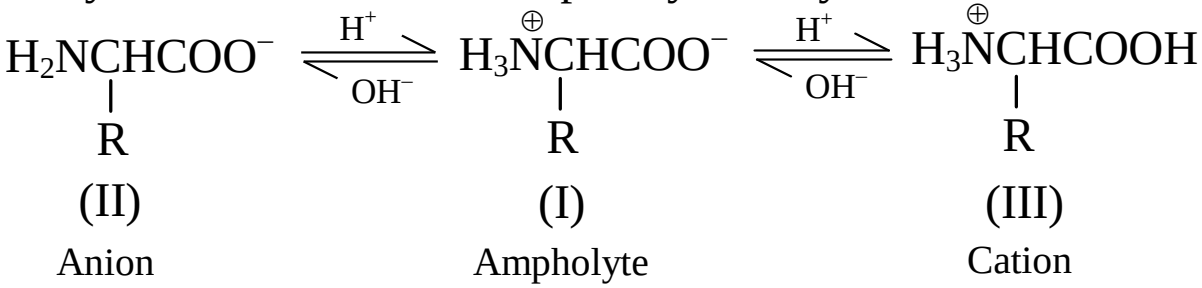
When the solution of an amino acid is made alkaline, the dipolar ion (I) is converted into the anion (II). The stronger base, hydroxide ion, removes a proton from the ammonium ion and displaces the weaker base, the amine.



In summary, the acidic group of a simple amino acid like glycine is $-NH_3^+$ not $-COOH$ and the basic group is $-COO^-$ not $-NH_2$.

We must keep in mind that ions (II) and (III), which contain a free $-NH_2$ or $-COOH$ group, are in equilibrium with dipolar ion (I). Consequently, amino acids undergo reactions characteristic of amines and carboxylic acids. As ion (II) is removed, by reaction with benzoyl chloride, for

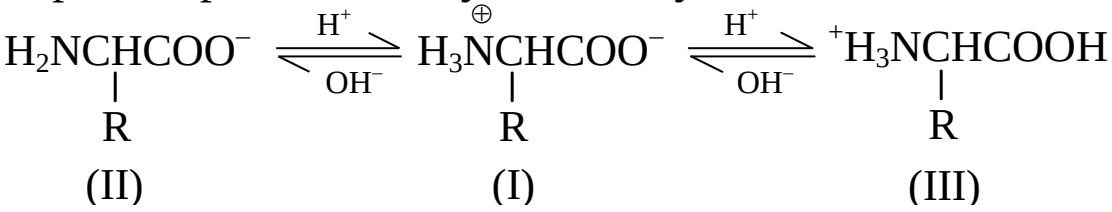
example, the equilibrium shifts to supply more of ion (II), so that eventually the amino acids is completely benzoylated.



Wherever feasible, we can speed up a desired reaction by adjusting the acidity or basicity of the solution in such a way as to increase the concentration of the reactive species.

15.3 Isoelectric Point of Amino Acids

What happens when a solution of an amino acid is placed in an electric field depends upon the acidity or basicity of the solution.



In quite alkaline solution, anions (II) exceed cations (III), and there is a net migration of amino acid toward the anode. In quite acidic solution, cations (III) are in excess, and there is a net migration of amino acid toward the cathode. If (II) and (III) are exactly balanced, there is no net migration; under such conditions any one molecule exists as a positive ion and as a negative ion for exactly the same amount of time, and any small movement in the direction of one electrode is subsequently cancelled by an equal movement back towards the other electrode. **The hydrogen ion concentration of the solution in which a particular amino acid does not migrate under the influence of an electric field is called the isoelectric point (pI) of that amino acid. The isoelectric point (pI) is the pH at which the amino acid exists only as a dipolar ion with net charge zero.**

A monoamino monocarboxylic acid, $^{+}H_3NCHRCOO^{-}$, is somewhat more acidic than basic (for example, glycine: $K_a = 1.6 \times 10^{-10}$ and $K_b = 2.5 \times 10^{-12}$). If crystals of such an amino acid are added to water, the resulting solution contains more of the anion (II), $H_2NCHRCOO^{-}$, than of the cation (III), $^{+}H_3NCHRCOOH$. This excess ionisation of ammonium ion to amino ($I \rightleftharpoons II + H^{+}$) must be repressed, by addition of acid, to reach the isoelectric point, which therefore lies somewhat on the side of neutrality (pH 7). For example, the isoelectric point of glycine is at pH 6.1.

An amino acid usually shows its lowest solubility in a solution at the isoelectric point, since here there is the highest concentration of the dipolar ion. As the solution is made more alkaline or more acidic, the concentration of one of the more soluble ions, (II) or (III), increases.

Illustration 1.

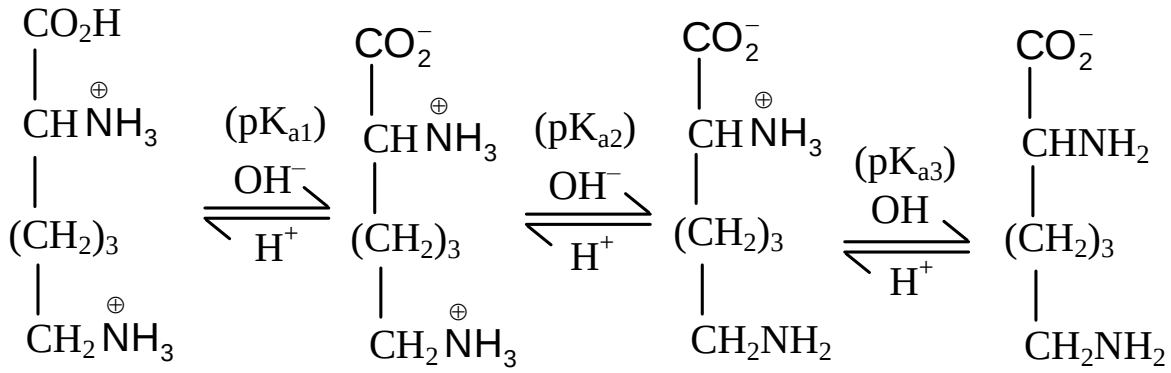
Calculate the isoelectric points of lysine, $CO_2^{-}CH(\overset{\oplus}{N}H_3)(CH_2)_4NH_2$ and aspartic acid, $CO_2^{-}CH(\overset{\oplus}{N}H_3)CH_2CO_2H$. The pKa_1, pKa_2 and pKa_3 of the di-cation of lysine are 2.18, 8.95 and 10.53 respectively. The pKa_1, pKa_2 and pKa_3 of the cation of aspartic acid are 1.88, 3.65 and 9.60 respectively.

Solution:

The isoelectric point (pI) is the pH at which the amino acid exists only as a dipolar ion with net charge zero.

At isoelectric point, for a neutral amino acid, $pI = \dfrac{(pK_{a_1} + pK_{a_2})}{2}$

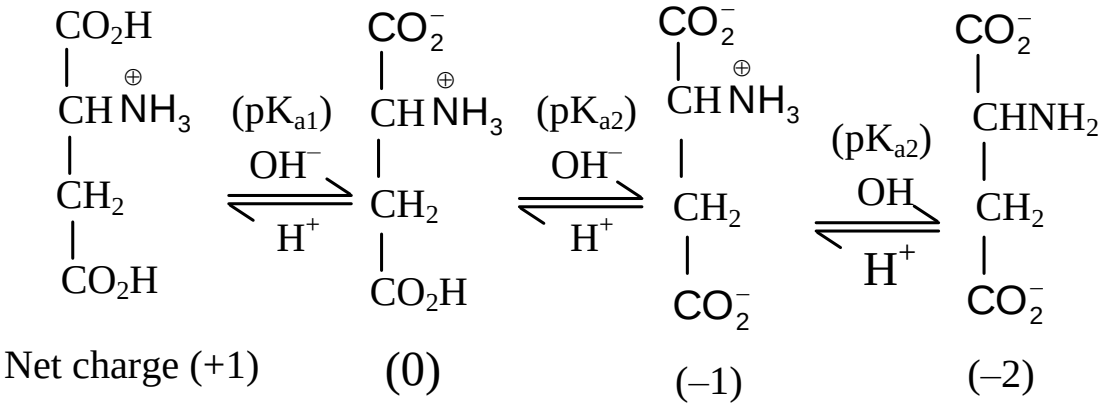
The dissociation of cationic form of lysine can be represented as



The species with zero net charge exists between species with (+1) and (–1) net charges.

$$pI = \frac{(pK_{a_2} + pK_{a_3})}{2} = \frac{(8.95 + 10.53)}{2} = 9.74$$

The dissociation of cationic form of aspartic acid can be shown as



$$pI = \frac{(pK_{a_1} + pK_{a_2})}{2} = \frac{(1.88 + 3.65)}{2} = 2.77$$

15.4 Electrophoresis

If a filter paper–strip moistened with a solution of a mixture of AA's is placed between two electrodes, the charged molecule will migrate to one electrode or the other at a rate that depends on its net charge and the applied voltage. The net charge depends on the pH. The strip is then stained with a reagent that reacts with the amino acid, thereby forming a color whose position on the strip is compared for identification with that

of a known sample. This process known as electrophoresis is used for identification of amino acids.

16. Preparation of Amino Acids

16.1 Amination of α–Halo Acids

Amination of α–halo acids of the many methods that have been developed for synthesizing amino acids, we shall take up only one: amination of α–halo acids. Considered in its various modifications, this method is probably the most generally useful, although, like any of the methods, it cannot be applied to the synthesis of all the amino acids.

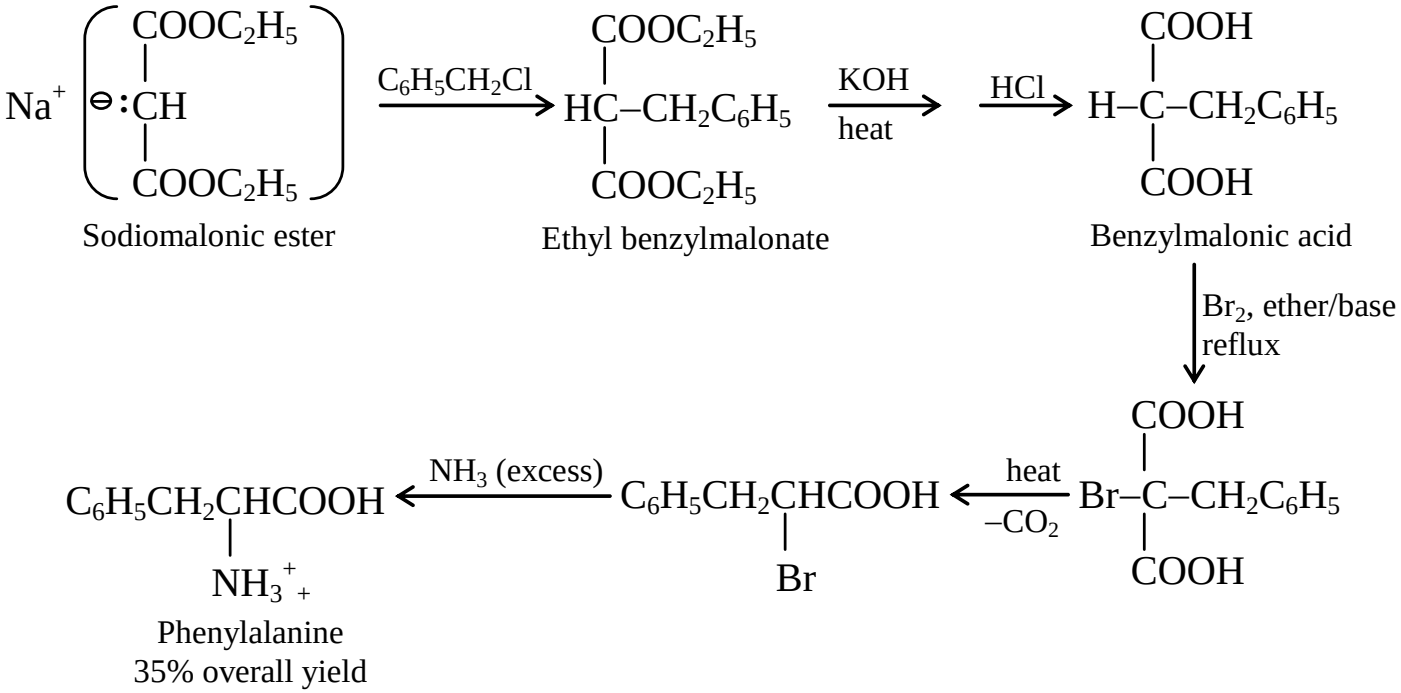
Sometimes an α–chloro or α–bromo acid is subjected to direct ammonolysis with a large excess (Why?) of concentrated aqueous ammonia. For example,

$$CH_3CH_2COOH \xrightarrow{Br_2\ ,\ P} \underset{\underset{Br}{|}}{CH_3CHCOOH} \xrightarrow{NH_3\ (excess)} \underset{\underset{\overset{\oplus}{NH_3}}{|}}{CH_3CHCOO^-}$$

α–Bromopropionic acid — Alanine

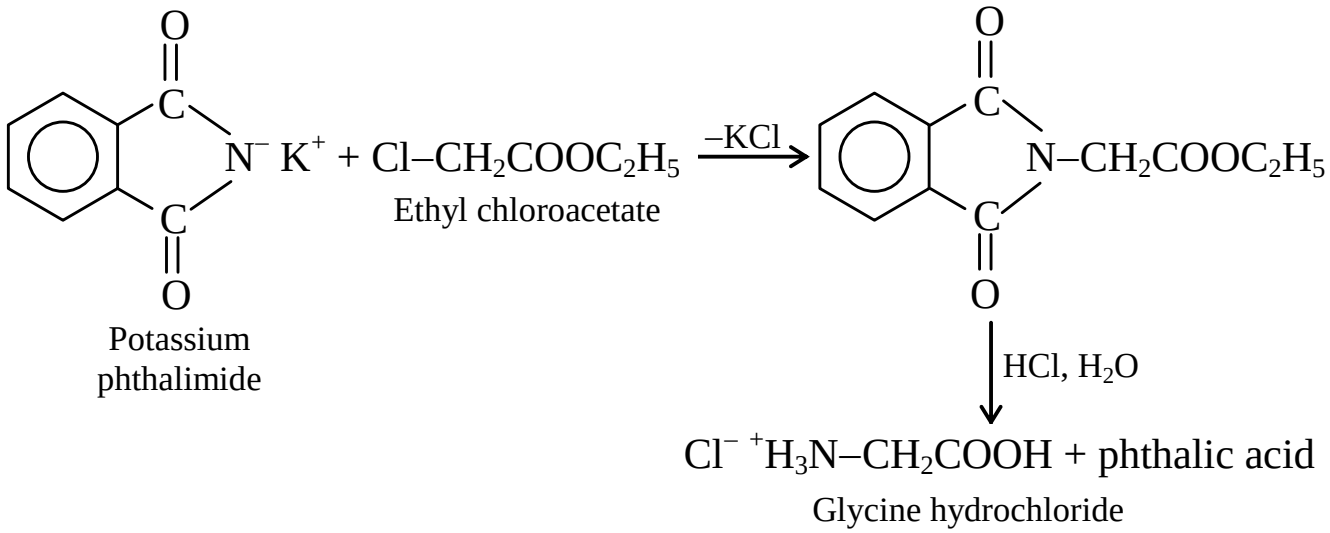
The necessary α–halo acids or esters can be prepared by the Hell–Volhard–Zelinsky halogenation of the unsubstituted acids, or by a modification of the malonic ester synthesis, the usual route to the unsubstituted acids. For example,

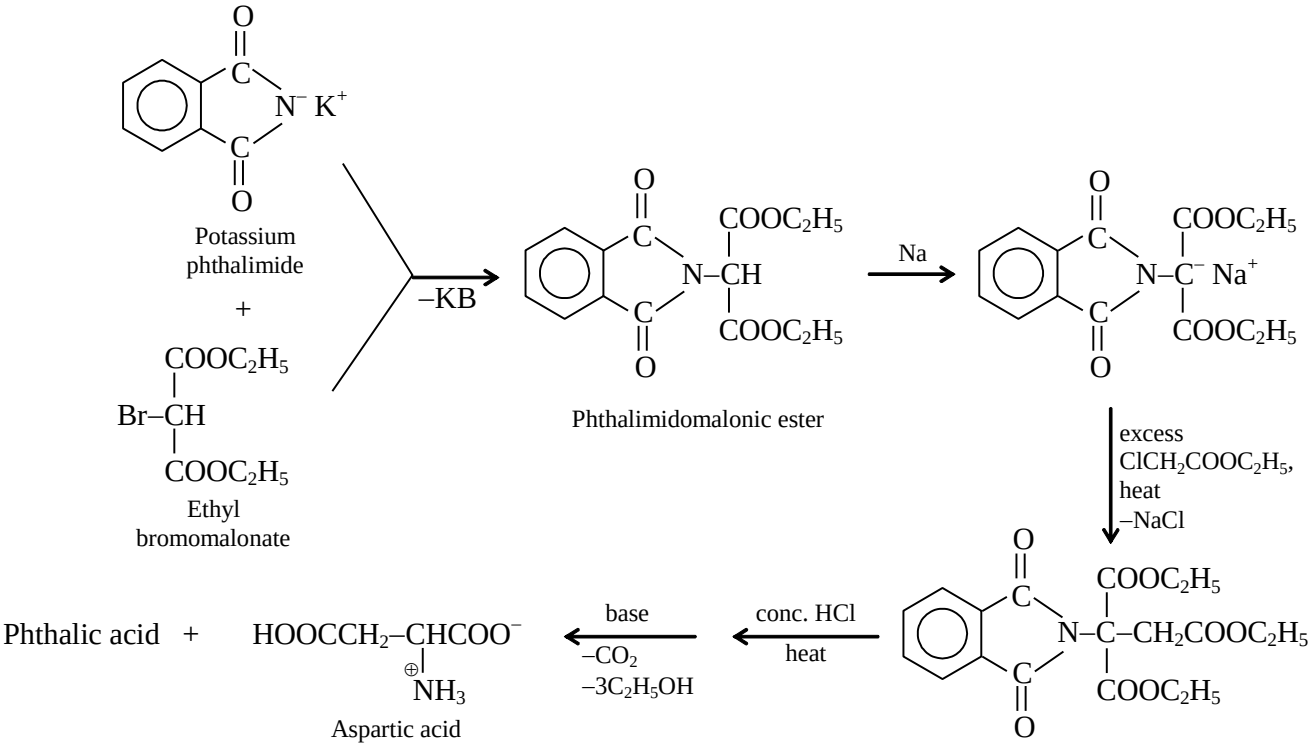
16.2 From Diethyl Malonate



16.3 Gabriel Phthalimide Synthesis

Better yields are generally obtained by the Gabriel phthalimide synthesis; the α–halo esters are used instead of α–halo acids (Why?). A further modification, the phthalimide malonic ester method, is a combined malonic ester–Gabriel synthesis.

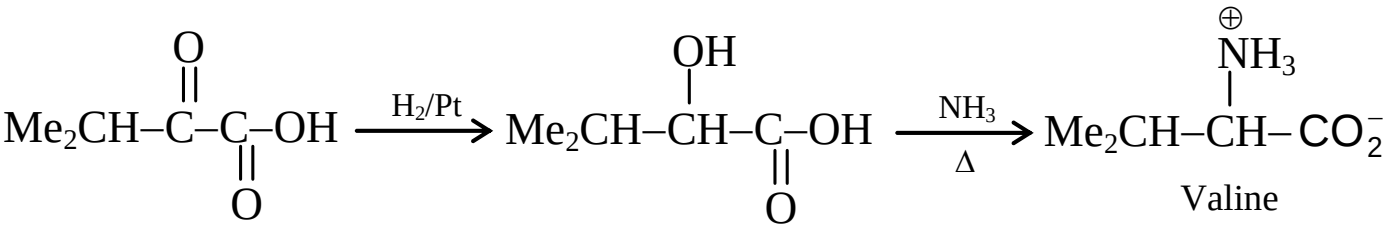




These synthetic amino acids are, of course, optically inactive, and must be resolved if the active materials are desired for comparison with the naturally occurring acids or for synthesis of peptides. There is growing interest in enantiotopic synthesis, which yield directly optically active amino acids; such preparation must be carried out in a chiral medium.

16.4 Reductive Amination

Another method of preparing α–amino acids is reductive amination of α–keto acids. For example,

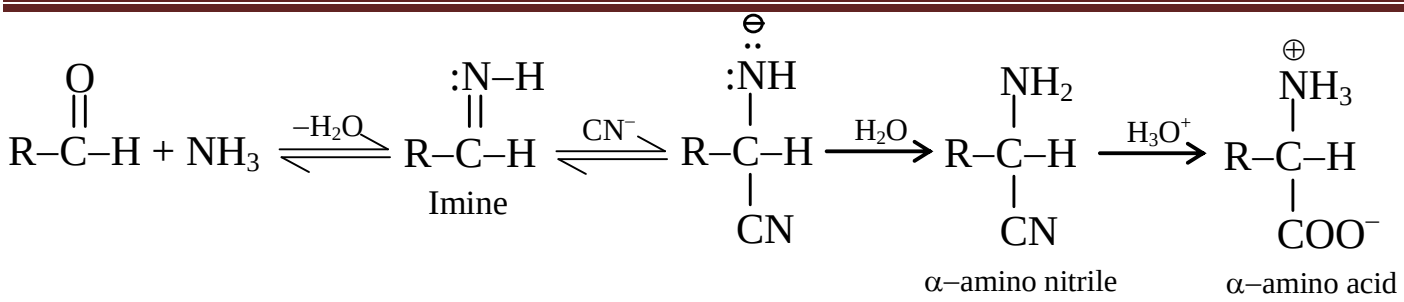


16.5 Strecker's Synthesis

Strecker's synthesis is also used for preparing α–amino acids.
in this reaction generally aldehyde is treated with mixture of ammonium chloride and KCN in aqueous solution which forms NH_3 and HCN,

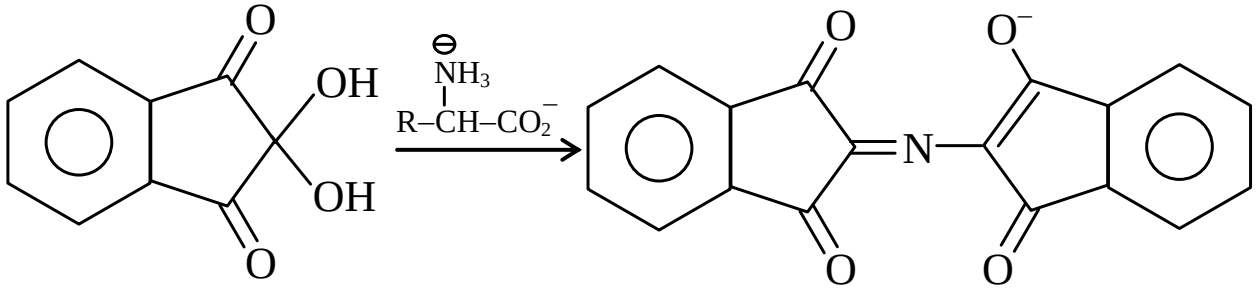
$$NH_4Cl + KCN \xrightarrow{\text{aqueous}} NH_4CN + KCl,$$

$$NH_4CN \xrightarrow{\text{aqueous}} NH_3 + HCN$$



17. Reactions of Amino Acids

Amino acids are detected by ninhydrin test. All amino acids give violet–coloured product with ninhydrin (triketo hydroindene hydrate) except proline and 4–hydroxy proline, which gives yellow colour with it.



The same violet coloured dye forms from all α–AA's with 1° amino groups because only their nitrogen is incorporated into it. The 2° amines proline and 4–hydroxyproline give different adducts that absorb light at a different wavelength and thus have a different yellow color.

When alanine $MeCH(\overset{\oplus}{N}H_3)COO^{\ominus}$ reacts with $(CH_3CO)_2O$, protection of amino group takes place and the product is N–acylated product.

$$CH_3CH(\overset{\oplus}{N}H_3)COO^{\ominus} + (CH_3CO)_2O \longrightarrow CH_3CONHCH(Me)COOH$$

N–acylation of alanine can also be carried out by treatment with PhCOCl in presence of NaOH.

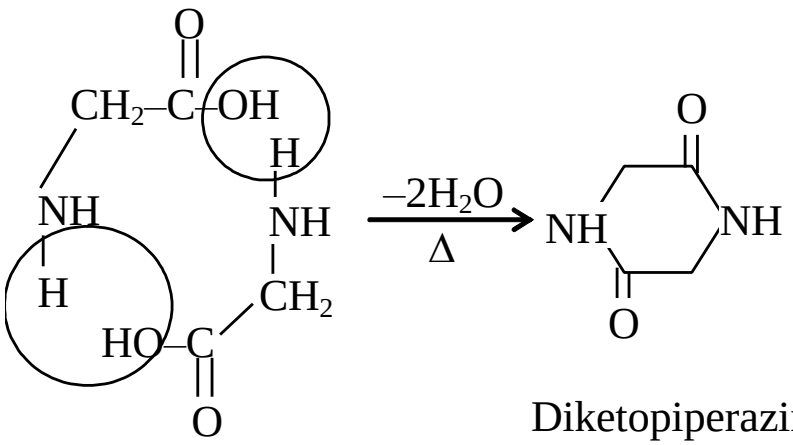
$$CH_3CH(\overset{\oplus}{N}H_3)COO^{\ominus} + PhCOCl + NaOH \longrightarrow PhCONHCH(CH_3)COO^{\ominus}\ Na^{\oplus}$$

Esterification is carried out by acid catalysis. Baryta treatment produces salt of the amino acid $(NH_2CH(Me)COO)_2Ba^{+2}$.

Nitrous acid produced in situ ($NaNO_2$+ HCl) on treatment with amino acid gives nitrogen and α–hydroxyacid.

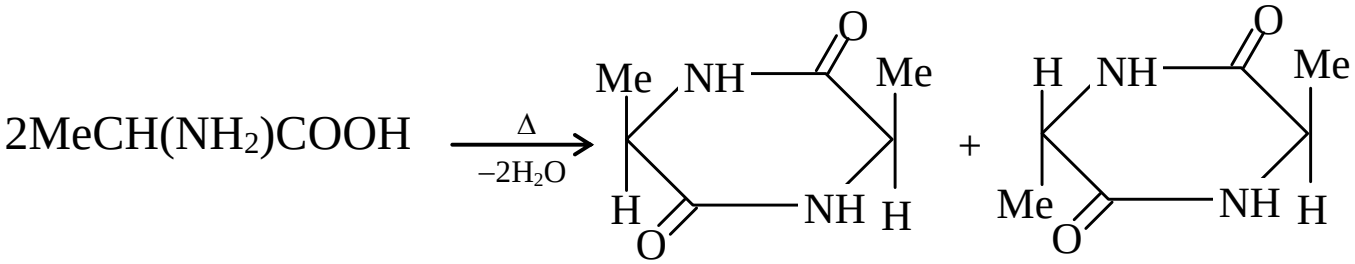
$$RCH(\overset{\oplus}{N}H_3)COO^{\ominus} + HNO_2 \longrightarrow RCH(OH)COOH + N_2\uparrow$$

Heating of amino acids leads to dehydration intermolecularly to form cyclic diamides.



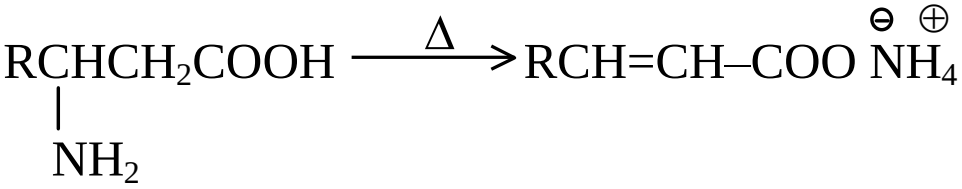
Diketopiperazine

When alanine is heated, then two diastereomers are obtained. One of them (trans) is not resolvable.



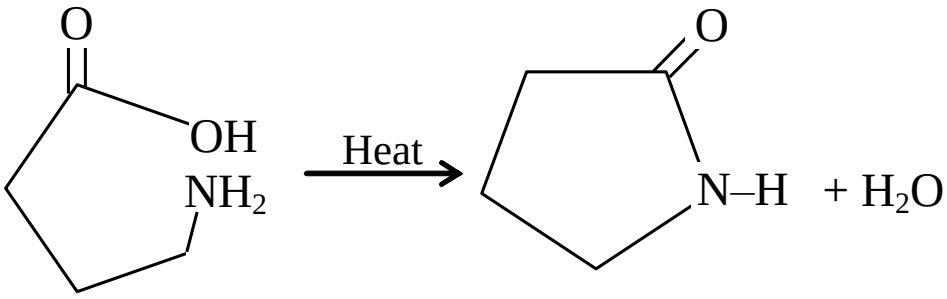
Cis (Racemic) Trans (Meso)

When β–amino acids are heated, α, β–unsaturated salt are formed.

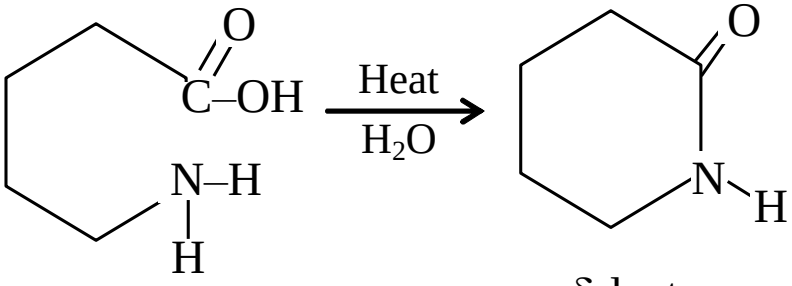


γ, δ, ε–amino acids when heated alone gives γ, δ–lactam and polymer respectively.

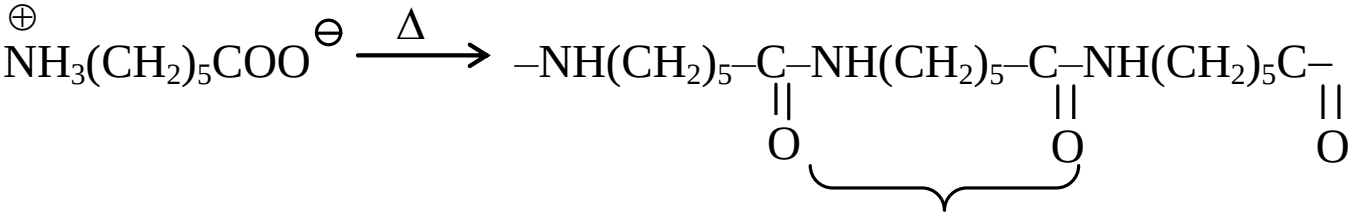
The reason for the formation of polymer is that when ε–amino cyclises intramolecularly, it leads to large angle strain within the compound.



γ-lactam

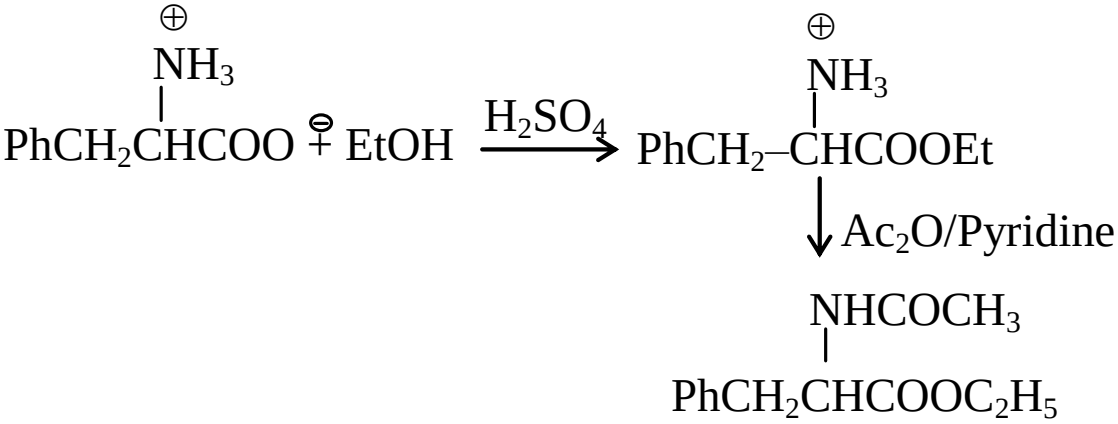


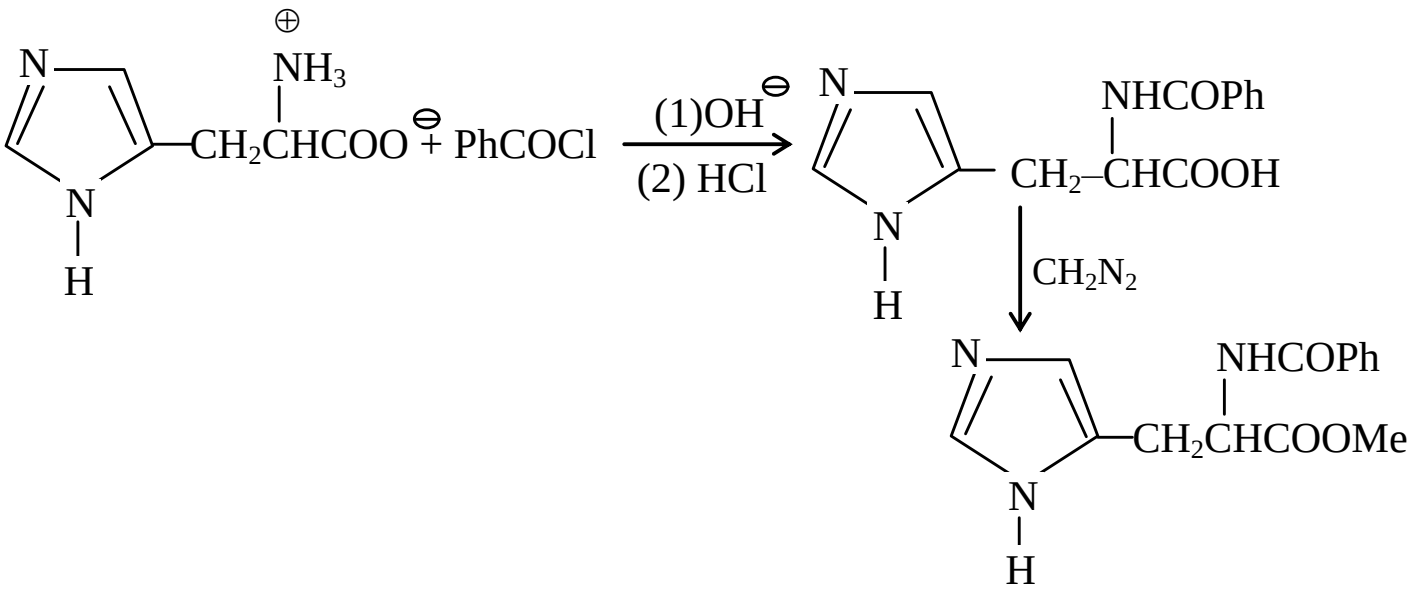
δ-lactam



mer of Nylon 6

N–acetyl phenyl alanine ethyl ester and N–benzoyl histidine methyl ester can be produced from the following amino acids.





Since Me esters can be made with CH_2N_2, acylation can occur first.

18. Peptide

A peptide is an amide formed by intermolecular reaction of the amino group of one amino acid and the carboxyl group of a second amino acid. Dipeptides are made from two amino acid's, tripeptides from three amino acid's, etc, which may be the same or different. If there are four to ten amino acid residues, the peptide is called an oligopeptide. A polypeptide is a chain made up of many amino acids. The terms peptide and polypeptide are often used interchangeably. A protein consists of one or more polypeptide chains and each chain can contain as many as several hundred amino acids. The total number of residues may vary from 50 to over 1000.

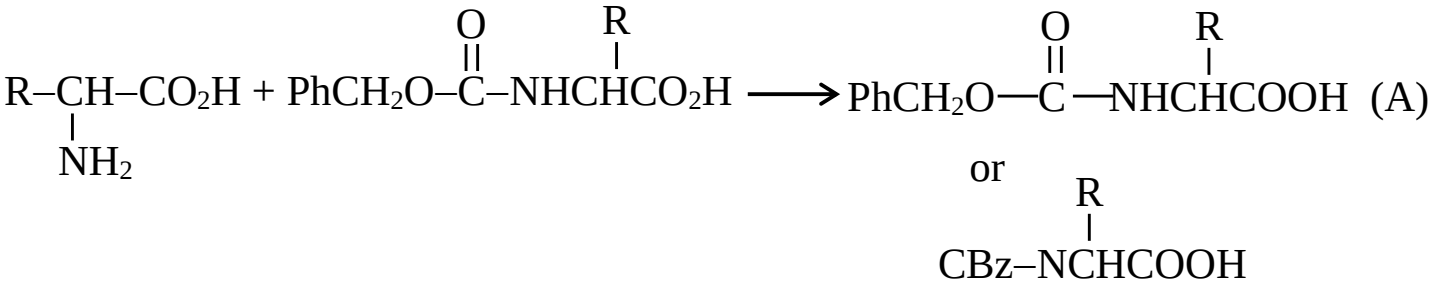
By convention, the amino acid with the free amino group (N–terminus) is written at the left end and the one with the unreacted carboxyl group (C–terminus) at the right end. The suffix –ine is replaced by –yl for each amino acid in the chain reading from left to right, followed by the full name of the C–terminal amino acid.

In peptides, the C–N bond has considerable double–bond characters arising from the delocalization of N's non–bonding e^-'s to the O of C=O.

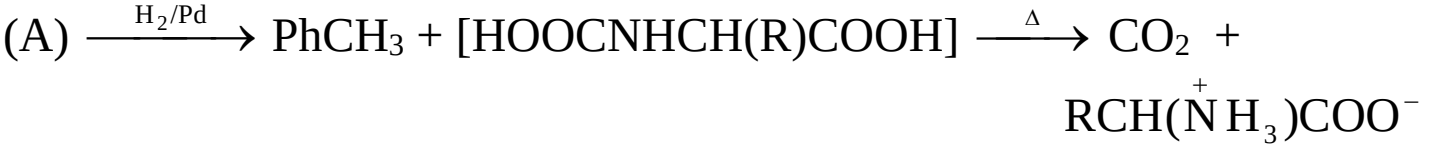
Firstly, the amino and carboxyl groups that are not to be linked in peptide bonds must be blocked so as to be unreactive. Then all other reactive

functional groups in the R's must also be protected, to prevent their participating in the coupling procedure. The coupling must be effected by a method that does not cause racemization or chemical alteration of the side chains. Finally, all protecting groups must be removed quantitatively by mild methods that do not cause rearrangements, racemization or cleavage of the peptide bonds.

Benzyl chlorocarbonate, $PhCH_2OCOCl$, also called carbobenzoxy chloride, (CBzCl), is a useful reagent for protecting the amino group of amino acid.

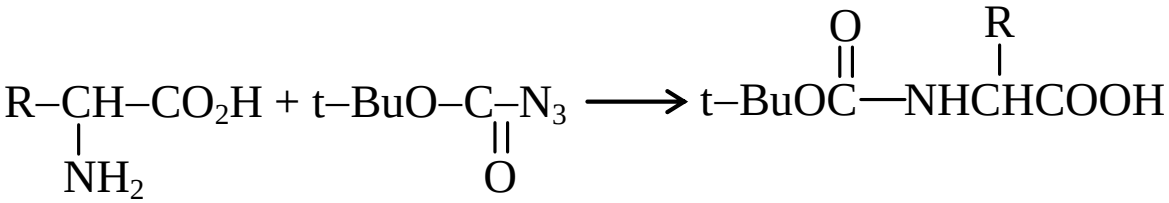


The product is a urethane (a carbamate), or half–ester and half–amide of carbonic acid. Catalytic hydrogenolysis cleaves the benzyl –O bond, forming an unstable carbamic acid, which decarboxylates.

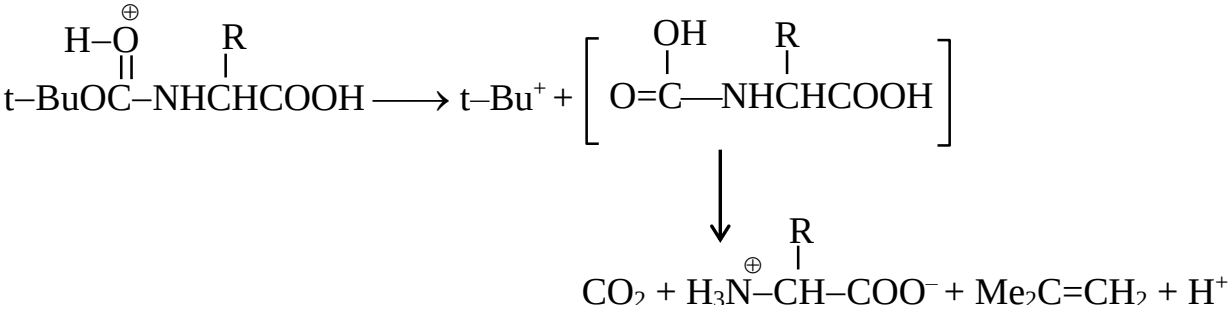


If the side chain contains S, the catalyst is poisoned and Na in NH_3 is used. HBr may also be used, provided it does not hydrolyse the peptide linkages.

Another protecting group for NH_2 is the *t*–butoxycarbonyl (Boc) group, $t\text{–}BuOC(=O)$–, introduced with t–butyl carbonate or t–butoxycarbonyl azide.



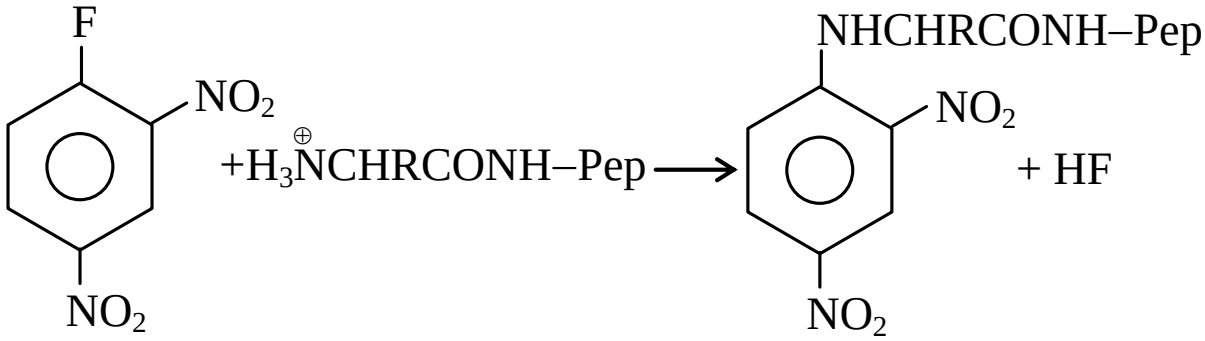
Since the group is a t–butyl ester, it is easily hydrolysed in anhydrous acid (CF_3COOH or HBr in HOAc). Cleavage occurs to give $t–Bu^+$ and a carbamic acid, which decomposes to CO_2 and the amino acid.



Heating with either strong acid or base hydrolyses all the peptide bonds, freeing the constituent amino acids. Selective partial hydrolysis may be affected by employing certain proteolytic (protein–breaking) enzymes. For example, trypsin only hydrolyses a peptide bond formed from the carboxyl group of lysine or arginine and chymotrypsin from the carboxyl group of phenylalanine, tyrosine and tryptophan.

Sanger reagent, 1–fluoro–2, 4–dinitrobenzene (DNFB), is a reagent that reacts with a peptide chain, $Pep–NHCOCHRNH_3^+$.

This reagent is used to identify the N–terminal AA of the peptide chain.



Acid hydrolysis of the N–DNP–peptide (where DNP is dinitrophenyl) gives individual amino acid's and an N–2, 4–dinitrophenylamine acid (N–DNP–AA, where AA is N–terminal), detected by its yellow color.

19. Proteins

Proteins are categorized according to (a) shape and (b) their biological function. Proteins according to shape are further classified as globular, somewhat spherical and fibrous, long fibres or planar sheets.

According to their biological action, they are classified as enzymes, hormones, antibodies, etc. A protein has, secondary, tertiary and quaternary structures. The primary structure is simply the amino acid sequence of the peptide chain. The secondary structure is a result of the different conformations that the chain can take. The tertiary structure is determined by any folding of the chain in on itself. A quaternary structure results when two or more peptide chains in some proteins are linked together by weak forces of attraction of their surface groups. Such proteins are called oligomers (dimers, trimers etc.).

The H–bonding in secondary structure exists between an N–H of one amino acid residue and the O=C of another properly situated amino acid residue. There are 3 types of secondary structures for protein. (1) The peptide sequence is coiled into a right–handed spiral in the α–helix, with the R groups positioned on the outside of the spiral. Each amide H–N bonds to the O=C on the next turn of the coil, four residues away by H–bonds, stabilizing this arrangement. (2) In the pleated sheet of β–structure, the peptide chains lie side–by–side in an open structure, with inter–chain amide H–bonding holding the chains together. Parallel pleated sheets have chains running in the same direction, all with their N–terminal residues starting at the same end. Antiparallel pleated sheets have their chains running in opposite directions. The α C's rotate slightly out of the plane of the sheet to minimize repulsions between their bulky R groups, giving rise to the crimps or pleats. In both cases, the R groups alternate positions above and below the sheet. (3) The random coil structure has no repeating geometric pattern; encompassed within it are sequences in a helical conformation, a pleated sheet conformation and regions that appear

to have no discernible repeating structure, but are actually not random conformations.

The unique three–dimensional shape of a protein in tertiary structure is the result of the intramolecular forces of attraction that cause bending and coiling in the helical coil. These forces are a function of the nature of the amino acid side chains within the molecule. Globular proteins have their non–polar R groups pointing to the interior (the hydrophobic or non–aqueous region) and their polar side chains projecting toward the aqueous environment, somewhat like a micelle. They are slightly water soluble. Fibrous proteins are insoluble in water. Their polypeptide chains are held together by inter–chain H–bonds. **The following are the attractive forces responsible for the tertiary structure:**

(1) Ionic: bonding between COO^- and NH_3^+ at different sites.
(2) H–bonding: mainly between side–chain NH_2 and COOH, also involving OH's (of serine, for example) and the N–H of tryptophan.
(3) Weakly hydrophobic van der Waal's attractive forces engendered by side–chain R groups and
(4) Disulfide cross linkages between loops of the polypeptide chain.

The same kind of attractive and repulsive forces responsible for the tertiary structure operate to hold together and stabilize the subunits of the quaternary structure.

Protein found in living system with definite configuration and biological activity is termed as native protein. If a native protein is subjected to physical or chemical treatment, which may disrupt its higher structures (conformations) without affecting its primary structure, the protein is said to be denatured. During denaturation, the protein molecule uncoils from an ordered and specific conformation into a more random conformation leading to precipitation. Thus denaturation leads to increase in entropy and loss of biological activity of the protein. The denaturation may be reversible or reversible. Thus, the coagulation of egg white on boiling of egg protein is an example of irreversible protein denaturation. However, in certain cases it is found that if the disruptive agent is removed the protein

recovers its original physical and chemical properties and biological activity the reverse of denaturation is known as renaturation.

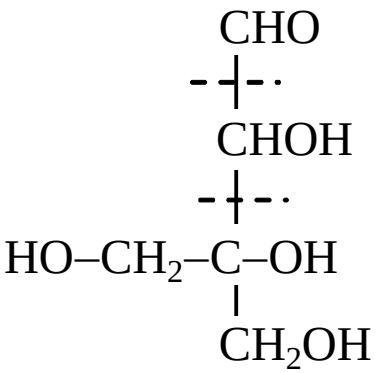
Fundamental Solved Examples

Example 1.

Compound (A) $C_5H_{10}O_5$, give a tetra–acetate with Ac_2O and oxidation of (A) with $Br_2–H_2O$ gives an acid, $C_5H_{10}O_6$. Reduction of (A) with HI and red phosphorous gives 2–methylbutane. What is structure of (A)?

Solution:

The formation of tetraacetate indicates presence of 4 OH groups and oxidation with bromine water indicates presence of CHO group. Reduction with red phosphorous and HI indicates presence of one carbon in the side chain. Thus, the structure of (A) would be



Example 2.

Compound (A) $C_5H_{10}O_4$, is oxidised by $Br_2–H_2O$ to the acid, $C_5H_{10}O_5$, which readily forms a lactone. (A) forms a triacetate with Ac_2O and an osazone with $PhNHNH_2$. (A) is oxidised by HIO_4, only one molecule of which is consumed. What is the structure of (A)?

Solution:

(A) contains three hydroxyl groups and an aldehyde group. Formation of a lactone shows that one hydroxyl group is in the γ– or δ–position with respect to the carboxyl group. Since (A) contains four oxygen atoms and these have been accounted for as three hydroxyl groups and an aldehyde group, the formation of an osazone shows the presence of the group

–CH(OH)CHO. Since only one molecule of periodic acid is consumed, (A) contains only one set of adjacent oxidisable groups. This must therefore be the CH(OH)CHO group and so the other CHOH groups must be 'separated' from each other and from the CHOH of CH(OH)CHO. Also, the absence of four hydroxyl groups in a five–carbon chain suggests (A) is a deoxy–compound. This 'deoxy–carbon atom' is therefore the one that 'separates' all the three CHOH groups. (A) structure which fits all the facts is

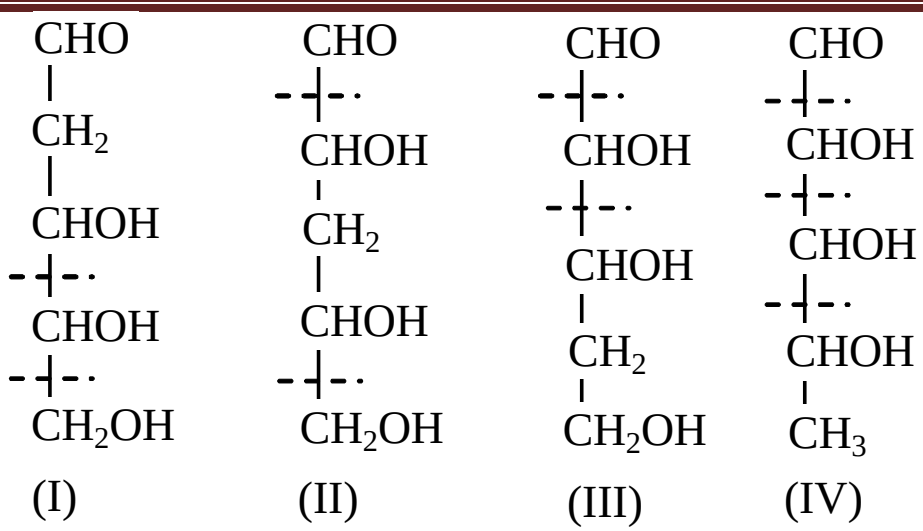
Example 3.

Compound (A) $C_5H_{10}O_4$, is oxidised by $Br_2–H_2O$ to the acid, $C_5H_{10}O_5$. (A) forms a triacetate (Ac_2O) and is reduced by HI to n–pentane. Oxidation of (A) with HIO_4 gives, among other products, 1 molecule of CH_2O and 1 molecule of HCO_2H. What are the possible structures of (A) and how could you distinguish between them?

Solution:

(A) is an aldehyde, contains three hydroxyl groups and the carbon skeleton consists of five carbon atoms in a straight chain. Also, the formula $C_5H_{10}O_4$ therefore suggests that (A) is a deoxy–sugar.

If we now try to work out the possibilities based directly on the periodic oxidation of (A), we shall find it difficult to get an answer. In a case like this, it is simpler to use what we have deduced so far and then see what can be deduced from the periodic acid oxidation. This approach is reasonable provided that the number of the possibilities is relatively small. Hence, we have (by systematically shifting the CH_2 group down the chain).



(I) (II) (III) (IV)

Now, the periodic acid oxidation produces one molecule of CH_2O and one molecule of HCO_2H. Inspection of the structures shows that (I) and (II), but not (III) and (IV), can give the required products. Hence, (A) is ether (I) or (II).

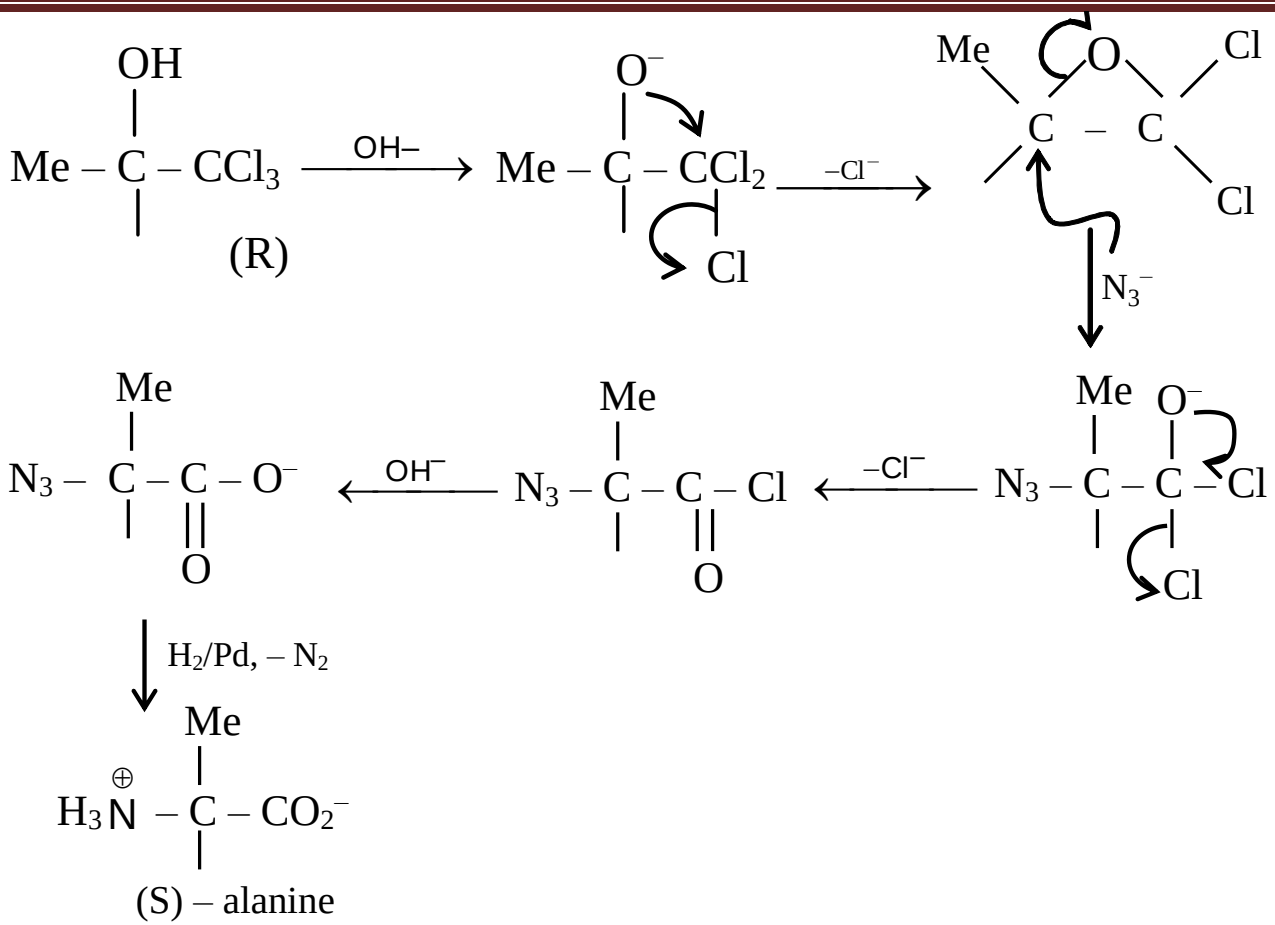
(I) and (II) may readily be distinguished by reaction with phenylhydrazine. (I) forms a phenylhydrazone, but (II) forms an osazone.

Example 4.

Treatment of (R)–$MeCH(OH)CCl_3$, first with alkaline NaN_3 and then reducing the product with H_2/Pd yields (S)–alanine, $CH_3CH(\overset{\oplus}{N}H_3)CO_2^-$. Explain.

Solution:

In alkaline solution, the OH will be converted to O^- which displaces Cl of adjacent carbon to form an oxirane ring. In the oxirane ring, the configuration of chiral carbon is still (R). The oxirane ring is then opened by nucleophilic attack of azide ion, which occurs with inversion of configuration i.e. configuration becomes (S). The acid chloride will be hydrolysed in alkaline conditions to give acid. This is followed by reduction of N_3 group to give (S)– alanine.



Example 5:

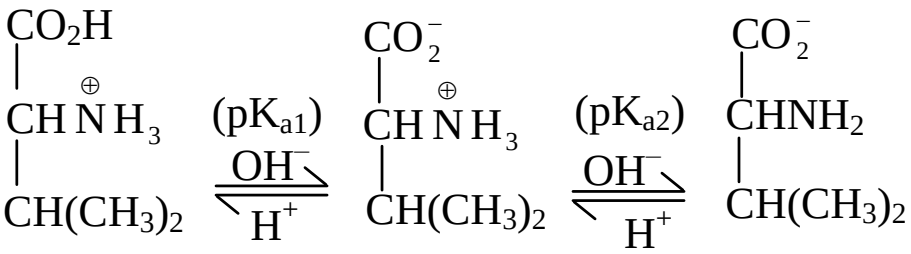
The pKa of the carboxyl group in an amino acid valine, $(CH_3)_2CHCH(NH_2)(COOH)$ is 2.31 and the pKa for the amino group of the same amino acid is 9.69. Compute the isoelectric point (pI) for valine and draw the structure of this amino acid when the pH of the solution equals to pI. Also draw the structures of valine that predominate at pH = 2 and pOH = 2.

Solution:

The isoelectric point (pI) is the pH at which the amino acid exists only as a dipolar ion with net charge zero.

At isoelectric point, for a neutral amino acid, $pI = \frac{(pK_{a_1} + pK_{a_2})}{2}$

The dissociation of cationic form of valine can be represented as

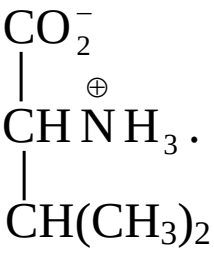


Net charge (+1) (0) (–1)

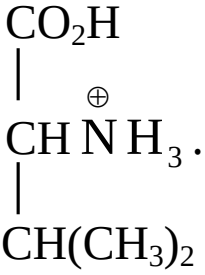
The species with zero net charge exists between species with (+1) and (–1) net charges.

$$pI = \frac{(pK_{a_1} + pK_{a_2})}{2} = \frac{9.69 + 2.31}{2} = 6$$

When the pH of the solution equals to pI, the structure of valine is



When the pH of the solution is two, the structure of valine is



When the pH of the solution is 12, the structure of valine is