NUCLEIC ACID

Nucleotides

These are the monomer units or building blocks of nucleic acids. They serve multiple additional functions. For example, they form a part of many coenzymes and serve as donors of phosphoryl groups (e.g. ATP or GTP).

PURINES, PYRIMIDINES, NUCLEOSIDES AND NUCLEOTIDES

Purines and pyrimidines are nitrogen-containing heterocycles, cyclic compounds whose rings contain both carbon and other elements (hetero atoms). Note that their six-atom rings are numbered in opposite direction (Figure 1). The planar character of purines and pyrimidines facilitates their close allocation or stacking which stabilizes double stranded DNA. The Oxo and amino groups of purines and pyrimidines exhibit heto-enol amine-imine tautomerism (Figure 2), but physiologic conditions strongly favour the amino and oxo forms.

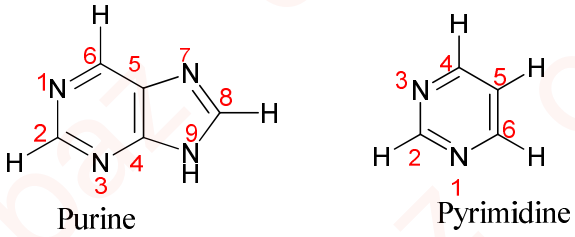


Figure 1: Purine and Pyrimidine atoms are numbered according to the international system.

Nucleosides and Nucleotides

Nucleosides

These are derivatives of purines and pyrimidines that have a sugar linked to a ring nitrogen numerals with a prime e.g. 2^1 or 3^1 to differentiate it from that of the heterocyclic base. For example, the sugar in ribonucleosides is D-ribose, and in that of deoxyribonucleosides is 2-deoxy-D-ribose. The sugar is linked to the heterocyclic base via a Beta-*N*-glycosidic bond, always to N-1 of a pyrimidine or to N-9 of a purine (Figure 3)

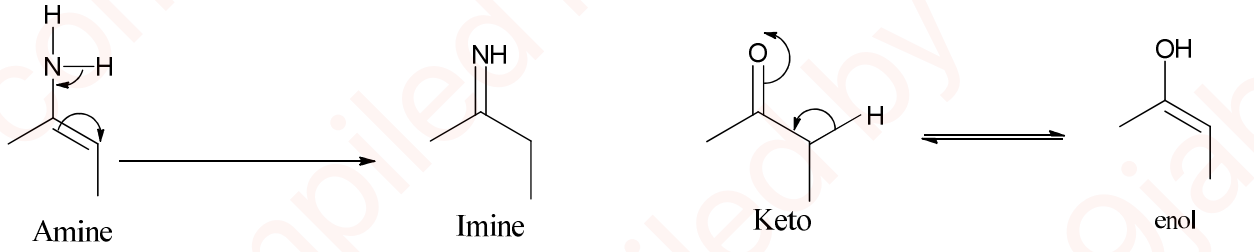


Figure 2: Tautomerism of the oxo and amino functional group of purines and pyrimidines

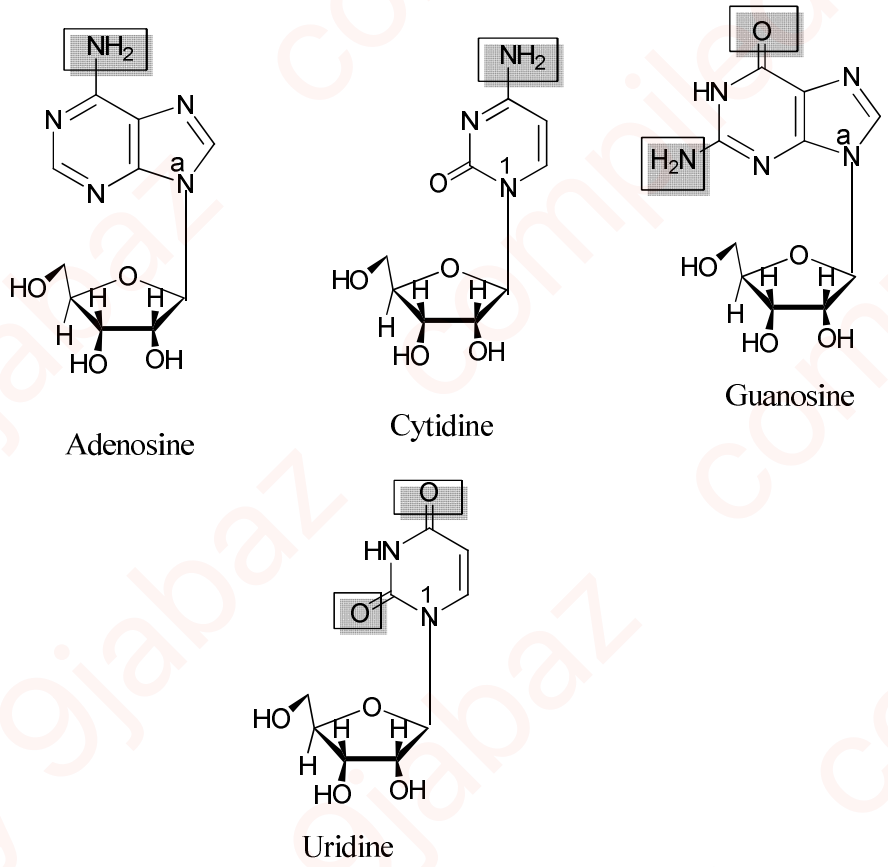


Figure 3: Ribonucleosides, drawn as the Syn conformers.

Nucleotides

Mononucleotides are nucleosides with a phosphoryl group esterified to hydroxy group of the sugar. For example, 3- and 5- nucleotides are nucleosides with a phosphoryl group on the 3^{1}-

or 5^1-hydroxy group of the sugar, respectively. Since most nucleotid76es are 5^1- is usually omitted from their names. Additional phosphoryl groups linked by acid anhydride bonds to the phosphoryl group of mononucleotide form nucleoside diphosphates and triphosphates (Fig 4)

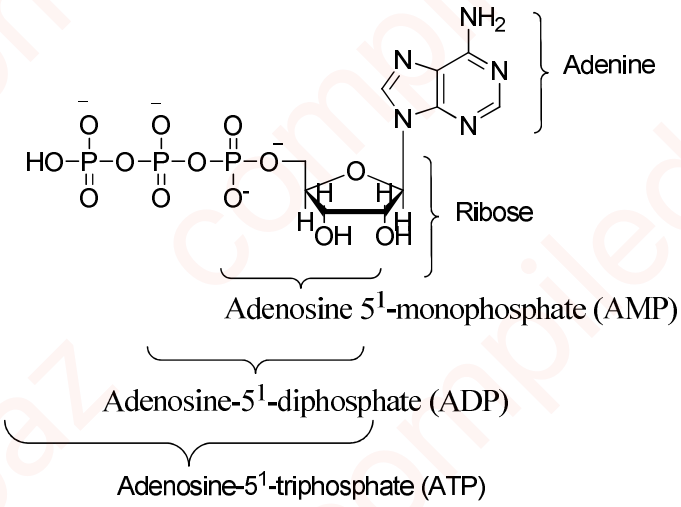


Figure 4: ATP, its di, tri and monophosphate**.**

Since steric hindrance by the base restricts rotation about the β-*N*-glycosidic bond of nucleosides and nucleotides both therefore exist as syn or anti conformers (Figure 5). Both conformers occur in nature but anti conformers predominate. The table below contain the major purines and pyrimidines and their nucleosides and nucleotides derivatives. The following abbreviations are used for bases Adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U), whether free or present in nucleosides or nucleotides. The prefix d' is (deoxy) indicates that the sugar is 2^i- deoxy-D-ribose (e.g. dGTP). (Figure 6)

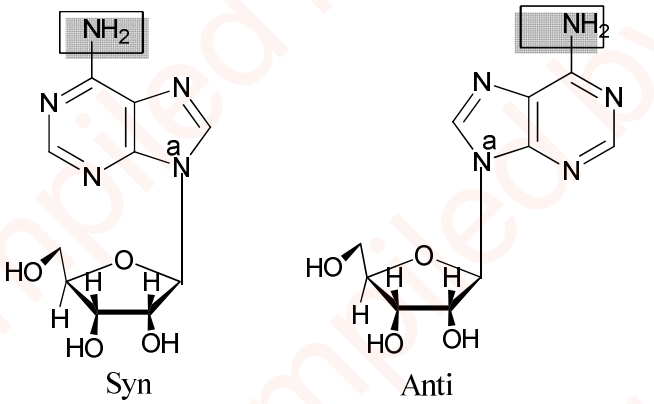
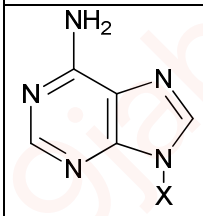
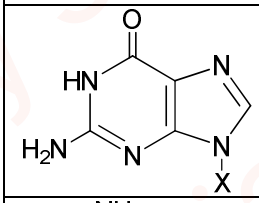
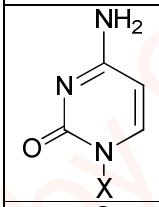
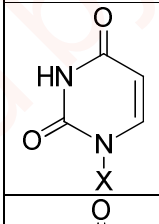
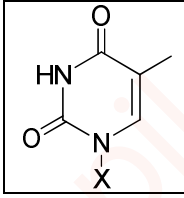


Figure 5: The Syn and anti-conformers of adenosine differ with respect to orientation about *N*-glycosidic bond.

Table 1: Bases nucleosides, and nucleotides

Base Formula	Base (X =H)	Nucleosides X= Ribose or Deoxyribose	Nucleotide, where X= Ribose phosphate
	Adenine (A)	Adenosine (A)	Adenosine monophosphate
	Guanine (G)	Guanosine (G)	Guanosine monophosphate (GMP)
	Cytosine (C)	Cytidine (C)	Cytidine monophosphate (CMP)
	Uracil (U)	Uridine (U)	Uridine monophosphate
	Thymine	Thymidine	Thymidine monophosphate (TMP)

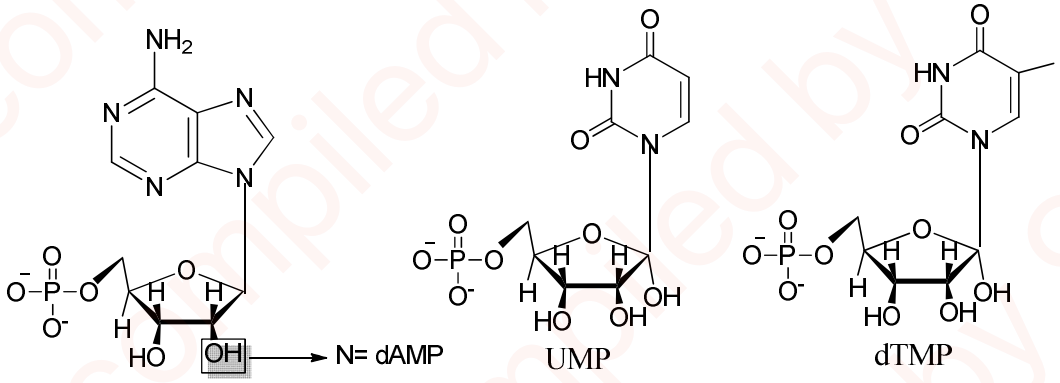


Figure 6: AMP, dAMP, UMP, dTMP

Additional Bases in Nucleic Acids.

Small quantities of additional purines and pyrimidine occur in DNA and RNA's. Examples are 5-methylcytosine of bacterial and human DNA, 5-hydroxymethylcytosine of bacterial and viral nucleic acids and mono- and di-N-methylated adenine and guanine of mammalian messenger RNAs (Figure 7)

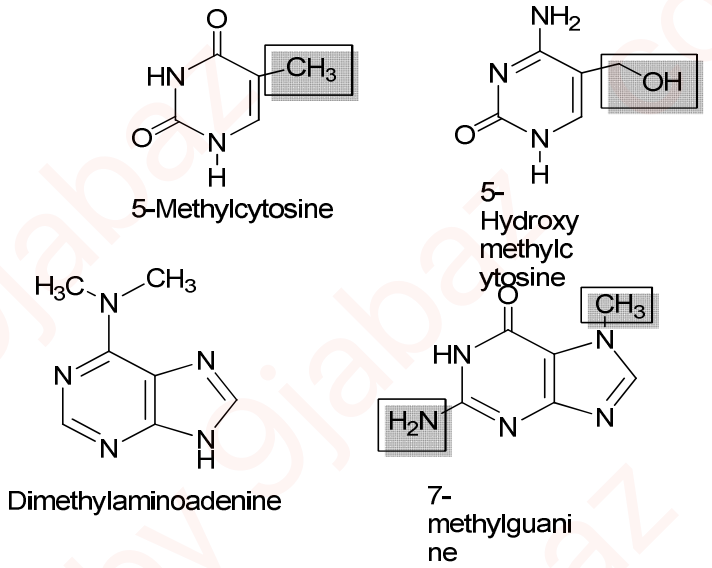


Figure 7: 4 common naturally occurring pyrimidines and purines

Physiological Functions of Nucleotides

They take part in reactions that fulfill physiological functions such as proteins synthesis nucleic acid synthesis, regulatory cascades and signal transduction pathways.

ATP serves as transducer of free energy adenosine 3^1-phosphate-5^1-phosphosulphate donor for sulphated proteoglycans and for sulphate conjugates of drugs and methyl group donor to 5-adenosylmethionine (Figure 11).

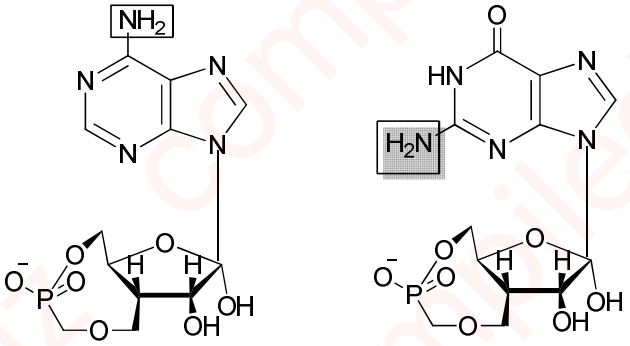


Figure 9: c AMP, $3^1,5^1$-cyclic AMP. And c GMP

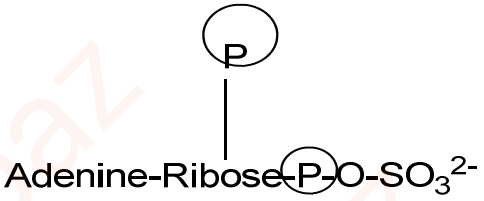


Figure 10: Adenine 3^1-phosphate-5^1-phosphosulphate.

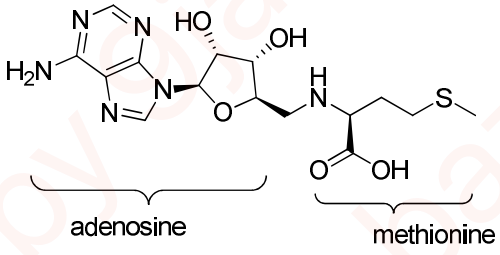


Figure 11: S-Adenosylmethionine

GTP serves as allosteric regulator as an energy source for protein synthesis, and cGMP serves as second messenger in response to nitric oxide (NO) during relaxation of smooth muscles.

UDP-sugar derivatives participate in sugar epimerization and biosynthesis of glycogen, glycosyldisaccharides and the oligosaccharides of glycoproteins and proteoglycans: UDP-glucuronic acid forms. The urinary glucuronide conjugates of bilirubin and of drugs such as aspirin.

CTP participates in biosynthesis of phosphoglycerides, sphingomyelin and other substituted sphingosines. Finally, many coenzymes incorporate nucleotides as well as structures similar to purines and pyrimidines nucleotides.

Properties of nucleotides

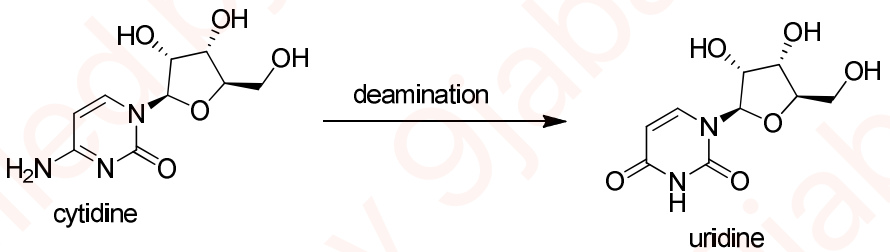
(1) They are more water soluble than their corresponding bases.

(2) They are relatively strong acids

(3) 5-phosphate group is relatively stable to acid hydrolysis; however, 5-positions can be easily hydrolysed by 5^1- nucleotilase.

(4) Tender components containing either the purine or pyrimidine bases can be easily detected because of strong absorption of uv light by these compound. Their mode of absorption obeys Beer Lamberts law and uv maximum for the based is about 260 nm

In general, purines bases nucleosides and nucleotides have stronger absorption at 260 nm than the pyrimidines and their derivatives. This differences can be used for the analysis of their compounds both quantitatively and qualitatively. For example, if cytosine is delaminated, a marked shift is caused in λ_{max} from 271 to 262 nm. (Figure 12)



λ 271 nm λ 262 nm

Figure 12

Synthetic Nucleotides Analogs-Their uses in Chemotherapy

Synthetic analogs of purines, pyrimidines nucleosides and nucleotides altered in either the heterocyclic ring or the sugar moiety have a numerous clinical application in medicine. They perform their functions by inhibiting enzymes essential of nucleic acids synthesis or their incorporating into nucleic acid resulting disruption of base pairing. Examples of synthetic pyrimidine and purine analogs used by oncologists are 5-fluro-, or any 5-iodouracil, 3-deoxyuridene, 6-thioguarines and 6-mercaptopurine, 5- or 6- azauridine or 5- or 6- cytidine, and 8-azaguanine (Figure 13). They are incorporated into DNA prior to cell division. for example, allopurinol an analog of purine is used in the treatment of hyperuricemia and gout where it inhibits purine biosynthesis and xanthine oxidase activity. Cytarabine is used in chemotherapy of cancer. Azathioprine which is catabolized to 6-mercaptopurine is used during organ transplantation to supposes immunologic rejection.

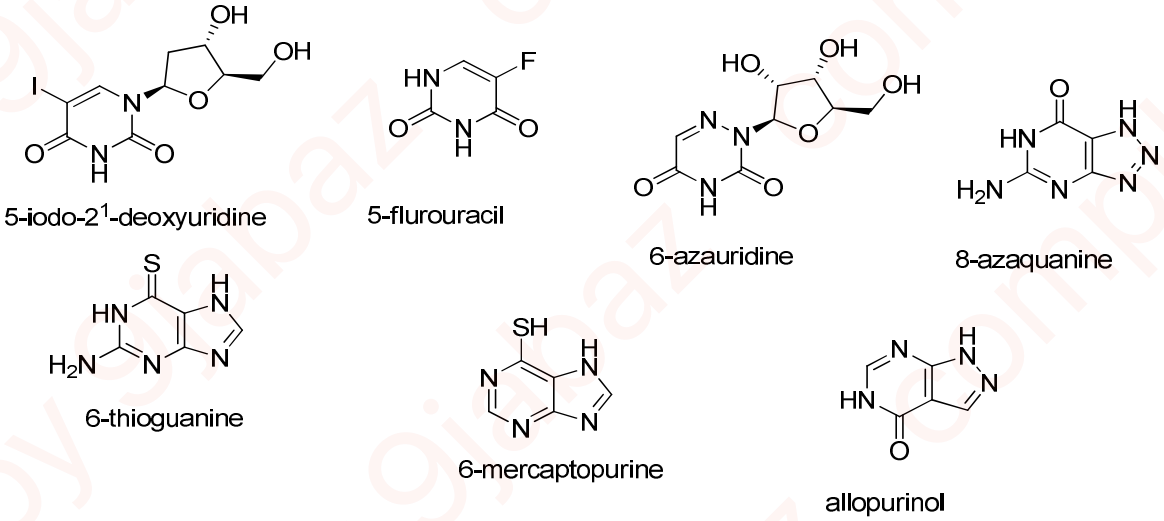


Figure 13: Selected synthetic pyrimidine and purine analogs

POLYNUCLEOTIDES

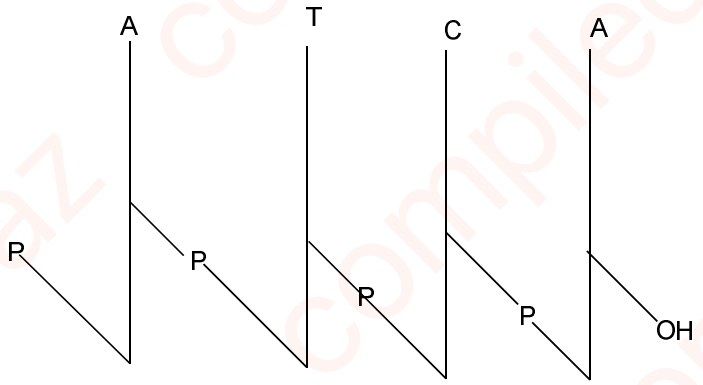
The 5^1-phosphoryl group of a mononucleotide can esterify a second – OH group, forming a phosphodiester. Most commonly, this second –OH groups is the 3^1 OH of the pentose of a second nucleotide. This leads to the formation of dinucleotide in which a sugar moieties

→

are linked by a 3^1 5^1 phosphodiester bond to form backbone of RNA and DNA. RNA's are far less stable than DNA since the 2^1- OH groups of RNA (absent from DNA) functions as a nucleophile during hydrolysis of $3^1,5^1$-phosphodiester bond.

The primary structure of polynucleotides

The base sequence that is primary structure of polynucleotide can be represented as given below. The phosphodiester bond is represented by P or p, bases by simple letter and pentose by a vertical line.



Here all the phosphodiester bonds are 5 3^1, a more compact notation is possible. This representation indicates that 5^1- hydroxy but not 3^1- hydroxy is phosphorylated. The most compact representation shows only the base sequence with the 5^1-end on the left and the 3^1-end on the right. The phosphoryl group are assumed but not shown.

G G A T C A

Nucleic Acid Structure and Function

Biomedical important

The discovery that genetic information is codded along the length of a polymeric molecule made up of four types of monomeric units was a major scientific breakthrough in the twenty century. This molecule is called DNA and is organized into genes, the fundamental units of genetic information's. The basic information's pathway i.e. DNA directs the synthesis of RNA which in turn direct the synthesis of protein has been

elucidated. Genes do not function autonomously but their replication and function are controlled by various gene products, often in collaboration with components of various signal transduction pathways knowledge of the structure and functions of nucleic acids is essential in understanding genetics and many aspects of pathophysiology as well as the genetic basic dieses

DNA contains the genetic information

The evidence that DNA contained the genetic information was first made in 1944 by Avery, MacLeod, and McCarty. All the experiment conducted revealed DNA to be donor of genetic information. DNA contain four Deoxynucleotides. The monomeric units of DNA are deoxyadenylate, deoxyguanylate, deoxycytidylate and thymidylate. These monomeric units of DNA are held in polymeric form by $3,5^{1}$-phosphodiester bridges constituting single strand (Figure 14). The information content of DNA (the genetic code) resides in the sequence in which these monomers- purine and pyrimidine deoxyribonucleosides are ordered. The polymers possess polarity one end had a 5^{1}-hydroxyl or phosphate terminal while the other has 3^{1}-phosphate or hydroxy terminal. Since the genetic information resides in the order of the monomeric units within the polymers, hence there must be a mechanism of reproducing or replicating this specific information with a high degree of fidelity. X-ray analysis of DNA revealed that the concentration of deoxyadenosine (A) nucleotide equals that of thymidine (T) nucleotide (A = T), while the concentration of deoxyguanosine (G) nucleotide equals that of deoxycytidine (C) nucleotide (G = C), this revelation led to the proposal of a model of a double stranded DNA molecule (Figure 15). The two strands of this double-stranded helix are held by hydrogen bonds between the purine and pyrimidine bases of the respective linear molecules. The pairing between the purine and pyrimidine nucleotide on the opposite stands are very specific and depended upon hydrogen bonding of A with T and

G with C (figure 16). DNA is right handed because the double helix is restricted to the following A&T only and G&C only.

This pairing explains the earlier observation that in a double stranded DNA molecule A = T and G = C. the two strands of the double helical molecule, each of which possess a polarity are anti-parallel i.e. one strand runs in 5^1 to 3^1 direction and other 3^1 to 5^1 direction.

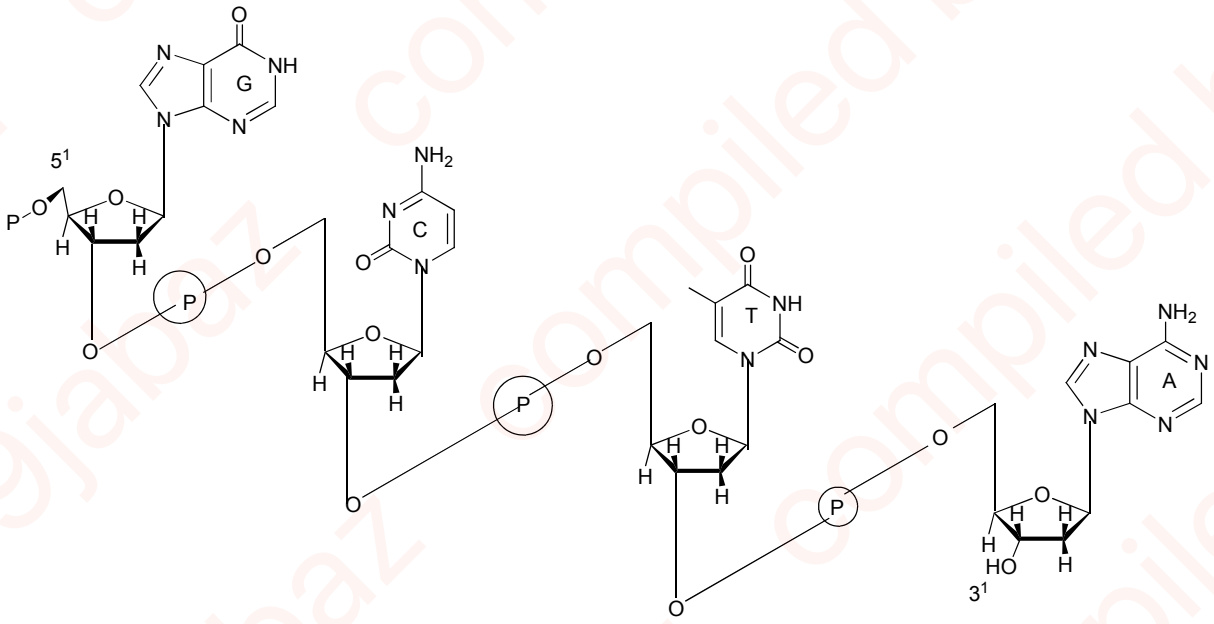


Figure 14: Segment of one strand of a DNA molecule in which purine and pyrimidine bases guanine (G), cytosine (C), thymine (T), and adenine (A) are held together by a phosphodiester back bone between 2^1-deoxyribosyl nucleotide attached to the nucleobases by a *N*-glycosidic bond.

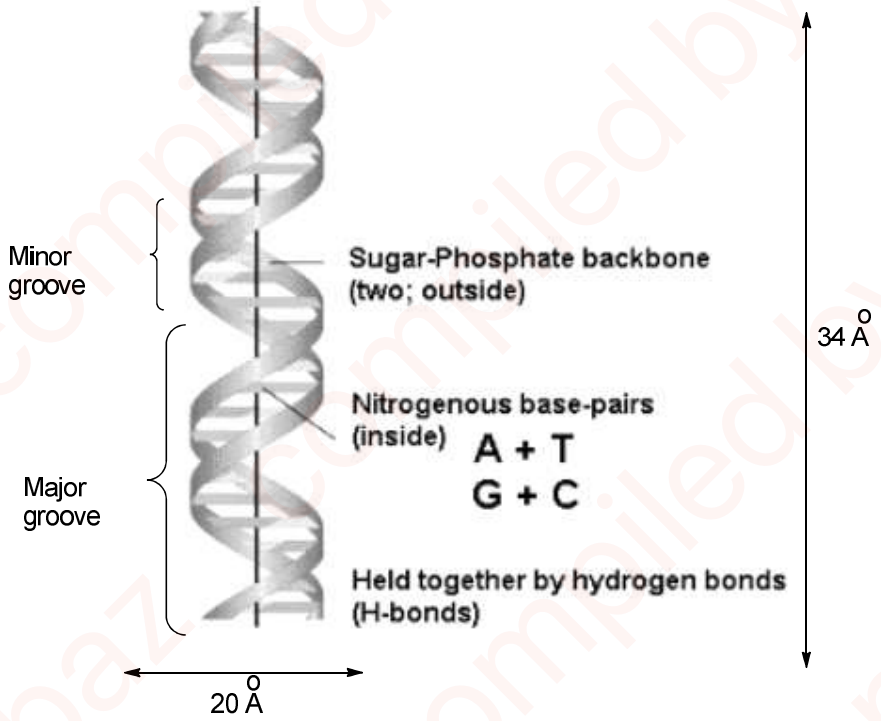


Figure 15: A diagrammatic representation of the Watson and Crick model of the double helical structure of the B form of DNA. The horizontal arrow indicates the width of the double helical helix and the vertical arrows indicates the distance spanned by one complete turn of the double helix (34 Å). One turn of B- DNA includes ten base pairs, so the rise in 3.4 Å per bp the central axis of the double helix is indicated by the vertical rod. The short arrows designate the polarity of the anti-parallel strands. The major and minor grooves are depicted. (A) adenine (C) cytosine. G guanine, T, thymine; P phosphate; S, sugar (deoxy ribose)

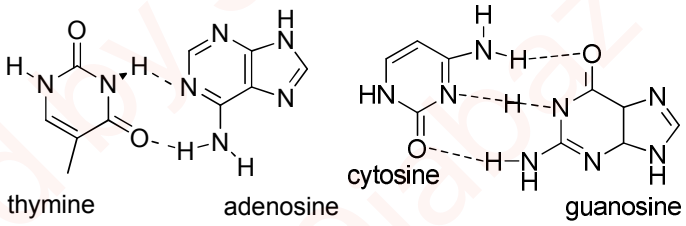


Figure 16: Bases pairing between deoxyadenosine and thymidine involves the formation of two hydrogen bonds. Three such bonds are formed between deoxycytidine and deoxyguanosine. The broken line represents hydrogen bonds.

This is analogous to two parallel streets, each running one way but carrying traffic in opposite direction. In the double stranded DNA molecules, the genetic information resides in the sequence of nucleotides one strand, the template strand. This template strand is copied during nucleic acid synthesis. It is sometimes referred to as the noncoding strand. The opposite strand is considered the coding strand because it matches the RNA transcript that encoded the protein.

Two strands are held together by hydrogen bonds between opposing bases. As seen in figure 16, three hydrogen bonds hold the deoxyguanosine nucleotide to the deoxycytidine nucleotide, whereas the other pair A-T pair is held together by two hydrogen bonds. Thus, G-C bond are more resistant to denaturation or melting than A-T rich regions.

Structural analysis of DNA by denaturation (melting)

The double stranded structure of DNA can be separated into two strands (melted) in solution by increasing the temperature or decreasing the salt concentration, this lead to the pulling apart of the two stacks of bases while the bases are still connected in the polymer by phosphodiester back bone. This denaturation of the DNA molecule leads to an increase in the optical absorbance of the purine and pyrimidine bases- a phenomenon referred to as hyperchromicity of denaturation. The double stranded DNA molecules exhibit properties of rigid rod and in solution is a viscous material that loses its viscosity upon denaturation. The separation of the strands of a given DNA molecule over a temperature range. The mid-point is called the melting temperature (T_m). it is affected by the base compositions of the DNA and the salt concentration of the solution. DNA and the salt concentration of the solution. DNA that is rich in G-C pair melts at a higher temperature than those rich in A-T pairs. A tenfold increase in monovalent cation concentration increases the Tm by

16.6 ^{o} C on the other hand destabilizes hydrogen bonding between bases, hence lowering the Tm. This allow the strands of DNA or DNA-RNA hybrids to be separated at much lower temperature there by reducing phosphodiester bond breakage that occurs at high temperatures.

DNA Function as a template for Replication and Transcription

The genetic information stored in the nucleotide sequence if DNA serves two purposes:

(1) It is the source of information for the synthesis of all pattern molecules of the cell and organisms and

(2) It provides the information inherited by daughter cells or offspring's. Both functions given above require that the DNA molecule serves as a template in the first case for the transcription of the information into RNA and in the second case for the replication of the information into daughter DNA molecules.

The complementary of double stranded of DNA suggest that replication of the DNA molecule occurs in a semi conservative manner. Thus when each strand of the double stranded parental DNA molecule separated to form its compliment during replication each serve as a template on which a Nero-complementary strand is synthesized (see figure 4). The two newly formed double stranded daughter DNA molecule each containing one strand (but complementary rather than identical) from the parent double-stranded DNA molecule (see figure 5). Each daughter cell contains DNA molecule with information identical to that which the parent possessed yet in each daughter cell the DNA molecule of the parent cell has been only semi conserved.

The chemical Nature of RNA differs from that DNA

RNA is a polymer of purine and pyrimidine ribonucleotides linked together by $3^{1},5^{1}$-phosphodiester bridges analogues to those DNA. It shares many features with DNA but it possesses served specific differences.

1 The sugar moiety in RNA to which the phosphate and bases are attached is ribose rather than 2^1-deoxyribose of DNA

2 The pyrimidine components of RNA differ from those of DNA. For examples DNA contains A, G, T and C while RNTA contains A G C t that instead of thymine, RNA contains ribonucleotide of uracil.

3 RNA exists as a single strand rather than double strand in DNA. However, given proper complementary base sequence with opposite polarity, the single strand of RNA is capable of folding back itself like hairpin and these acquiring double-stranded characteristics

4 Since the RNA is a single strand its G does not necessary equal its C content nor does it's A equal it content.

5 RNA can be hydrolysed by alkali to $2^1 3^1$ cyclic diesters Fig 17 mononucleotide compounds that cannot be formed from alkali-treated DNA because of the absence of a 2^1-hydroxyl group. The alkali lability at RNA is useful both diagnostically and analytically.

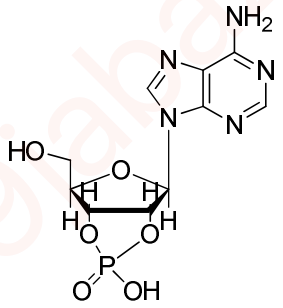


Figure 17: Adenosine-2^1,3^1-cyclic phosphoric acid

RNA and Protein Synthesis

RNA and Protein Synthesis

The cytoplasmic RNA molecules that serve as templates for protein synthesis (i.e. those tracts transfer genetic information from DNA to the protein synthesizing machinery are called messenger RNAs, (mRNA). Many other cytoplasmic RNA molecules (ribosomal RNAs, i.e. mRNAs) have structural roles wherein they contribute to the formation and function of ribosomes (the organelle machinery involves in protein synthesis) or serve as adapter molecules for the translation of RNA information into specific sequences of polymerized amino acids

Hydrolysis of Polynucleotides

Nucleic acids can be hydrolysed by treatment with enzyme, acid or alkali. It is possible to identify nucleic acids (RNA or DNA) in the base of their sensitively to above regents.

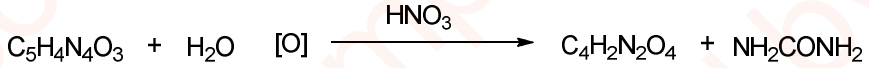
(a) Acid and base hydrolysis

In the case of DNA, gently hydrolyses with 0.1M HCl at pH 3.0 causes selective hydrolytic removal of its purine bases without affecting the N-glycosidic bond of pyrimidine bases or phosphodiesters bonds of the back bone. The resulting DNA derivatives devoid of the purine bases is called apurinic acid. Selective removal of pyrimidine bases by different chemical conditions produces a pyrymidinic acid. Heating of DNA at 110 degrees in 1.0 M HCl for 1 hr gives purine and pyrimidine bases, deoxyribose and phosphate. DNA is not hydrolysed by bases (0.3M NaOH), but RNA does. Research shown that OH group of 2^1 of D-ribose is required for alkaline hydrolysis of RNA. The presence of base attacks the P-O-C_2 and P-O-C_3 linkages equally yielding an equimolar mixture of $2^1 3^1$-nucleoside monophosphate. The cyclic monophosphate appears to be intermediate in alkaline hydrolysis of RNA and Since DNA has no 2^1-OH group hence cannot form 2^1 and 3^1-cyclic monophosphate hence cannot be hydrolysed by pancreatic ribonuclease.

THE SYNTHESES OF SOME PURINES

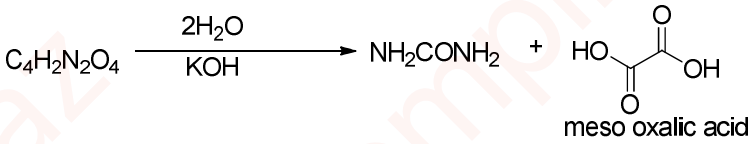
1. Uric acid

This acid is a derivatives of purine found in the human urine and dropping of some birds. Its molecular formula is $C_5H_4N_4O_3$. Its oxidation with HNO_3 gives equal amount of alloxan and urea.

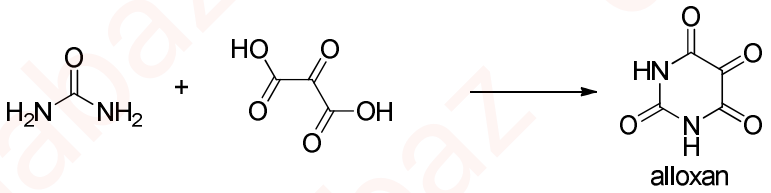


Proof of the structure of alloxan ($C_4H_2N_2O_4$)

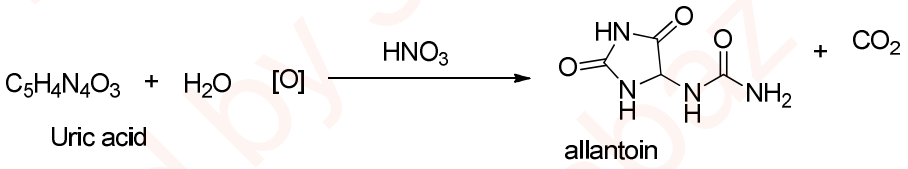
Hydrolysis with alkali



Since alloxan has no free NH_2 and –COOH groups, the product of alkali hydrolysis above shows that it is mesoxaly urea. This was confirmed by the reaction between mexooxalic acid

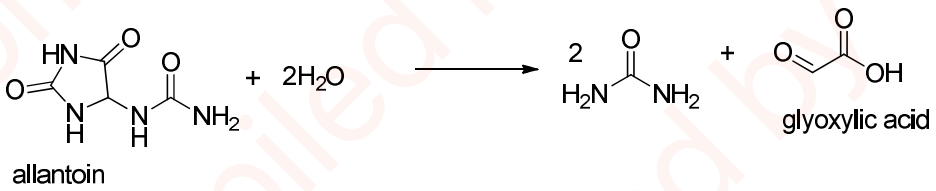


When uric acid is oxidized in aqueous solution of lead dioxide, allantoin and cabondioxide are obtained

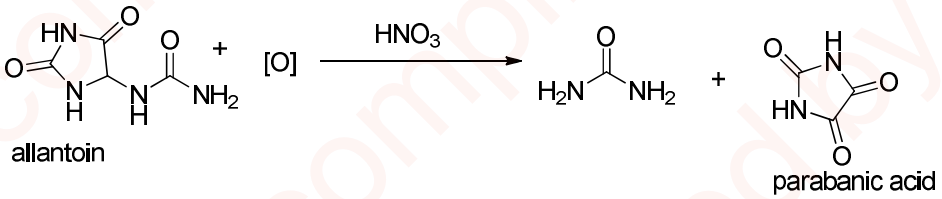


Structure of allantoin in ($C_4H6N_4O_3$)

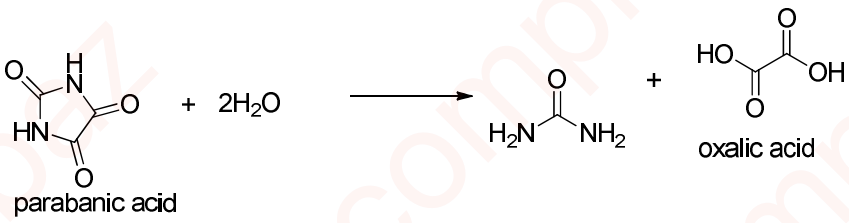
i. Alkali hydrolysis of allantoin gives:



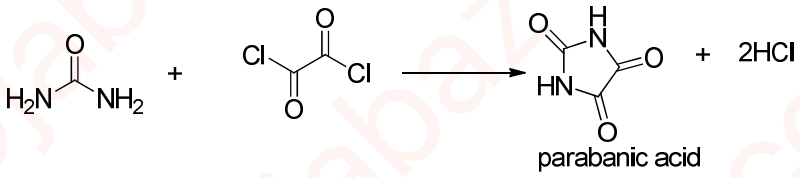
ii. Its oxidation with HNO_3 gives:



iii. Hydrolysis of parabanic acid gives:

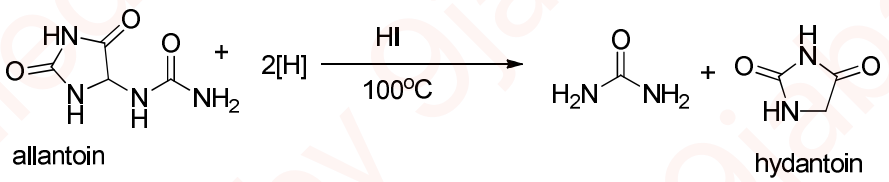


The structure of parabanic acid was confirmed by its synthesis from the condensation reaction between oxaly chloride and urea.

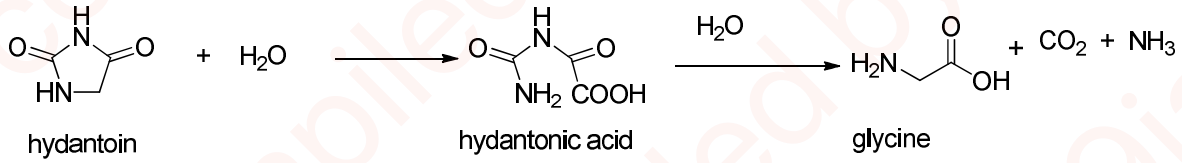


The above information showed that allantoin contains parabanic acid joined to a molecule of urea. The point of the attachment is obtained by the following experimental evidence:

Reduction with HI

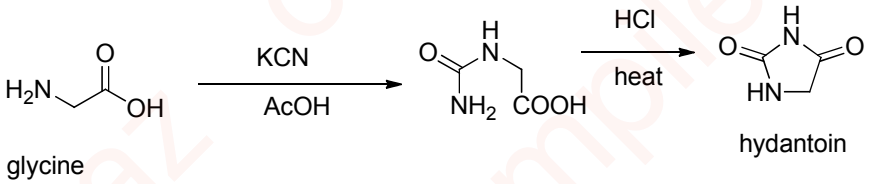


iv. Controlled hydrolysis of hydantoin gives:

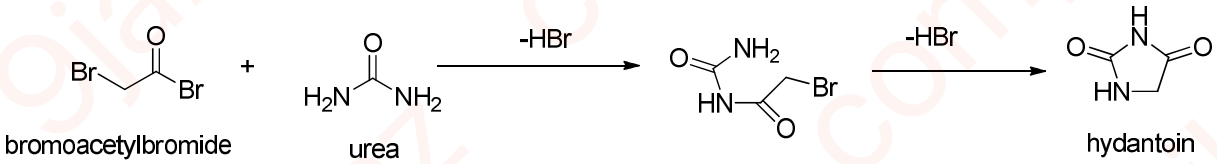


+

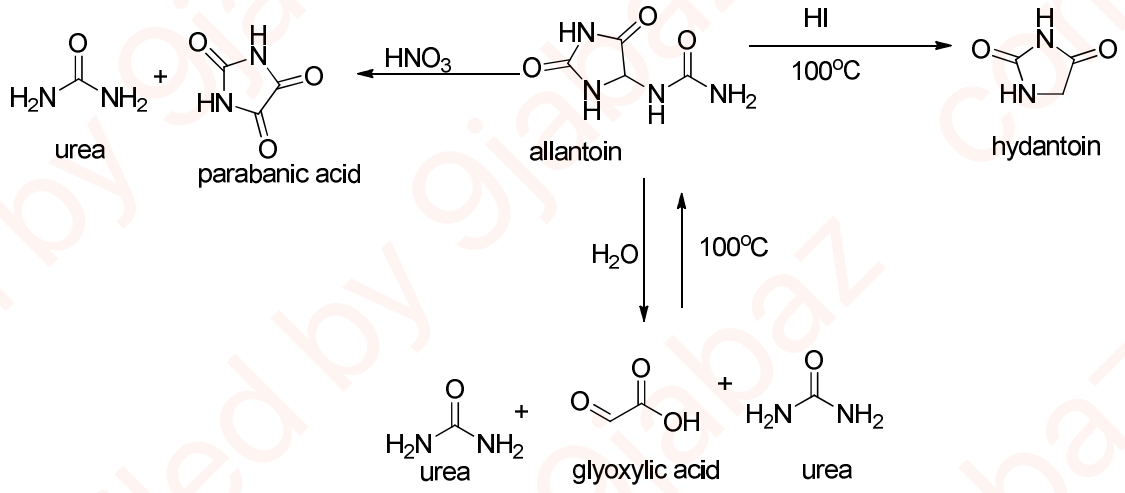
This shows that hydantoin is glycollylurea. The synthesis of hydantoin by West (1918) has been used to confirm its structure thus:



v. It can also be prepared from the reaction between bromoacetylbromide and urea:

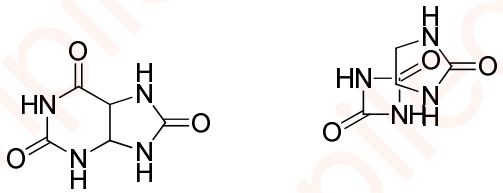


vi. All the above reaction and the reactions at allantoin will account for its structure.



vii. The oxidation of uric acid gives allantoin where uric acid lost one carbon atom as CO_2. To know the structure of uric acid we need to fix the one carbon back. The structure given to

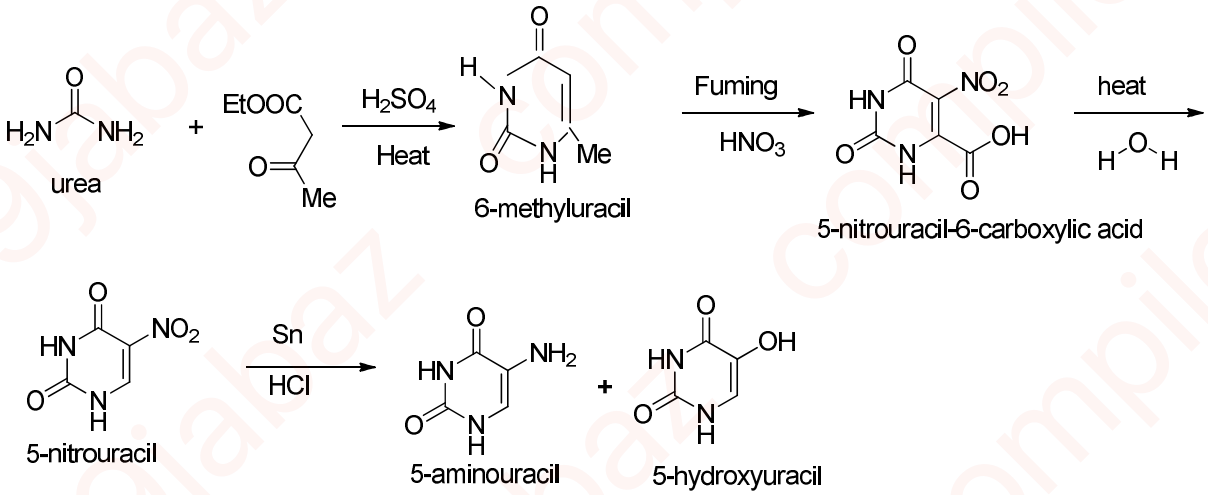
uric acid must also include alloxan skeleton since its oxidation with HNO_3 above gave alloxan:



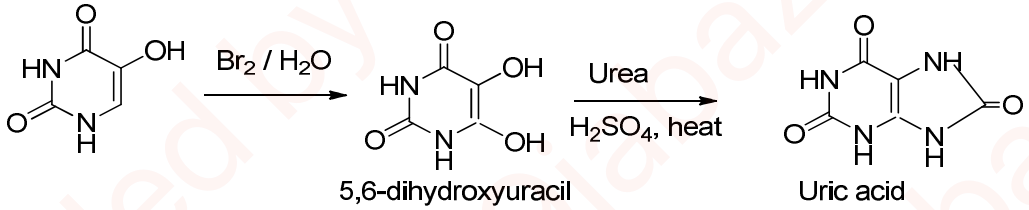
Medicus fomula 1875 Fittig formula 1878

Studies by other workers e.g. Fischer (1884) supported Medicus formula while Fittig formula was shown to be untenable. The proof of the Medicus formula was achieved by the synthesis of uric acid. Three methods are given below.

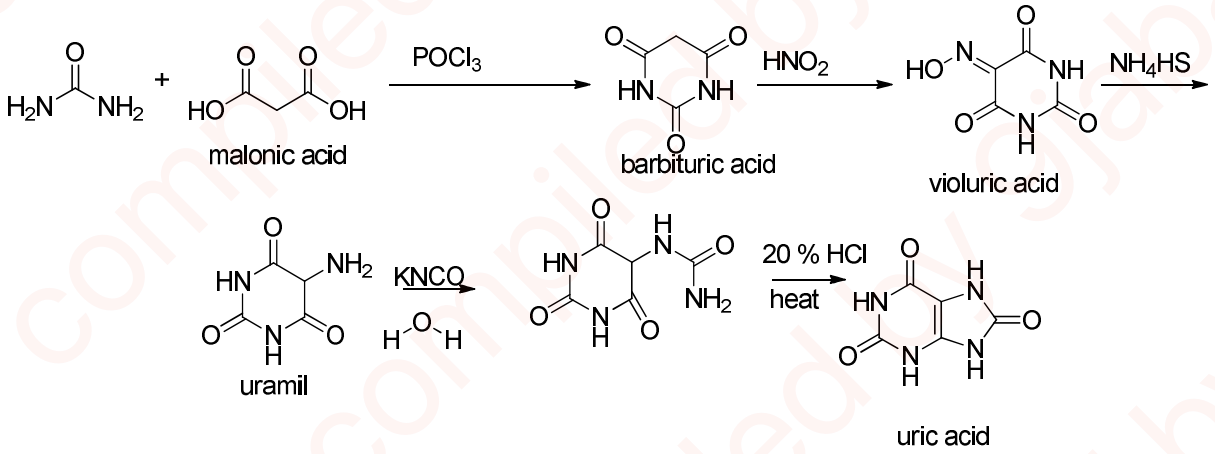
(1) Behrend and Roosen (1888) synthesis of uric acid



viii. The reduction section led to the conversion of some aminouracil into hydroxyuracil. Nitrous acid was used to convert 5-aminouracil into 5-hydroxyuracil.



(2) Fischer (1885) synthesis of uric acid



1.0 CHEMISTRY OF CARBOHYDRATES (SECTION ONE)

This section gives a simple treatment of the Chemistry of Carbohydrates. It provides concise information on this class of Chemistry and suitable for beginners.

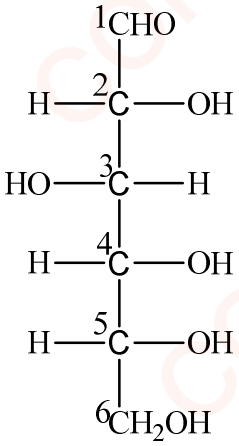
Carbohydrates are natural products with general a formula $Cn(H_2O)n$. Other names for carbohydrates are also saccharides. This very large family includes monomers - called simple sugars or monosaccharides up to very large molecules (complex) called polymers which are made up of these simple sugars. The polymers are also called polysaccharides such as starch, cellulose, dextrin and glycogen.

1.1 *Classification and nomenclature of monosaccharide*

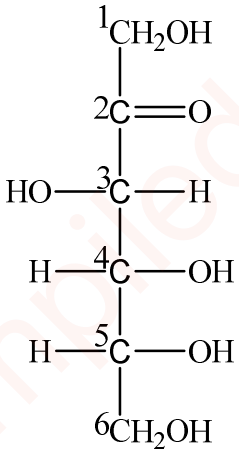
1.1.1 *Monosaccharides*

Monosaccharides are chiral polyhydroxyalkanals or polyhydroxyalkanones which are the cylic hemiacetal forms of these sugars. This class of saccharide is divided into two major groups depending on whether their acyclic forms contain an aldehyde functional group or a keto functional group which are aldoses or ketoses, respectively.

Aldoses and Ketoses: All monosaccharides have their names end with ***"ose"*** hence, they are divided into aldoses that is aldehyde plus ose) and ketoses (ketone plus ose). For example, glucose is an, aldose while fructose is a ketose (Figures 1.0 a & b).



Glucose "a" (an aldose)



Fructose "b" (a ketose)

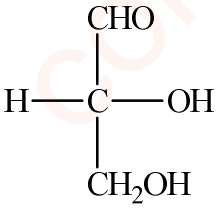
Figure 1.1 a & b: Structures of D-glucose and D-fructose

The above structures are the ***Fischer projections*** of these monosaccharides. The aldehyde functional group (carboxaldehyde carbon) of an aldose is assigned number 1 and the primary alcohol group (-CH_2OH) assigned the last number.

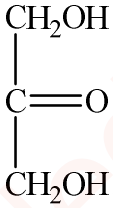
The simple sugars can also be classified according to the number of carbons that they contain. Both glucose and fructose given in Figure 1.1 a & b above contain six carbons each, hence they are both **hexoses**. If both classifications (functional groups and number of carbons) are combined, then glucose will become an **aldohexose** and fructose a **ketohexose**.

1.2 *Glyceraldehyde and dihydroxyacetone*

The simplest carbohydrates - glyceraldehyde and dihydroxyacetone, which are **aldotriose** and a **ketotriose**, respectively (Figure 1.2 a & b). All series of aldoses and ketoses are built from glyceraldehyde and dihydroxyacetone, respectively.



glyceraldehyde "a"
(an aldotriose)

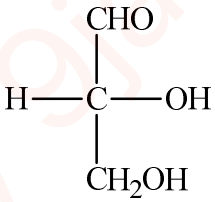


dihydroxyacetone "b"
(a ketotriose)

Figure 1.2 a & b: Structures of a glyceraldehyde and dihydroxyacetone

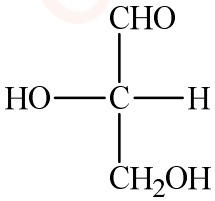
1.3 *D - and L - Monosaccharides*

Using Fischer projection, the first carbon is a carbonyl carbon for aldose and the second carbon is carbonyl for ketose. If the last **chiral carbon** has its hydroxyl group on the right, the sugar is designated **D - sugar** but if it is on the left, it is a **L - sugar**. See Figure 1.3.1 a & b below:



(+) - D - glyceraldehyde "a"

(+) - (R) - glyceraldehyde



(-) - L - glyceraldehyde "b"

(-) - (S) - glyceraldehyde

Fig 1.3.1 a & b: Structures of D- and L - glucose

The Figure 1.3 a & b shows that L - Glucose is an enantiomer of D - Glucose.

The simplest sugar is glyceraldehyde ($C_3H_6O_3$). Because it contains three carbons, it is a triose, and it is an aldotriose because its first carbon is an alkanal.

Emil Fischer assigned the configuration (-) - glyceraldehyde to glyceraldehyde drawn using Fischer projection if the hydroxyl group of the only chiral carbon is on the left which called **"L"** (*laevo*). When the hydroxyl group is on the right of the chiral carbon, he called it **"D"** (*dextro*).

These two enantiomers of glyceraldehyde were later assigned **"S"** (*sinister*) for L - configuration and **"R"** (*recutum*) for D - configutation.

Any molecule that contains n chiral centres will have 2^n stereoisomers provided no meso compounds are present. For example, if there two chiral centres, hence $2^2 = 4$, which means four carbon sugars (aldotetrose). Their structures are given in Figure 1.3.2 a, b, c & d below:

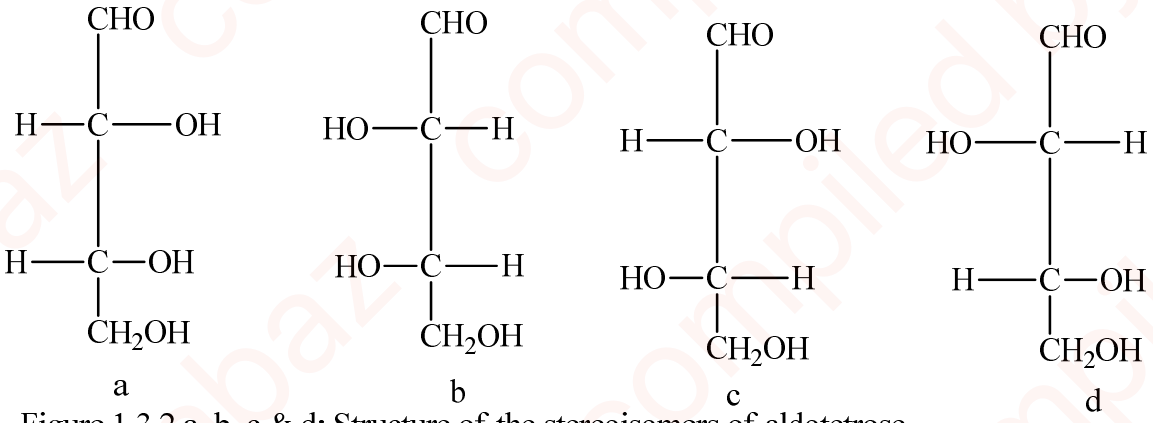


Figure 1.3.2 a, b, c & d: Structure of the stereoisomers of aldotetrose

These four aldotetroses were obtained by inserting **-HCOH-** between carbonyl carbon and the next chiral carbon of both L - glyceraldehyde and D - glyceraldehyde and the mirror image of each will give the other two compounds to make four aldotetroses given above.

1.3 *Epimers*

An epimer is one of a pair of **diastereomers.** Epimer occurs when two molecules have different configuration at only one chiral centre. That is all other chiral centres in the molecule are the same except one chiral centre. Examples are D - Glucose and D - Mannose (Figure 1.4 a & b).

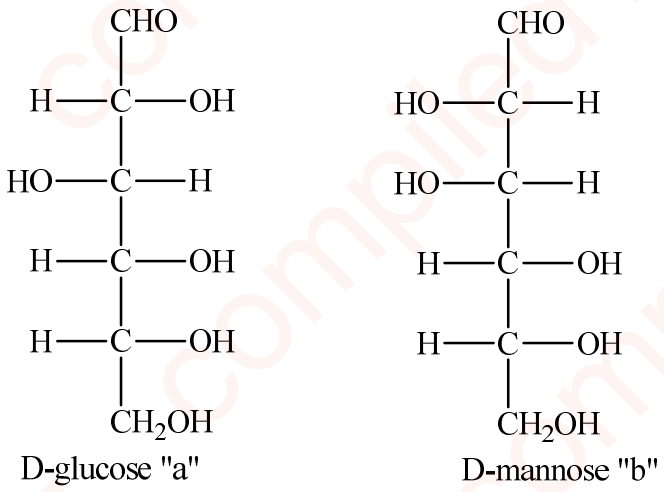


Figure 1.4 a & b: D-glucose and D-mannose

Diastereomer

This term is used to describe two molecules that are stereoisomers with the same formula, connectivity but different arrangement of atoms in space but are not enantiomers. That is the two molecules are not mirror images of each other or non-identical stereoisomers. They occur when two or more stereoisomers of compound for example sugar have different arrangement of atoms at one or more chiral centres but not at all centres which makes them not to be superimposable. If this different is at only one chiral centre, they are called ***epimers***.

The simplest ketose, dihydroxyacetone (Figure 1.5) lacks a chiral centre. Insertion of **-CH(OH)-** unit between the carbonyl carbon (carbon number 2) and the third carbon creates erythrulose (Figure 1.6). The D isomer of erythrulose is the bases of the D-ketose sugar series.

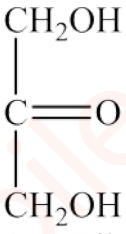


Figure 1.5: Dihydroxyacetone

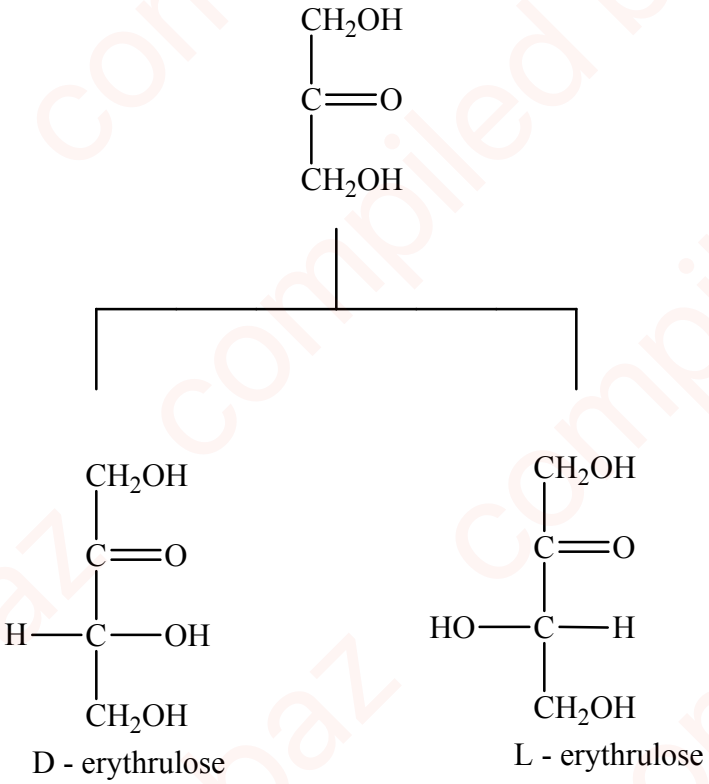


Figure 1.6: Formation of erythrulose from dihydroxyacetone

Ring structure of Monosaccharides

Glucose and other sugars can exist as cyclic hemiacetals or hemiketals. They are formed from intra-molecular reaction of a hydroxy group with a carboxyl group. Glucose and other aldohexoses form their most stable acetal by using the hydroxy group on carbon number 5, and the six-membered ring compound obtained is called the pyranose because it resembles tetrahydropyran (Figure 1.7). Hence, the cyclic form of D-glucose is called D-glucopyranose.

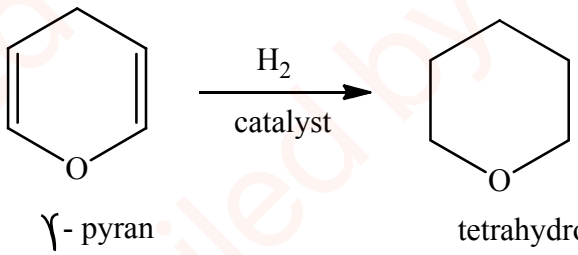


Figure 1.7: Terahydropyran from hydrogenation of γ-pyran

The hemiacetal formation or ***anomeric reaction*** creates a new chiral centre called ***anomeric carbon or anomeric centre*** that is carbon number one (Figure 1.8). This reaction results in two diastereomeric products that differed in configuration on the anomeric carbon. The two products are also called ***Anomers***. These anomers are designated – α or - β depending upon the relative configuration of the anomeric carbon (Figure 1.9 a and b).

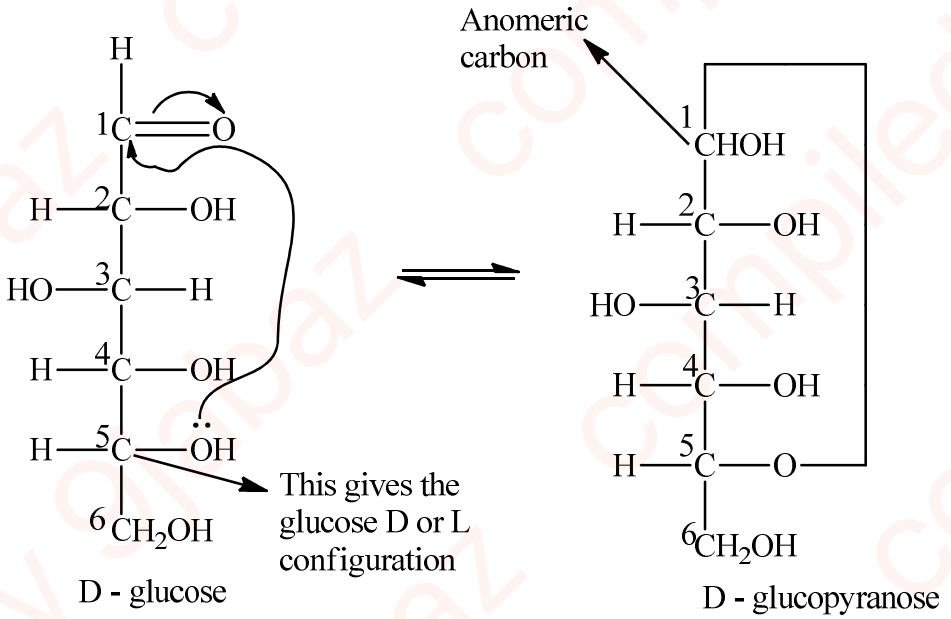


Figure 1.8: Anomeric reaction in D-Glucose (anomerization reaction)

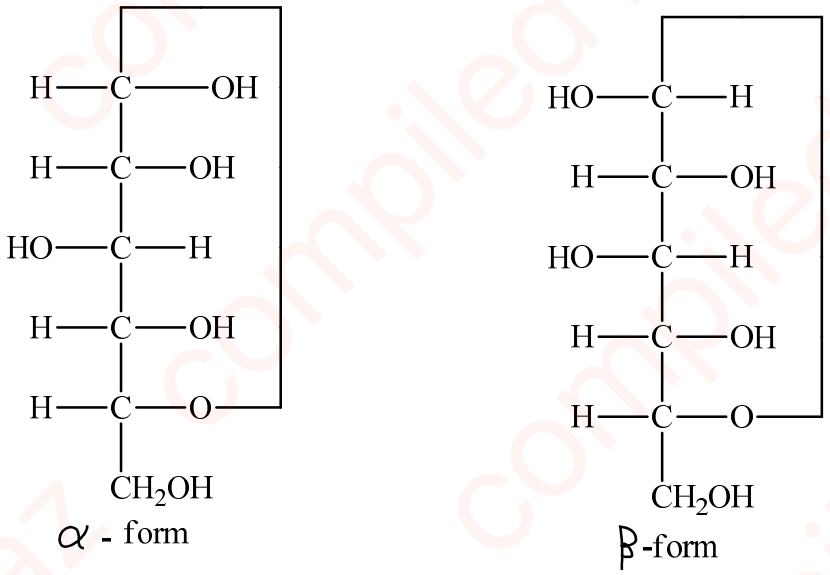


Figure 1.9: The α-form and the β-form of the glucose in ring forms

The structures in the Figure 1.9 above resemble the hydrogenated γ-Pyran (Figure 1.7) above, hence, D-glucose in ring form is named after pyran that is α- or β- D – glucopyranose (Figure 1.10 a and b).

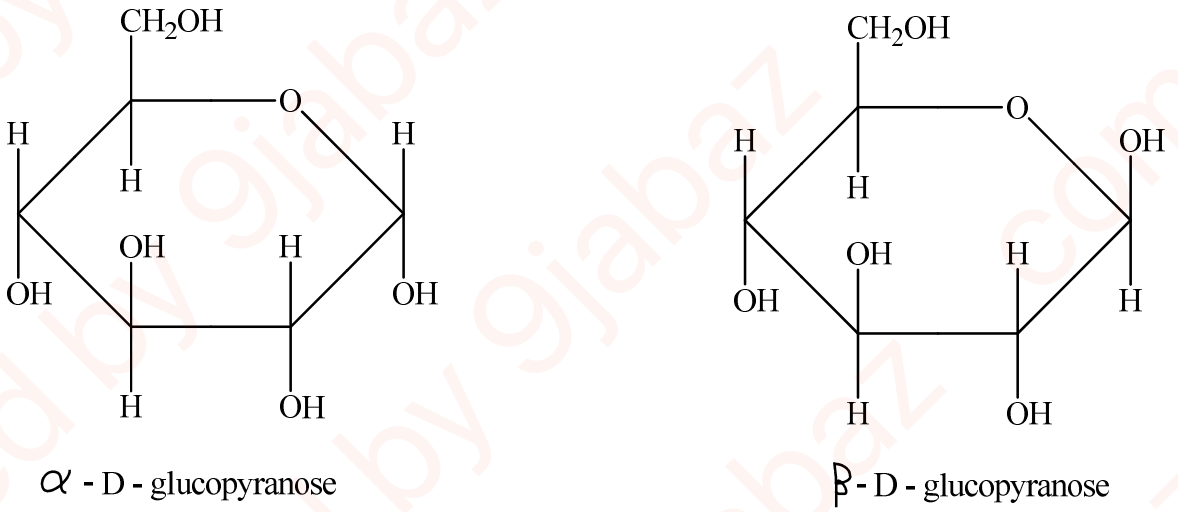


Figure 1.10 a & b: α- D – glucopyranose and β- D – glucopyranose

Ketohexoses also undergo the same intramolecular reaction described above for aldohexose using hydroxy on carbon number 5 (Figure 1.11). This anomeric reaction leads to the formation

of five membered ring compound named after tetrahydrofuran because it resemblances hydrogenated Furan (Figure 1.12). The reaction leads to the formation of two products which are also called ***Anomers***. These anomers like glucopyranose are designated - α or - β depending upon the relative configuration of the anomeric carbon. The ring forms of D-Fructose resemble hydrogenated Furan (Figure 1.12), hence, the ring structure of D-Fructose is named after Furan that is α - or – β- D-Fructofuranose (Figure 1.13 a and b).

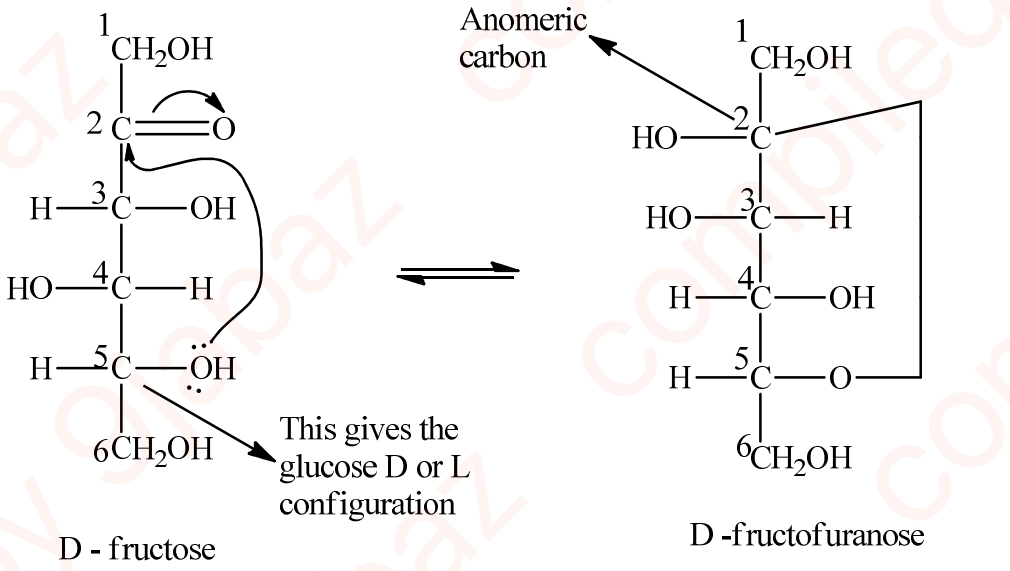


Figure 1.11: Anomeric reaction in D-Fructose (anomerization reaction)

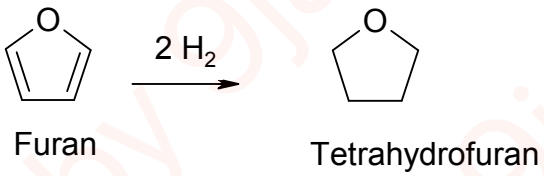
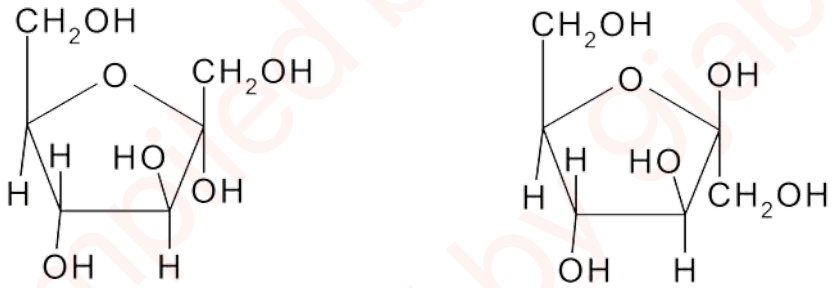


Figure 1.12: Hydrogenation of Furan



α - D-Fructofuranose (a) **β – Fructofuranose (b)**

Figure 1.13 a and b: α - or – β- D-Fructofuranose

The ratio of the α - D- glucopyranose to β - D- glucopyranose is 36 : 64% in nature. This is because the beta form is more stable than the alpha anomer. This stability can be explained using the ***anomeric effect***.

This effect arises from the orbital interaction between oxygen (heteroatom) and the anomeric carbon that is the CO bond. This reaction occurs when the anomeric hydroxy is in the ***axial position*** (Figure 1.14) that is **α-form** but this destabilizing reaction is not possible with the OH group in the ***equatorial position*** (Figure 1.15) that is **β-form**.

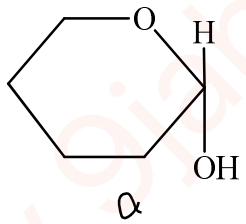


Figure 1.14: Figure showing OH group drawn in the axial position

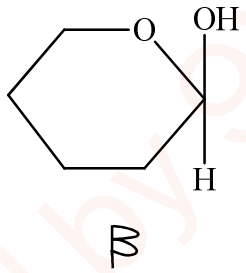


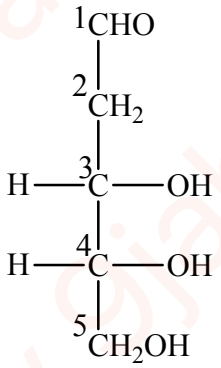
Figure 1.15: Figure showing OH group drawn in the equatorial position

Any ring substituents that are drawn perpendicularly to this axis of symmetry are called ***equatorial*** while those drawn parallel are referred to as ***axial***. For example, the OH group and other substituents on the β - D - glucopyranose are perpendicular to this axis. This reduces steric

hindrance, hence, the β - form is more stable than the α - form. This is another explanation for the reason why β - and α - forms are 64 and 36 % in nature, respectively.

Deoxy and Amino Sugars

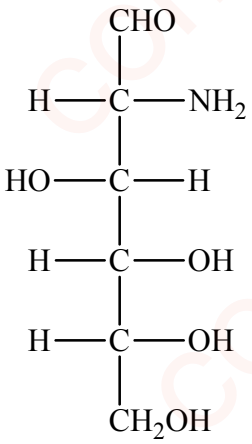
In nature, sugars can have one or more of their OH group(s) replaced by some substituents. Of these, substituents, H and -NH_2 are the most common. A Deoxy sugar has - CH_2 - group is place of a - CH(OH) - group. The most common deoxy sugar in nature is 2-deoxy—D—ribose (Figure 1.16) which is the sugar moiety of deoxyribonucleic acid (DNA).



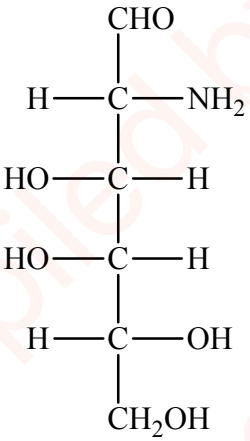
2 - deoxy - D - ribose

Figure 1.16: 2–deoxy-D-ribose

Amino sugar on the other hand has a – $CH(NH_2)$ – group replacing a - CH (OH) - group. Examples of important amino sugars are D - galactosamine and D - glucosamine (Figure 1.17 a & b).



D - glucosamine
(2 - amino - 2 - deoxy - D - glucose)



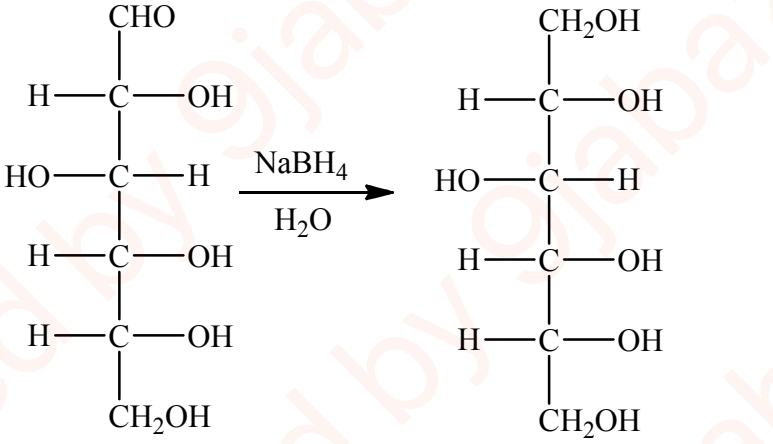
D - galactosamine
(2 - amino - 2 - deoxy - D - galactose)

Figure 1.17 a & b: D-glucosamine and D-galactosamine

1.3 *SOME REACTIONS OF MONOSACCARIDES*

1.3.1 *Reduction to glucitols*

Aldose monosaccharides such as D-glucose can be reduced glycitols. For example, D-glucose can be reduced to D-glucitol (Sorbitol) using a reducing agent such as Sodium borohydride in water ($NaBH_4/H_2O$) (Figure 1.18).



D - glucose

D - glucitol (sorbitol)

Figure 1.18: Reduction of D-glucose to D-Glucitol

1.3.2 *Oxidation to gluconic acids*

Aldoses are oxidized to glyconic acids using mild oxidants such as bromine water. For example, D-glucose is oxidized to D-gluconic acid (Figure 1.19).

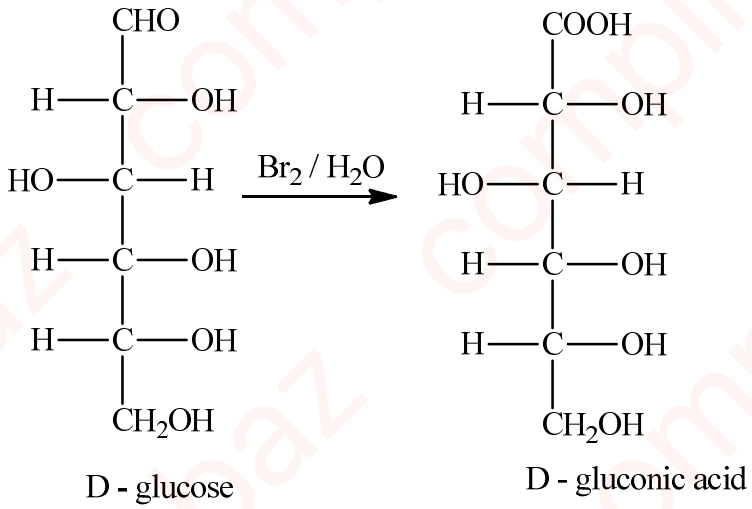


Figure 1.19: Oxidation of D-glucosewith mild oxidant to D-gluconic acid

1.3.3 *Oxidation to glucaric acids*

The oxidation of aldoses to glucaric acids can only be carried out using strong oxidants such as HNO_3. If D-glucose is oxidized with strong oxidant, it will give D-glucaric acid (Figure 1.20).

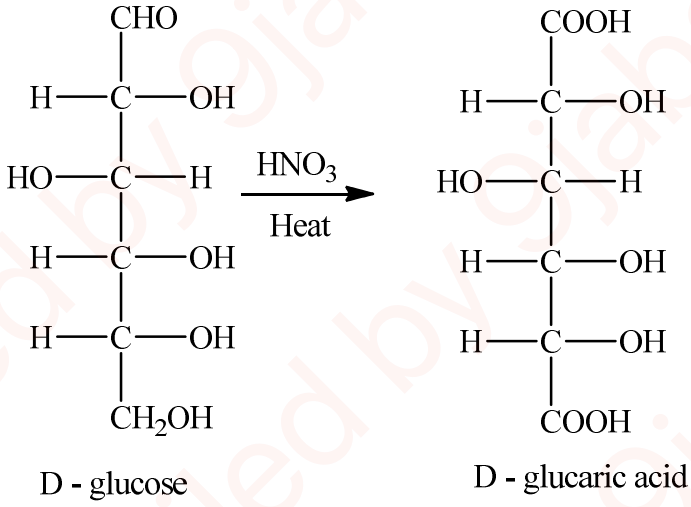


Figure 1.20: Oxidation of D-glucosewith strong oxidant to D-glucaric acid

1.3.4 (i) ***Reaction with Tollens' reagent***

The Tollens' reagent is prepared according the chemical equations (1 & 2) given below:

$2\ AgNO_3 + 2\ NaOH \longrightarrow Ag_2O\ (s) + 2\ NaNO_3 + H_2O$ ---------- (1)

The brown Silver (I) oxide precipitate formed is then dissolved in excess aqueous ammonia to give a clear solution of diammoniasilver (I) complex according to equation 2 given below:

$Ag_2O\ (s) + 4\ NH_3 + 2\ NaNO_3 + H_2O \longrightarrow 2[Ag(NH_3)_2]NO_3 + 2\ NaOH$ -----. (2)

The reaction between D-glucose and Tollens' reagent is given in equation 3 below:

$Ag(NH_3)_2^+ + \text{D-glucose} \longrightarrow \text{D-gluconic acid} + Ag\ (s)$

This reaction is the popular mirror image test used for the confirmation of alkanal.

1.3.4 (ii) ***Reaction with Fehling's and Benedict's Solutions***

Fehling's solution is made up of the following reagents: copper (II) ions complexed with tartrate ions. Both reagents are prepared in NaOH solution. On the other hand, Benedict's solution contains copper (II) ions with citrate ions instead of tartrate ions. It is prepared with Na_2CO_3 solution as against NaOH solution used for the Fehling's solution.

Both Fehling's and Benedict's solutions provide hydroxy ions that reacts with Cu^{2+} in complex form (equation 1). These hydroxy ions are provide by the NaOH and Na_2CO_3 used in the preparation Fehling's and Benedict's solutions, respectively. In the case of Benedict's solution, OH^- is obtained when CO_3^{2-} reversibly reacts with H_2O as follows:

$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^-$

$2Cu^{2+} + 2OH^- + 2e^- \rightarrow Cu_2O + H_2O$ …….. (1)

$RCHO + 3OH^- \rightarrow RCOO^- + 2H_2O + 2e^-$ …… (2)

(1) + (2) gives the overall chemical equation for the oxidation of the alkanal

$$RCHO + 5OH^- + 2Cu^{2+} \rightarrow RCOO^- + 3H_2O + Cu_2O\ (red\ ppt)$$

Only reducing sugars (aldoses) like D-glucose can be positive to Tollens', Fehling's and Benedict's tests. But it was observed that Fructose, a non-reducing sugar gives positive Tollens' test. This was made possible because a base catalyzed equilibrium takes place between glucose, mannose and fructose. The alkaline nature of this reagent provides this base catalyst and the rearrangement is called ***Lobry de Bruyn-Van Ekenstein rearrangment***. The mechanism for the conversation is given by the following chemical equations (Figure 1.21).

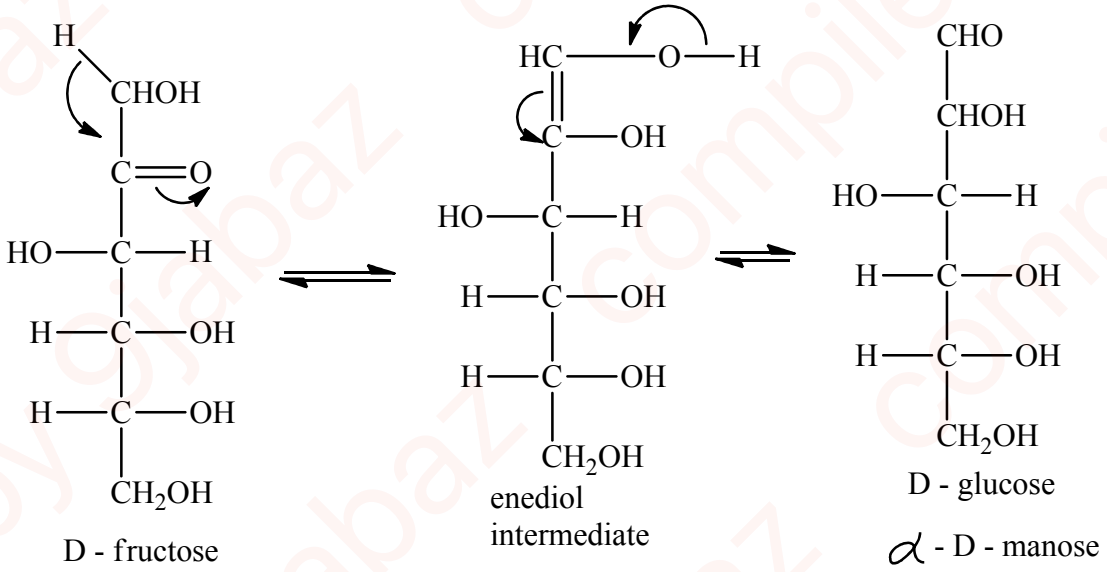


Figure 1.21: The base catalyzed equilibrium between glucose, mannose and fructose

1.3.5 *Reaction with hydrogen cyanide (HCN)*

Hydrogen cyanide reacts with reducing sugars the same way it reacts with alkanals (aldehydes) by attacking the carbonyl carbon to give a hydroxynitrile compound. An example of this reaction is given below (Figure 1.22):

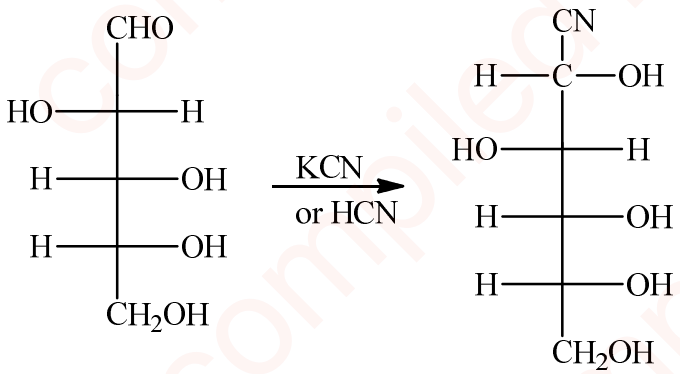


Figure 1.22: Reaction of reducing sugar with Cyanide to form hydroxynitrile compound

It should be noted that hydrogen cyanide is extremely poisonous gas; hence, it is not used directly. The aldehyde / ketone is reacted with a solution of sodium or potassium salt of cyanide in water along with little sulphuric acid to give a solution with a pH of between 4 and 5.

The Mechanisms of the Reaction

The mechanism is through nucleophilic addition. The mechanism is given below (Figure 1.23):

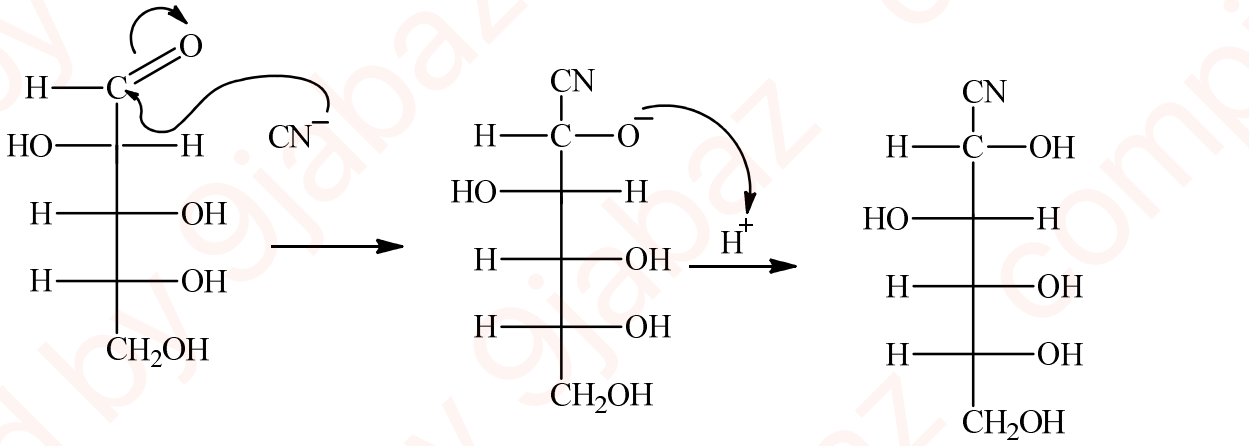


Figure 1.23: Mechanism for the reaction of reducing sugar with Cyanide

A typical example of this reaction is the reaction of D-arabinose with the solution of sodium cyanide given below. The reaction is called ***cyanohydrin***. This reaction is also used in

carbohydrate synthesis for increasing the chain length of a sugar by one carbon for example the mixture of D-glucose and D-mannose from D-arabonise (Figure 1.24).

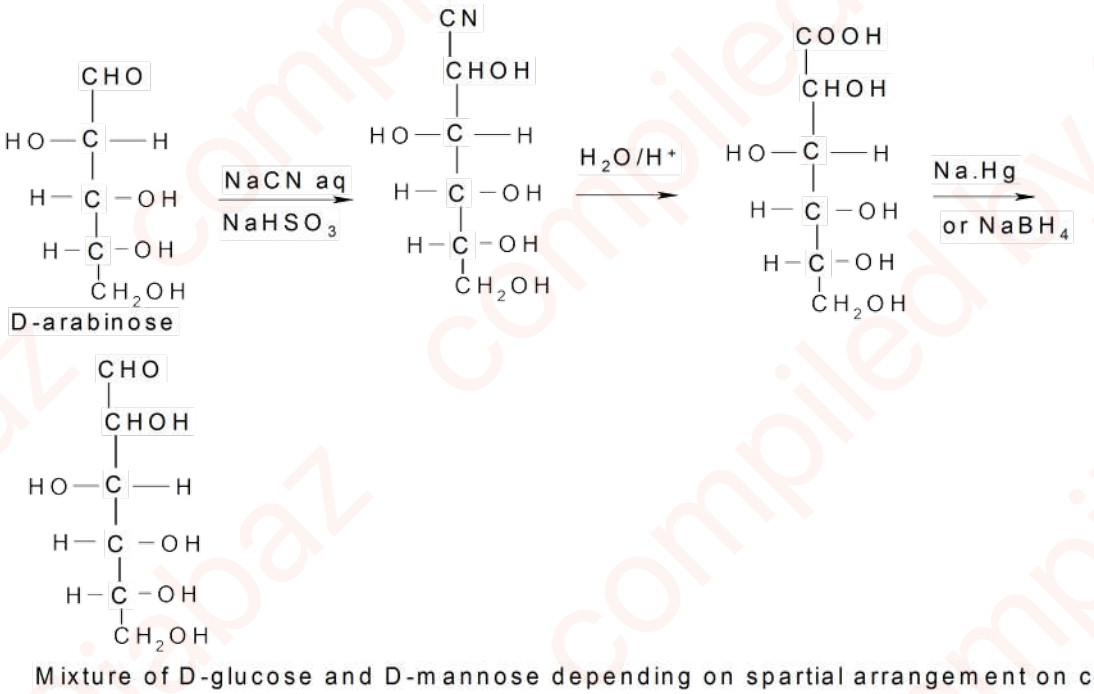


Figure 1.24: Synthesis of D-glucose and D-mannose from D-arabinose

1.3.6 *Ruff Degradation*

Ruff degradation is a method used for reducing the chain length of a carbohydrate by one carbon. In this method, the sugar to be shortened is first converted to gluconic acid using bromine water followed by decarboxylation (removal of CO_2) using ferric salt. Example is the conversion of D-glucose to D-arabinose (Figure 1.25)

Questions

1. Show using a suitable chemical equations how D-fructose forms hemiacetal.
2. Draw the structures of the following sugars: (a) D-ribose, (b) D–arabinose (c) D-mannose and (d) D-galactose.

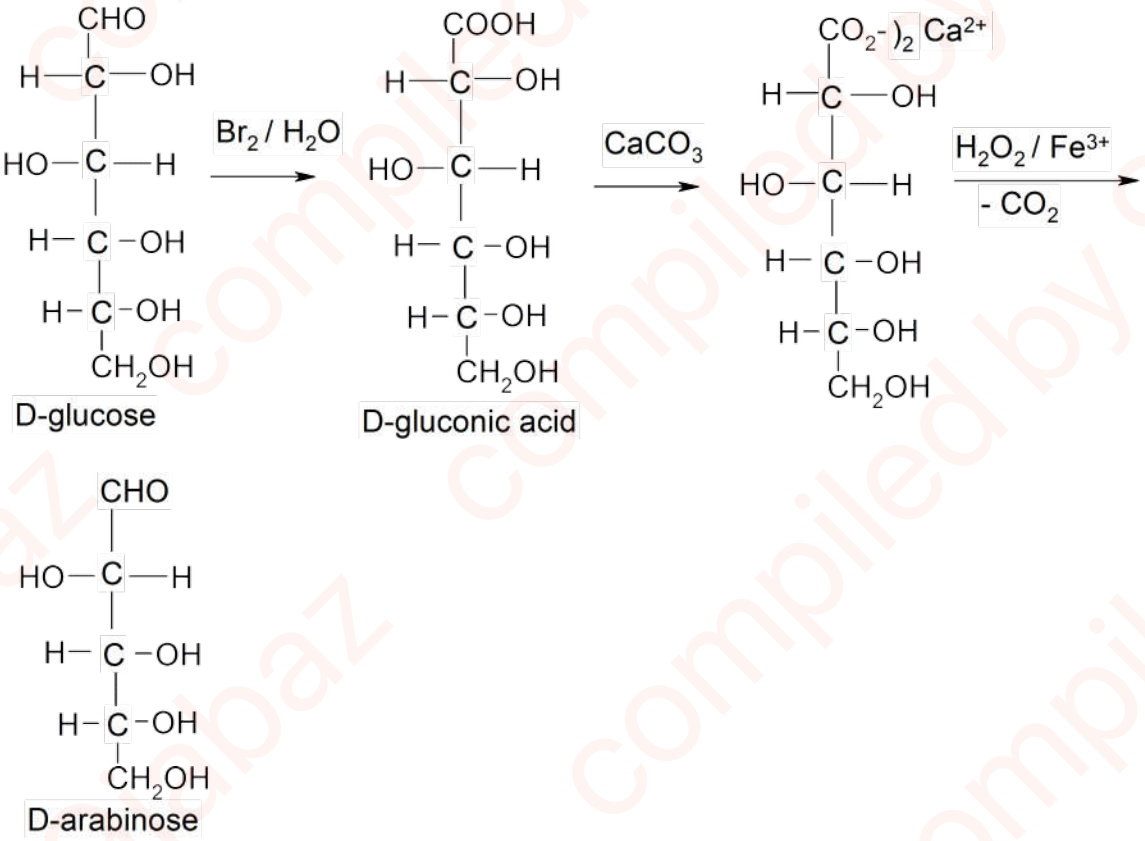
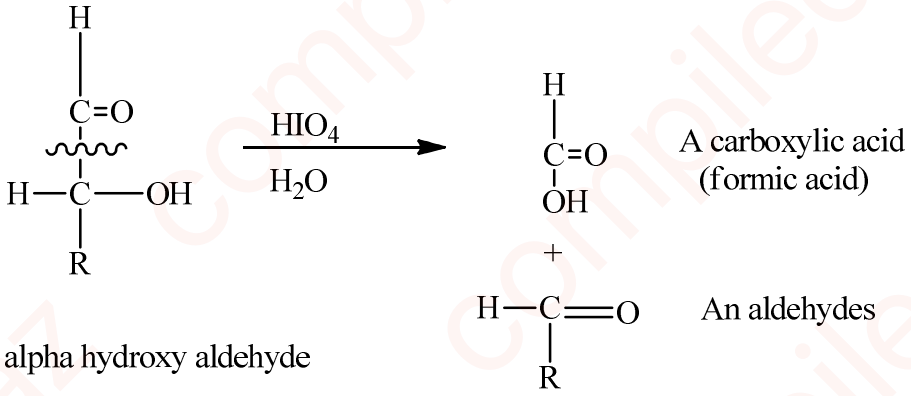


Figure: 1.25: Ruff degradation reaction for converting D-glucose to D-arabinose

1.3.7 Periodic acid oxidation

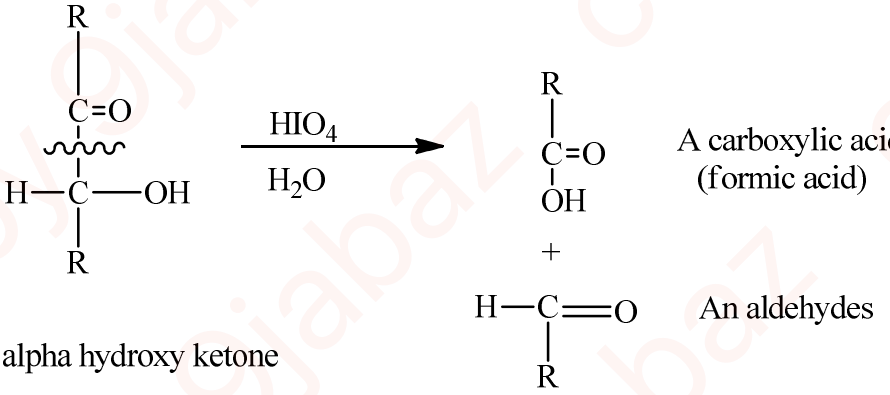
This is another method for determining the ring size of glycosides. It proceeds stoichiometrically and is a measure of the number of adjacent free hydroxyl groups. In this method, the moles of periodic acid (or sodium metaperiodate) consumed and the moles of formaldehyde and formic acid produced during the oxidation of a known weight of the saccharide under investigation are determined. One mole of the oxidant is reduced (consumed when two adjacent OH groups are oxidized with cleavage of the C-C bond joining them to yield two aldehyde groups. Terminal hydroxyl group yields formaldehyde while secondary hydroxyl group yields another aldehyde or form C acid if the 2^{0-} hydroxyl group is flanked on both sides by hydroxy groups (i.e. is oxidized twice). From the results, it is possible to determine the number and type (1^0 or $_Z{}^0$) of adjacent

hydroxyl groups present in a molecule. The general reactions are given below (Figures 2.6 A to F)



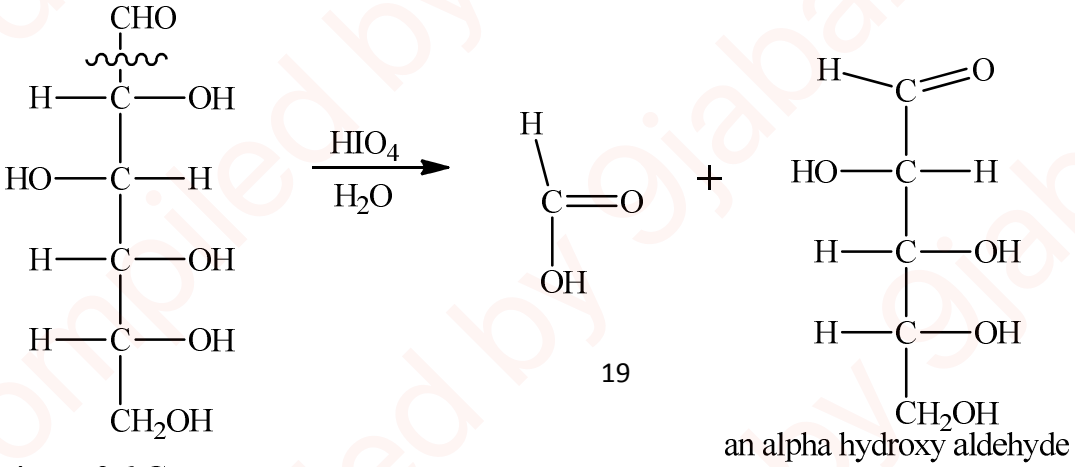
alpha hydroxy aldehyde

Figure 2.6 A



alpha hydroxy ketone

Figure 2.6 B



an alpha hydroxy aldehyde

Figure 2.6 C

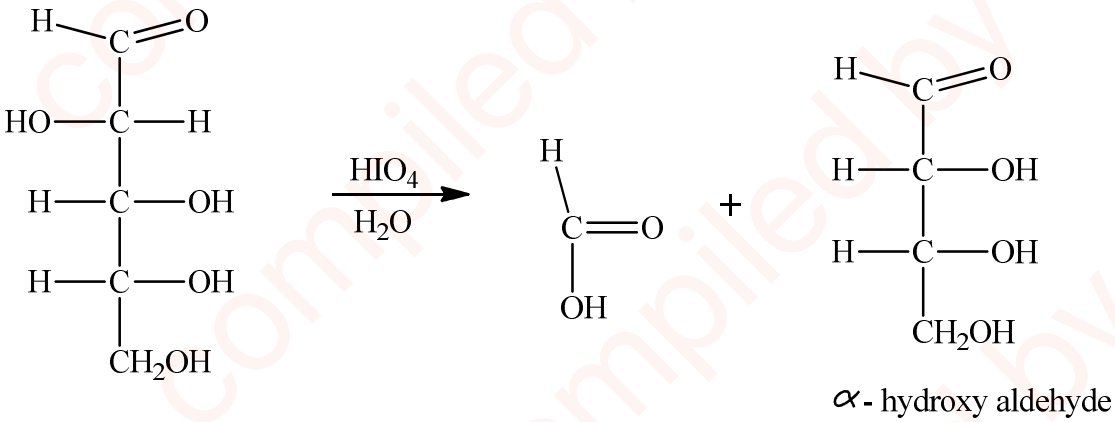


Figure 2.6 D

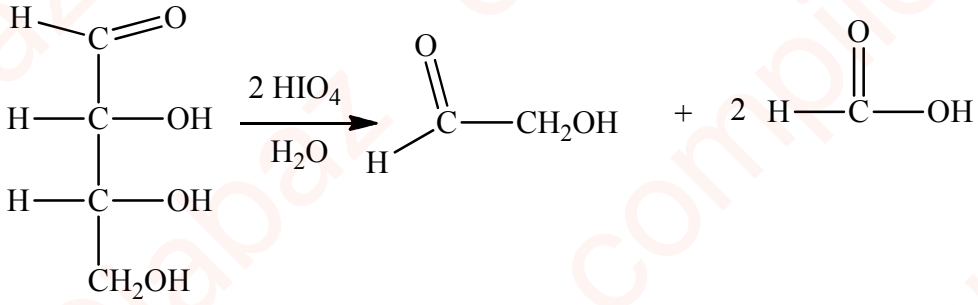


Figure 2.6 E

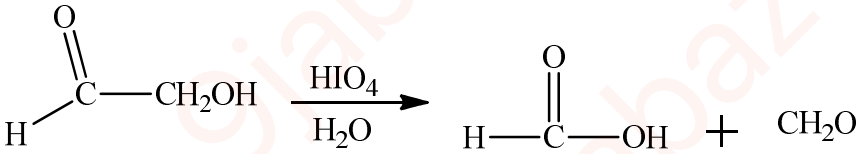


Figure 2.6 F

When all the steps are added up glucose will produce five times as much methanoic acid as methanal.

This type of degradation has played a vital role in understanding the structures of many carbohydrates. The degradation of α-D-glucopyranose by periodic acid is given below (Figure 2.7).

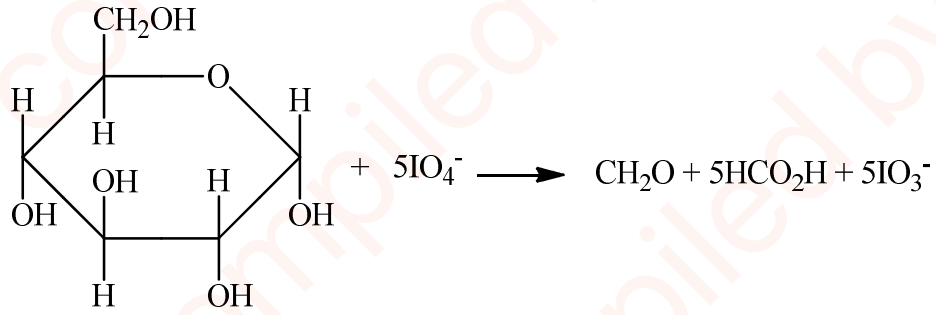
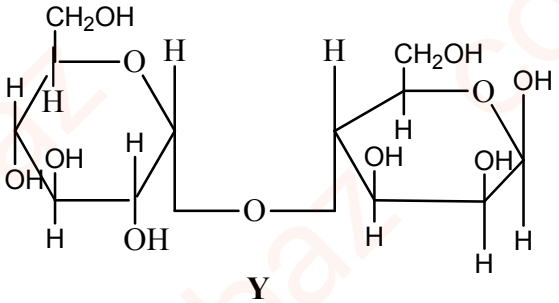


Figure 2.7: The degradation of Alpha - D - glucopyranose by periodic acid

1.4 *Disaccharides*

According to the name, they all consist of two simple sugars held together by ***a glycosidic bond***. Like monosaccharides, disaccharides are also simple sugars which are water soluble. Some typical examples of disaccharide are maltose, sucrose, lactose, cellobiose, getiobiose etc.

They have two simple sugars held together by a glycosidic bond. The most common involves the anomeric carbon of one sugar and non – anomeric carbon of the other (Compound Y)..



Y

MALTOSE

β – Maltose carries the impressive systematic name O – α – glucopyranosyl – (1,4) – β – D – glucopyranose. The disaccharide contains two latent carbonyl carbons. The one in the ring at the upper left is tied up in the glycosidic linkage. The other is in the ring at the lower right and is still a hemiacetal. The position of the free hemiacetal hydroxy group determines if it is β or α, which must appear in any name of the compound.

See the structure given in the class

Note, the structure of maltose is compound Y above.

<u>Cellobiose and Gentiobiose</u>

These are disaccharides that contains β – D glucopyranose bonded to a second β – D glucopyranose residue via a glycosidic bond. Cellobiose possesses a glycosidic bond between C1 and C4 of two simple sugars, while gentiobiose has a glycosidic between C1 and C6 of two simple sugars.

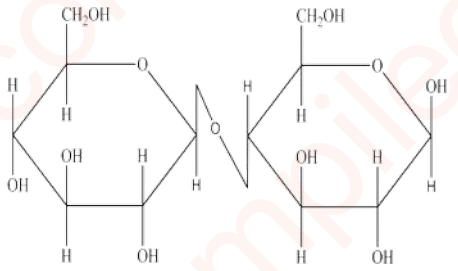


Figure 3.4: β - D - glucopyranosyl-1,β- D - glucopyranose(β - Cellobiose)

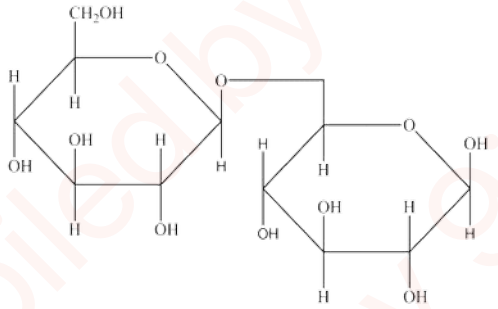
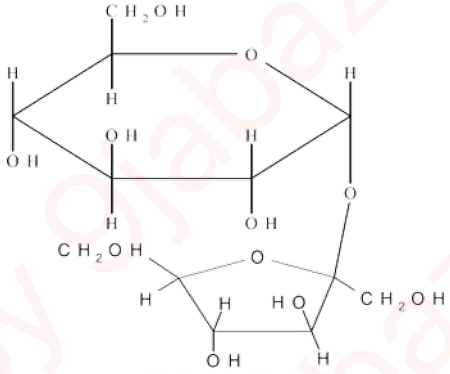


Figure 3.5: β - D - glucopyranosyl-1,β- D - glucopyranose(β - Gentiobiose)

SUCROSE

The alternative names of sucrose are cane sugar or beet sugar. It is the common table sugar, o – α – D – glucopyranosyl – (1,2) – β – D – fructofuranoside. This sugar is different from other disaccharides, thus, far studied because its glycoside linkage involves the anomeric centre of both sugars.



Alpha-D-glucophyranosyl-1,2-beta-D-fructofuranoside [Sucrose]

Sucrose, unlike maltose, Lactose, Cellobiose, and gentiobiose, doesn't exist as readily – inter – converted α and β – forms.

The fructose position of the name ends in "oside" in order to indicate that C2 of fructose is involved in the glycoside bond,

Because both anomeric carbons are involved in acetal formation, sucrose is a non-reducing sugar. It is negative to Tollen's reagent and Fehling's solution or Benedict's solution. Moreover, sucrose does not form an osazone, does not exist on anomeric forms and does not show mutarotation in solution. All these facts indicate that sucrose does not contain a "free" aldehyde or ketone group. When sucrose is hydrolysed by dilute aqueous acid or enzyme invertase (from yeast), equal amounts of α – D glucopyranose and β - D – fructofuranose are produced.

POLYSACCHARIDES

Polysaccharides are polymers containing as many as several thousands monosaccharide unit per molecule. As with other saccharides, it is important to know the following:

(a) Which specific monomer are involved in the polymer formation

(b) The method of linkage between the monomer

(c) The gross structure of the polymer.

If the polymer contains more than one type of monosaccharides, then the sequence of the sugars is also important.

Of all the naturally occurring polysaccharides, starch and cellulose are the most important. Both are products of photosynthesis. Cellulose play a tremendous role in our society. As wood, cellulose provides shelter, as pulp, it is the major constituent of cotton which is a natural fibre. Starch is the mainstay of many diets since it is the major compound in rice, potatoes, wheat and corn.

STARCH

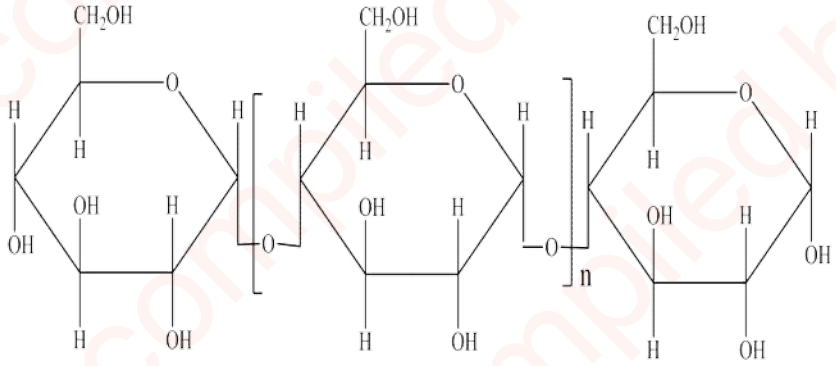
This is the major source of energy in plant cells. When intact, starch granules are insoluble in cold water; if the outer membrane has been broken by grinding, the granules swell in cold water and for a gel. When the intact granule is treated with warm water, a soluble portion of the starch diffuse through the granule wall, in hot water the granules swell and then burst

Starch contains two major fractions: amylose ($\approx 20\%$) and amylopectin ($\approx 80\%$). Both can be hydrolysed in acidic medium to give on D – glucose, since both polymer contains the same monomer, the important differences between them must exist in the bonding within these polymers.

AMYLOSE

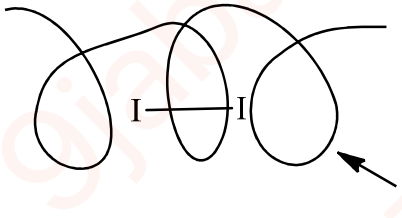
Maltose is the only disaccharide produced upon hydrolysis of amylose. The absence of cellobiose suggests that amylose is a linear polymer of D – glucose molecule, each bonded by an α – glycosidic linkage to C 4 of the adjacent glucose unit. If cellobiose had been produced, the β – glycosidic linkage would have been produced.

Amylose then is believed to be made up of long chains, each containing 1000 or more α – D glucopyranose units joined together by α – linkage as in maltose.



Amylose

α – Amylose is the fraction of starch that gives the intense blue colour with iodine. X – ray analysis shows that the chain coiled in the form of helix (spiral staircase) inside which is just enough to accommodate iodine molecule; the blue colour is due to entrapped iodine molecules.



Blue amylose iodine complex

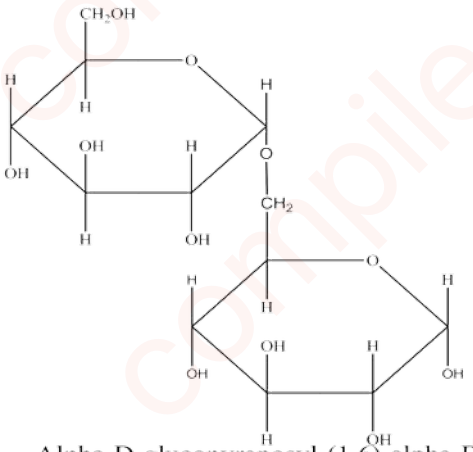
amylose chain

AMYLOPECTIN

Amylopectin is a branched polymer containing about 1000 D – glucose units. The main chain consists of an α – 1, 4 – D glycosidic linkage while branching occurs with an α – 1, 6 – D glycosidic bond. Branching is moderate with perhaps twenty five α – D glucopyranose units occurring between branching points.

The partial hydrolysis of amylopectin produces large molecules called dextrins. Dextrins are used to prepare mucilage, pastes and fabric sizing. (sizes are materials used to fill pores in cloth, paper; etc.). printing inks is often thickened by the addition of dextrins.

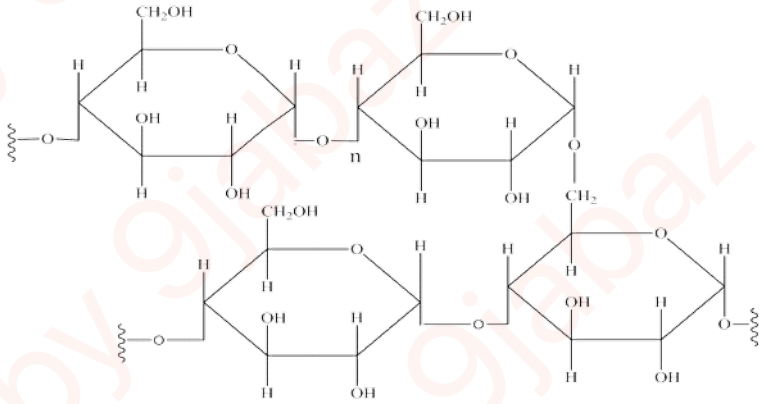
The major disaccharide produced by the hydrolysis of amylopectin is maltose, the glucose unit at each point has C – 1 and C – 6 – OH groups involved in glycosidic linkages. This leads to small amount of isomaltose upon hydrolysis.



Alpha-D-glucopyranosyl-(1,6)-alpha-D-glucopyranose (Alpha Isomaltose)

Structure of amylopectin

Amylopectin is hydrolysed to a single disaccharide maltose; the sequence of methylation and hydrolysis yields chiefly 2,3,6 – tri – o – methyl – D – glucose. Like amylose, amylopectin is made up of chains of α – D glucopyranose units, each unit joined by alpha – glucosidic linkage to C – 4 of the next one. However, its structure is more complex than that of amylose.

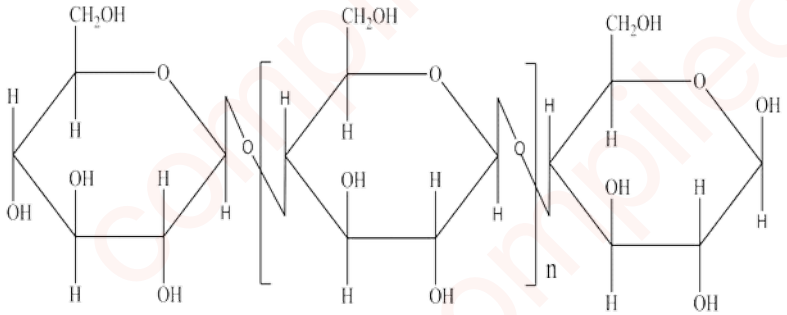


Amylopectin

CELLULOSE

Cellulose is an unbranched polymer of β – D – glucose which occurs in most plants. Most animals, including man and cattle, cannot hydrolyse the β – glycosidic link in cellulose

Cotton which is the most important natural fibre, is about 98% cellulose. Acetal linkage in cellulose are hydrolysed by acids but not by bases.



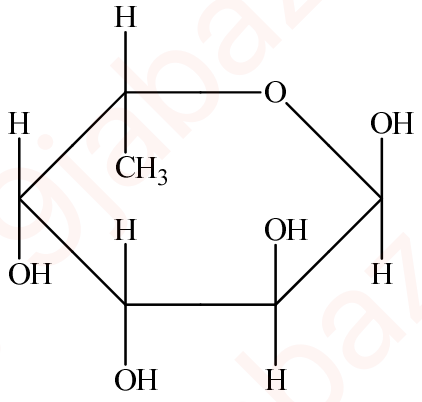
β-Cellulose

3.0 DISACCHARIDES, OLIGOSACCHARIDES AND POLYSACCHARIDES

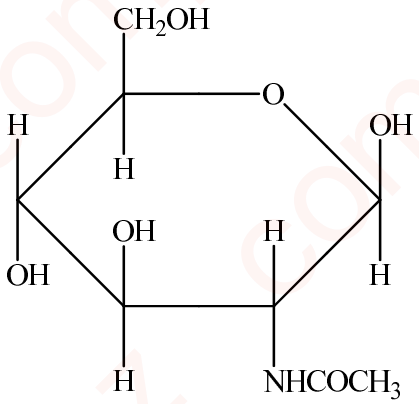
Students are expected to revise the Carbohydrate Chemistry Note under CHM 202.

In nature, carbohydrates are often found as oligosaccharides (polymers containing 2 to 10 monosaccharide units) or polysaccharides (polymers containing more than 10 – monomers). The polymers arise from a reaction between the OH – group on the hemiacetal carbon of one monosaccharide and a OH – group on the second monosaccharide unit. The bands usually involve C1 of one aldose and C4 of a second; others are between C1 and C2, between C1 and C3, and between C1 and C6.

There are few oligosaccharides in animals compared to plant tissues. The major one is Lactose; a disaccharide found in mammalian milk. Human milk differs in composition from cow's milk. For example, human milk contains L – fucose (6 – deoxy – L – galactose) or N – acetyl – D – glucosamine or both (Figure 2.53). The commonest is the tri – saccharide, L – fucosyl lactose (Figure 2.54).



L - Fucose



N - acetyl - D - glucosamine

Figure 2.53: The sugar content of human milk

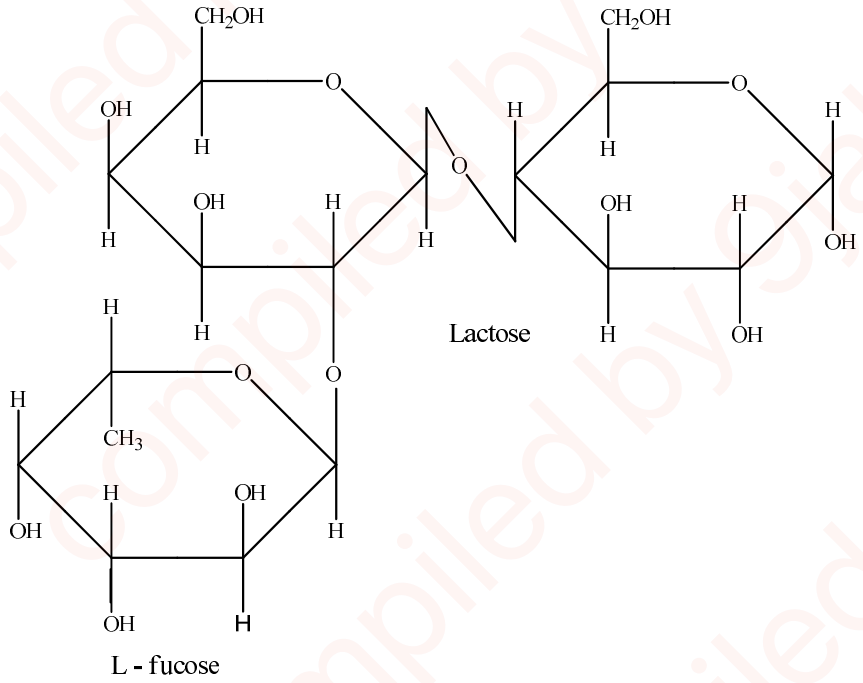


Figure 2.54: The Trisaccharide content of human milk (L - fucosyl lactose)

There are two types of polysaccharides: homopolysaccharides (or homoglycans) composed mainly of one monosaccharide and heteropolysaccharides (heteroglycans) which contain mainly of two or more different monosaccharides. Polysaccharides stored in plant and animals are homoglycans. Those that are used for support purposes in the plant kingdom include both homo – and heteroglycans while those in the animal kingdom are always heteroglycans.

Oligosaccharides are also grouped into simple (or true) oligosaccharides, which on depolymerization yield monosaccharides only, and conjugate oligosaccharides, which are linked to such nonsaccharides as peptides and lipids and on depolymerization yield monosaccharides and aglycans. They are further classified according to degree of polymerization, into disaccharides, trisaccharides, tetrasaccharides, etc.

The complete structure of oligosaccharide is established when the following points are determined:

(a) The degree of polymerization i.e. the number of monosaccharide units present in the oligomer molecule;

(b) the nature of the monosaccharide monomer(s);

(c) in the case of hetero – oligosaccharides, the monosaccharide sequence;

(d) the ring size (pyranose or furanose) and the position of linkage of the different monosaccharide (1 – ?) and

(e) the anomeric configuration (α or β) and the conformation of the monosaccharide units.

3.1 Disaccharides

According to the name, they all consist of two simple sugars held together by ***a glycosidic bond***. Like monosaccharides, disaccharides are also simple sugars which are water soluble. Some typical examples of disaccharide are maltose, sucrose, lactose, cellobiose, getiobiose etc. The most common involve the anomeric carbon of one sugar and non – anomeric carbon of the other (Figure 3.1).

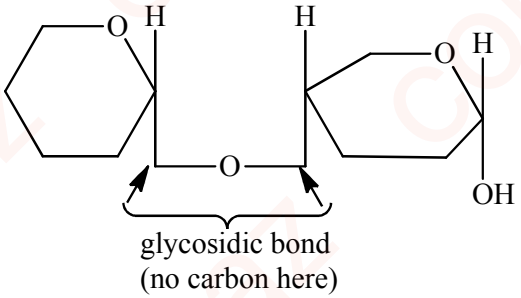
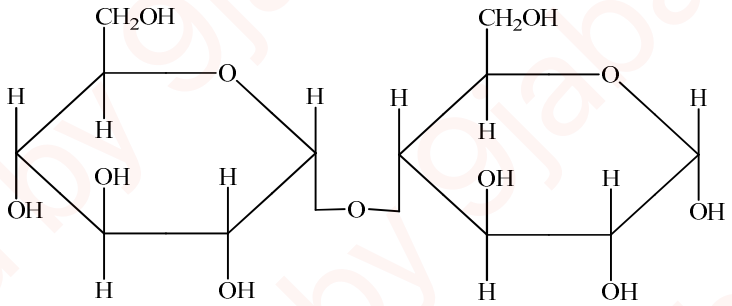


Figure 3.1: A typical disaccharide showing glycosidic a bond

3.1.1 Maltose

β – Maltose (Figure 3.2) carries the impressive systematic name O – α – D - glucopyranosyl – (1,4) – β – D – glucopyranose. The disaccharide contains two latent carbonyl carbons. The one in the ring at the upper left is tied up in the glycosidic linkage. The other is in the ring at the lower right and is still a hemiacetal. The position of the free hemiacetal hydroxy group determines if it is β or α, which must appear in any name of the compound.



α - D - glucopyranosyl-1,4-α - D - glucopyranose (α - Maltose)

The sugar in the left is an α – D – glucopyranose ring. This is bonded to a β – D – glucopyranose ring. The "(1,4)" indicates that C1 of the "first" ring is bonded to the C4 of the second "ring". The bridging atom is oxygen. This is indicated by the prefix O. Since the ring

on the left is viewed as a substituent attached to the ring on the right, the former is named "osyl" while the latter is named "ose".

Maltose can be obtained along with other sugars, by the partial hydrolysis of starch in aqueous acid. It is also formed in one stage of the fermentation of starch to ethyl alcohol. Hence, hydrolysis is catalyzed by the enzyme called diastase.

Facts leading to the deduction of the structure of maltose are as follows:

- It has the molecular formula $C_{12}H_{22}O_{11}$. It is oxidized by Tollen's and Fehling's reagents. It is a reducing sugar.
- It reacts with phenyl hydrazine to yield an osazone $C_{12}H_{20}O_9$ $(=NNHC_6H_5)_2$
- It is oxidized by bromine water to a monocarboxylic $(C_{11}H_{21}O_{10})COOH$, maltobionic acid
- It exists in alpha ($[\alpha] = +168^0$) and beta ($[\alpha] = +112^0$) forms which undergo mutarotation in solution (equilibrium $[\alpha] = +136^0$)

All the facts indicate the same thing: maltose contains a carbonyl group that exists in the reactive hemiacetal form. It contains only one such free carbonyl group since (1) the osazone contains only two phenyl hydrazine residue (2) oxidation by bromine water produces only a monocarboxylic acid.

When it is hydrolysed in aqueous acid or with maltase enzyme, it is converted into D – glucose which implies that it is made up of two D – glucose units.

$$2C_6H_{12}O_6 \xrightarrow{-H_2O} C_{12}H_{22}O_{11}$$

Two questions are to be assumed which are (i) which – OH group is involved and (ii) what are the sizes of the rings in the two units? The answers to the two questions are given by the sequence of oxidation, methylation, and hydrolysis shown in the figure below (Figure 3.3).

- Oxidation by bromine water converts maltose into the monocarboxylic acid i.e. D – maltobionic acid
- Treatment of the acid with dimethylsulphate and NaOH gives Octa – o – methyl – D – maltobionic acid
- Hydrolysis in acidic solution of the methylated acid gives two products, 2, 3, 5, 6 – tetra – o – methyl – D – gluconic acid and 2, 3, 4, 6 – tetra – o – methyl – D – glucopyranose

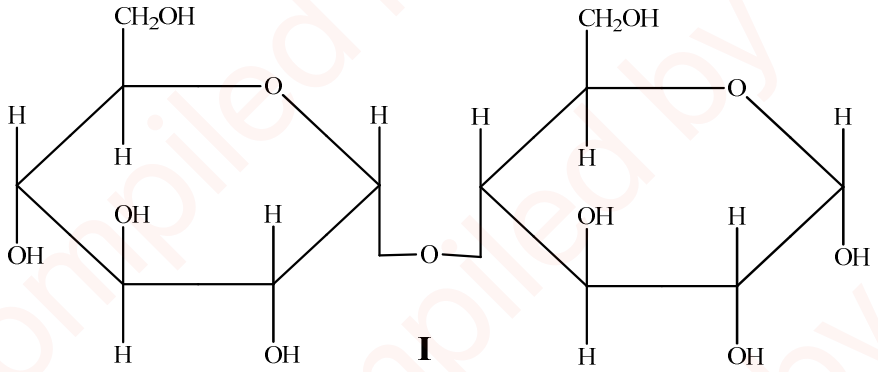
  These facts show that maltose has the structure I (Figure 3.3) with the name O – (α – D – glucopyranosyl – (1,4) – α – D – glucopyranose).

The proof of the structure I:

- The initial oxidation labels (with a COOH groups) involves the D – glucose unit with the free aldehyde group
- Methylation labels (as – OCH_3) involve every free – OH group.
- Finally, upon hydrolysis, the absence of a methoxyl group shows which – OH groups were not free.

The oxidized product, 2, 3, 5, 6 – tetra – o – methyl – D – gluconic acid, must have arisen from the reducing (oxidizable) D – glucose unit. The presence of – OH group on C – 4 shows that the position was not available for methylation at the maltobionic acid stage; hence it is the – OH on C – 4 that was involved in the glycoside linkage. This leaves only the – OH group on C -5 to be involved in ring formation of the reducing unit of the disaccharide. On these bases, one glucose unit is designated 4 – O – substituted – D – glucopyranose.

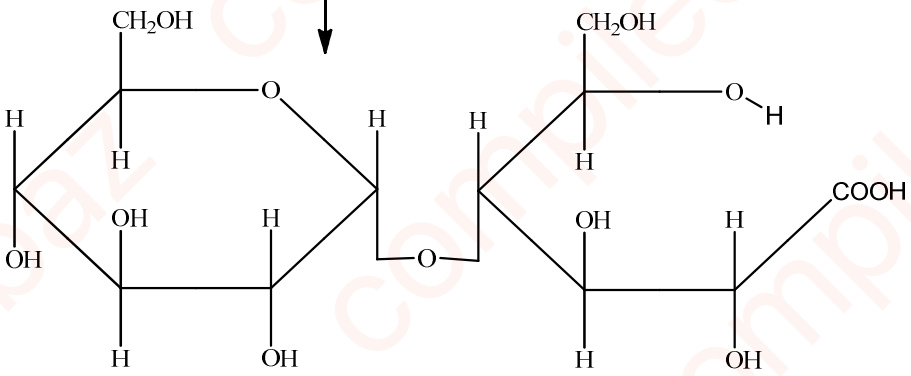
The unoxidized product , 2, 3, 5, 6 – tetra – o – methyl – D – glucopyranose have been from the non – reducing (non – oxidizable) D – glucose unit. The presence of the free – OH group at C – 5 implies that it escapes methylation, hence it was involved in the ring formation. On the basis of this second sugar is designated an α – D – glucopyranosyl group.



I

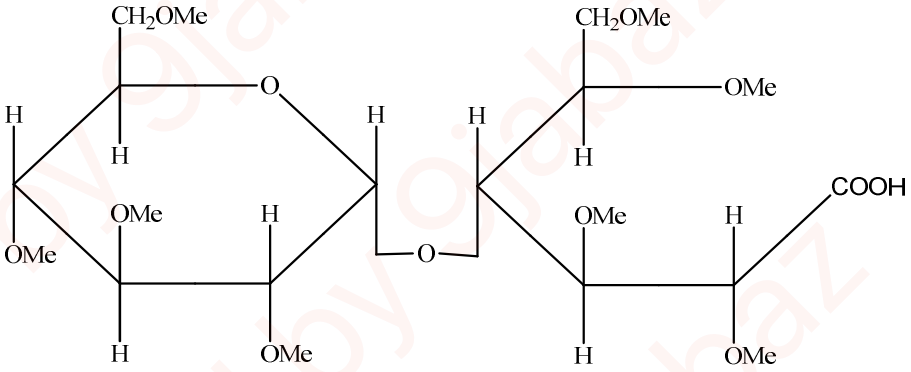
α - D - glucopyranosyl-1,4- α - D - glucopyranose (α - Maltose)

Br_2, H_2O (Oxidation)



D-Maltobionic acid

Me_2SO_4.NaOH Methylation



Octa - o- methyl- D- maltobionic acid

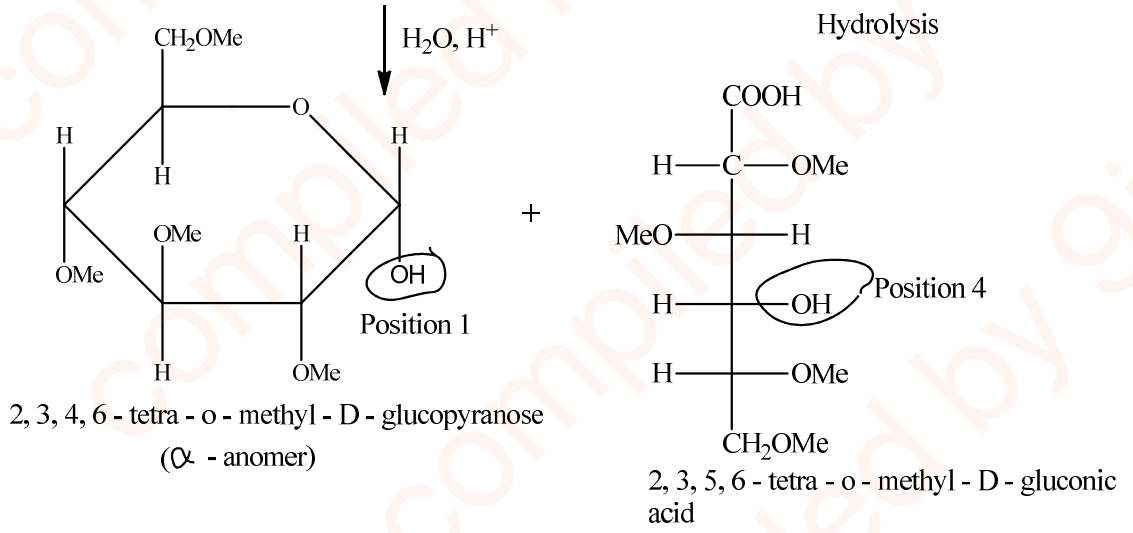


Figure 3.3: Sequence of oxidation, methylation, and hydrolysis shows that Maltose is α – D – glucopyranosyl – (1,4) – α – glucopyranose.

3.1.2 Cellobiose and Gentiobiose

These are disaccharides that contain β – D glucopyranose bonded to a second D – glucopyranose residue via a glycosidic bond. Cellobiose (Figure 3.4) possesses a glycosidic bond between C1 and C4 of two simple sugars, while Gentiobiose (Figure 3.5) has a glycosidic between C1 and C6 of two simple sugars.

When cellulose (cotton fibres) is treated for several days with H_2SO_4 and acetic anhydrides, a combination of acetylation and hydrolysis takes place; forming octaacetate of cellobiose. Alkaline hydrolysis of octaacetate produces cellobiose itself.

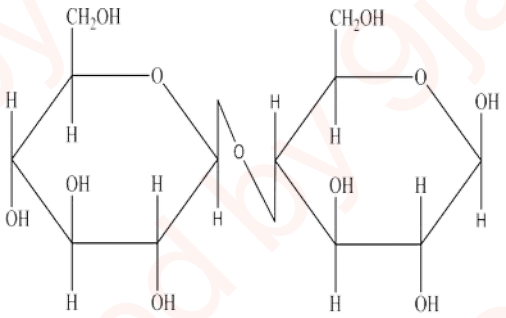


Figure 3.4: β - D - glucopyranosyl-1,4- β - D - glucopyranose (β - Cellobiose)

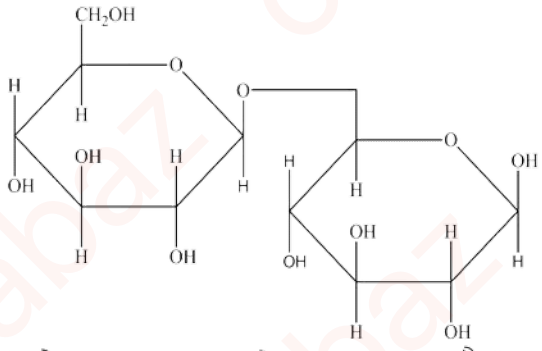


Figure 3.5: β - D - glucopyranosyl-1,6- β - D - glucopyranose (β - Gentiobiose)

Like maltose, cellobiose has the molecular formula $C_{12}H_{22}O_{11}$ and the following properties:

- It is a reducing sugar
- It forms an osazone.
- It exists in alpha and beta forms that undergo mutarotation
- It can be hydrolysed to two molecules of D – glucose
- The sequence of oxidation, methylation and hydrolysis (as described for maltose) shows that cellobiose contains two pyranose rings and a glycoside linkage to – OH group on C – 4
- It differs from maltose in one respect: it is hydrolysed by the enzyme emulsin (from bitter almonds), not by maltase. Since emulsin is known to hydrolyse only the β – glucoside linkage, it can be concluded that the structure of cellobiose differs from that of maltose in only one respect. The D – glucose units are joined by a beta linkage rather than by an alpha linkage.

Questions

(1) Write equations for the sequence of oxidation, methylation and hydrolysis as applied to cellobiose

(2) Show that when octa – o – methyl cellobionic acid is hydrolysed, the products are the same as those formed by the hydrolysis of octa – o – methyl – D – maltobionic acid.

3.1.3 Sucrose

The alternative names of sucrose are cane sugar or beet sugar. It is the common table sugar, o – α – D – glucopyranosyl – (1,2) – β – D – fructofuranoside. This sugar (Figure 3.6) is different from other disaccharides, thus, far studied because its glycoside linkage involves the anomeric centre of both sugars.

Sucrose, unlike maltose, Lactose, Cellobiose, and Gentiobiose, doesn't exist as readily – inter – converted α and β – forms. The fructose position of the name ends in "oside" in order to indicate that C2 of fructose is involved in the glycoside bond,

Because both anomeric carbons are involved in acetal formation, sucrose is a non-reducing sugar. It is negative to Tollen's reagent and Fehling's solution or Benedict's solution. Moreover, sucrose does not form an osazone, does not exist on anomeric forms and does not show mutarotation in solution. All these facts indicate that sucrose does not contain a "free" aldehyde or ketone group. When sucrose is hydrolysed by dilute aqueous acid or enzyme invertase (from yeast), equal amounts of D – glucose and D – fructose are produced. This hydrolysis is

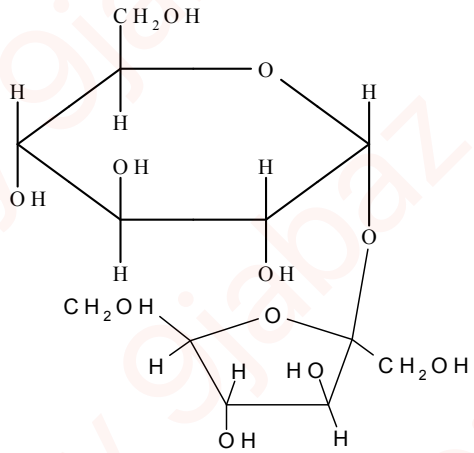


Figure 3.6: Alpha-D-glucophyranosyl-1,2-beta-D-fructofuranoside [Sucrose]

accompanied by a change in the sign of rotation from positive to negative; it is therefore the inversion of sucrose, and the Levorotatory mixture of D – glucose and D – fructose obtained has been called ***invert sugar*** and they are called ***dextrose*** and ***levulose***, respectively.

3.1.4 LACTOSE

Milk sugar or lactose (Figure 3.11), is synthesized in the mammary glands from D – glucose. It is present at a level of about 5% in the milk of mammals. α – Lactose bears the systematic name O – β – D – galactopyranosyl – (1,4) – α – D – glucopyranose. Lactose can also form a β – anomer.

3.2 POLYSACCHARIDES

Polysaccharides are polymers containing as many as several thousands monosaccharide units per molecule. As with other saccharides, it is important to know the following:

(a) Which specific monomer are involved in the polymer formation?

(b) The method of linkage between the monomer.

(c) The gross structure of the polymer.

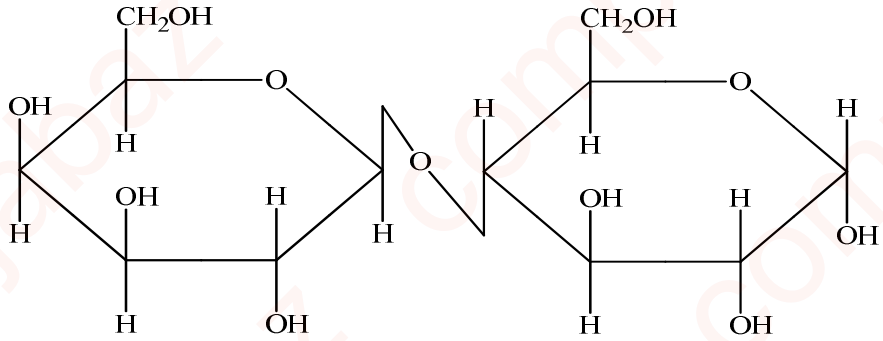


Figure 3.11: β - D - galactopyranosyl - (1,4) - α - D - glucopyranose (α - lactose)

The following reactions provide information about the structure of lactose (Figure 3.12).
If the polymer contains more than one type of monosaccharides, then the sequence of the sugars is also important.

Of all the naturally occurring polysaccharides, starch and cellulose are the most important. Both are products of photosynthesis. Cellulose plays a tremendous role in our society. As wood, cellulose provides shelter, as pulp, it is the major constituent of cotton which is a natural fibre. Starch is the mainstay of many diets since it is the major compound in rice, potatoes, wheat and corn.

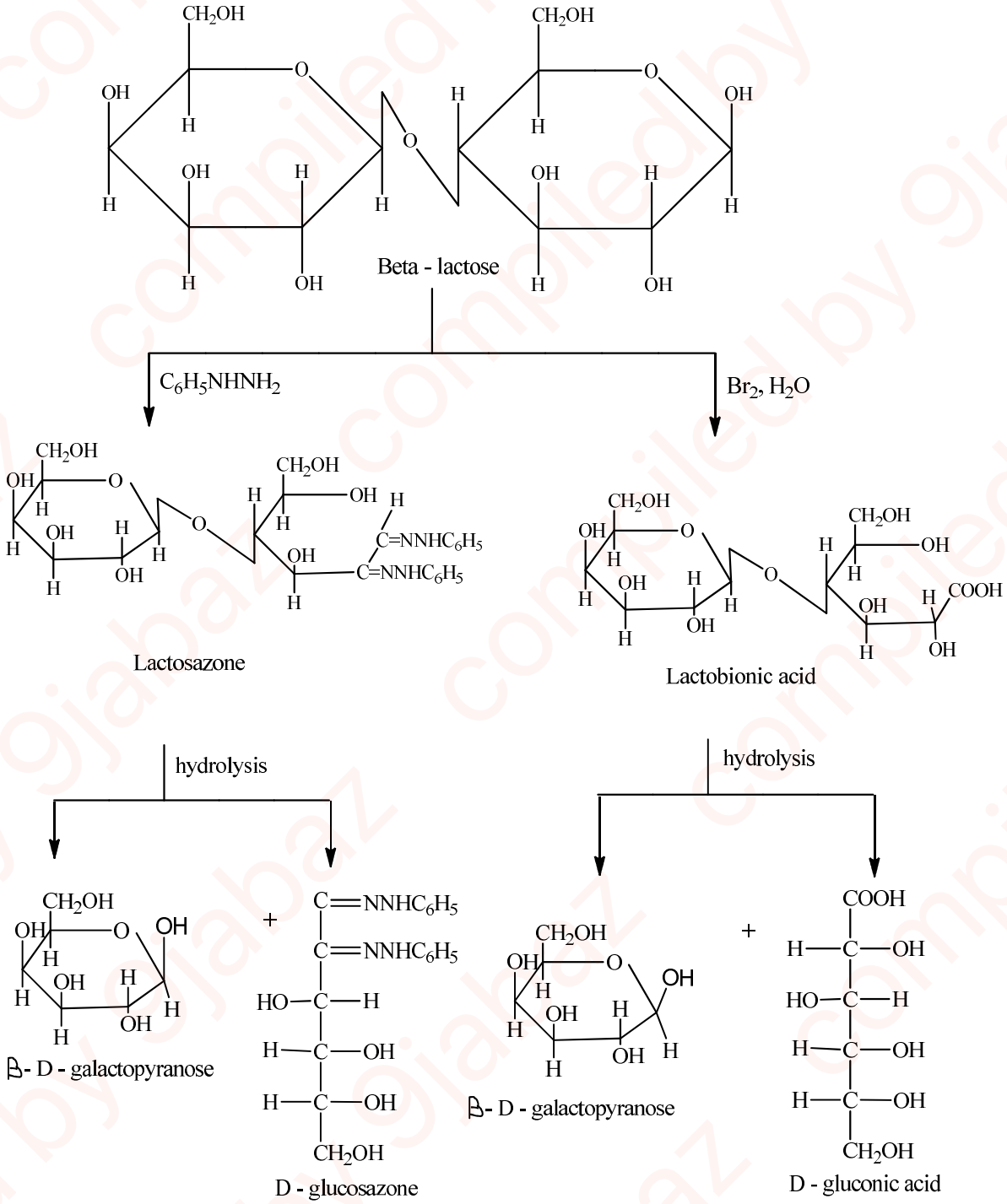


Figure 3.12: Hydrolysis of Lactose derivatives shows that glucose is the reducing unit

3.2.1 STARCH

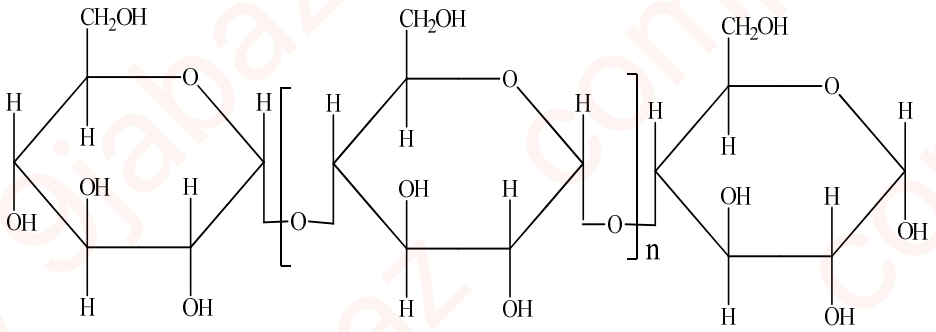
This is the major source of energy in plant cells. When intact, starch granules are insoluble in cold water; if the outer membrane has been broken by grinding, the granules swell in cold

water and for a gel. When the intact granule is treated with warm water, a soluble portion of the starch diffuse through the granule wall, in hot water the granules swell and then burst.

Starch contains two major fractions: amylose (≈ 20%) and amylopectin (≈ 80%). Both can be hydrolysed in acidic medium to give on D – glucose, since both polymer contain the same monomer, the important differences between them must exist in the bonding within these polymers.

3.2.1.1 AMYLOSE

Maltose is the only disaccharide produced upon partial hydrolysis of amylose. The absence of Cellobiose suggests that amylose (Figure 3.13) is a linear polymer of α – D – glucopyranose molecule, each bonded by an α – glycosidic linkage to C 4 of the adjacent glucose unit. If Cellobiose had been produced, the β – glycosidic linkage would have been produced.



Amylose

Amylose is believed to be made up of long chains, each containing 1000 or more α – D – glucopyranose units joined together by α – linkage as in maltose with little or no branching of the chain. α – Amylose is the fraction of starch that gives the intense blue colour with iodine. X – ray analysis shows that the chain coiled in the form of helix (spiral staircase) (Figure 3.16) inside which is just enough to accommodate iodine molecule; the blue colour is due to entrapped iodine molecules.

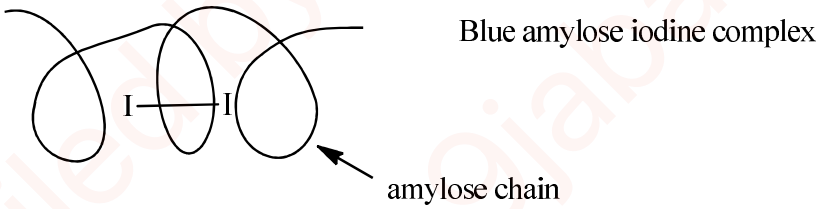


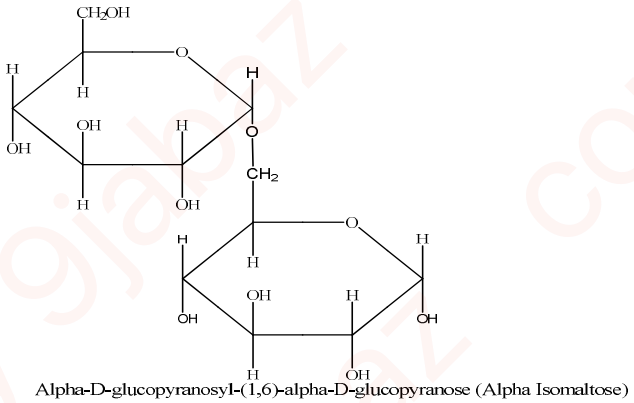
Figure 3.16: Amylose chain coiled in the form of helix with iodine molecule trapped within the chain

3.2.1.2 AMYLOPECTIN

Amylopectin is a branched polymer containing about 1000 α – D – glucopyranose units. The main chain consists of an α – 1, 4 – D glycosidic linkage while branching occurs with an α – 1, 6 – D glycosidic bond. Branching is moderate with perhaps twenty – five glucose occurring between branching points.

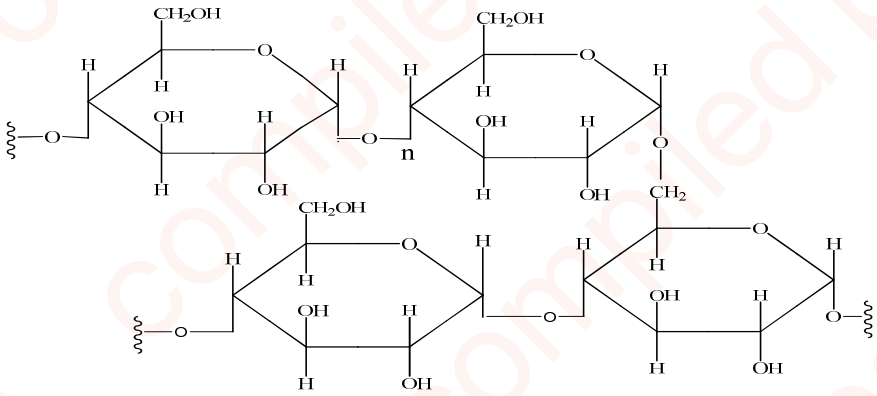
The partial hydrolysis of amylopectin produces large molecules called dextrins. Dextrins are used to prepare mucilage, pastes and fabric sizing. (Sizes are materials used to fill pores in cloth, paper; etc.). Printing inks is often thickened by the addition of dextrins.

The major disaccharide produced by the hydrolysis of amylopectin is maltose, the glucose unit at each point has C – 1 and C – 6 – OH groups involved in glycosidic linkages. This leads to small amount of ***isomaltose*** (Figure 3.17) upon hydrolysis.



Alpha-D-glucopyranosyl-(1,6)-alpha-D-glucopyranose (Alpha Isomaltose)

Structure of amylopectin



Amylopectin

3.2.3 CELLULOSE

Cellulose is an unbranched polymer of β – D – glucopyranose (Figure 3.20) which occurs in most plants. Most animals, including man and cattle, cannot hydrolyse the β – glycosidic link in cellulose.

Cotton which is the most important natural fibre is about 98% cellulose. Acetal linkages in cellulose are hydrolysed by acids but not by bases.

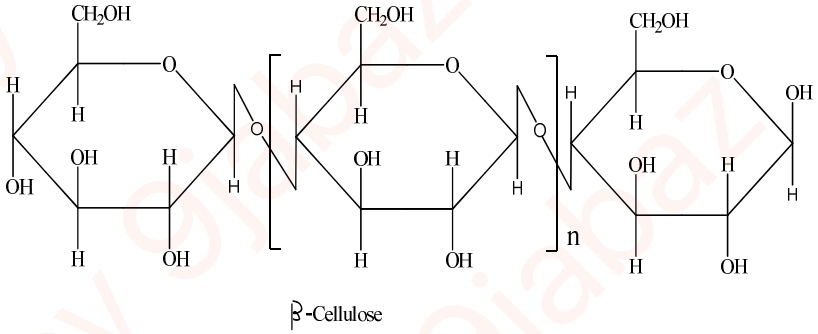
A partially methylated OH – group (methocel), is used as a thickner e.g. in shampoo or lotion.

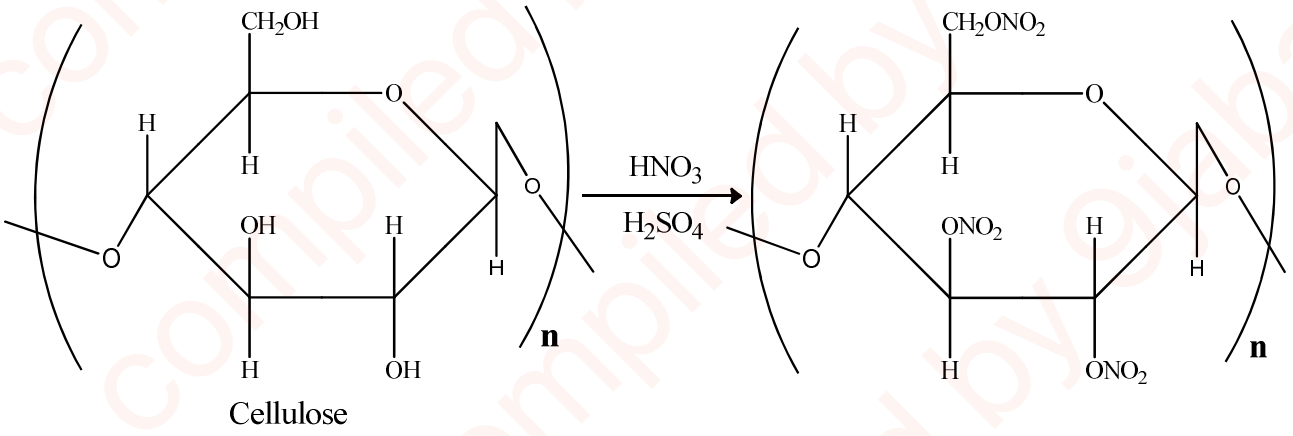
Cellulose that has many of its OH – groups converted to methoxy groups is used in varnishes, enamels, and packaging films.

Gun cotton – the trinitrate of cellulose (Figure: 3.21), looks like ordinary cotton but is extremely explosive. When mixed with nitroglycerin in right proportions, it is used in high – powered explosives and as propellant for artillery shells

Cellulose is insoluble in water, tasteless and a non – reducing carbohydrate. These properties are due in part to its extremely high molecular weight.

It has the formula $(C_6H_{10}O_5)_n$ and complete hydrolysis by acid yields β – D – glucopyranose.





Cellulose

Cellulose trinitrate

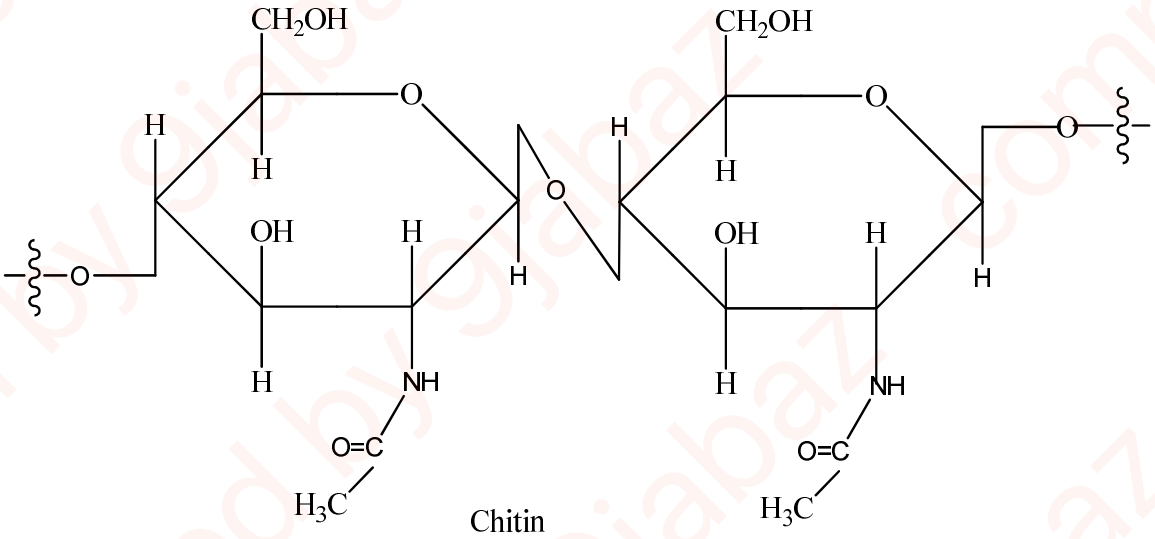
Figure 3.21: Trinitrate of cellulose

3.2.4 AMINO SUGARS

Amino sugars are important to many animals including mammals and anthropoids.

CHITIN: Crustaceans are characterised by a hard, crusty exoskeleton. This covering is made up of mainly chitin, a polymer of 2 – acetamido – 2 – deoxy – D – glucose.

Crab shell serves as an excellent source of 2 – amino – 2 – deoxy – β – D – glucopyranose. This amino sugar is produced ($\approx 70\%\ yield$) upon hydrolysis in concentrated HCl. From Chitin structure (Figure 3.22), it can be seen that it is similar to cellulose.



Chitin

Figure 3.22: Structure of Chitin

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PROTEINS

Polymers formed by condensation of α-amino acids. Hydrolysis of proteins with acids, bases or enzymes produces a mixture of α-amino acids. In proteins the amino acid units are linked by peptide linkages (–CO–NH–).

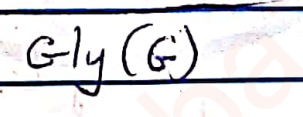
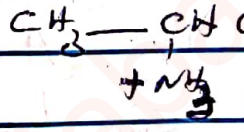
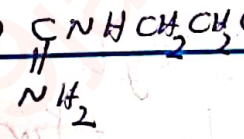
Amino acids

There are twenty three amino acids of general occurrence in all proteins.

Structures of amino acids

The general structure is given below:

The structures of some of these amino acids are given below:

	Name	Abbreviation	Formula
1	Glycine	Gly (G)	
2	Alanine	Ala (A)	
3	Arginine	Arg (R)	

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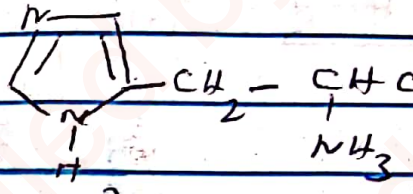
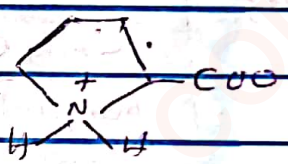
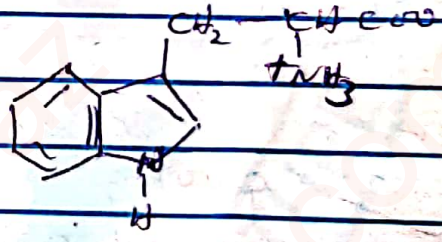
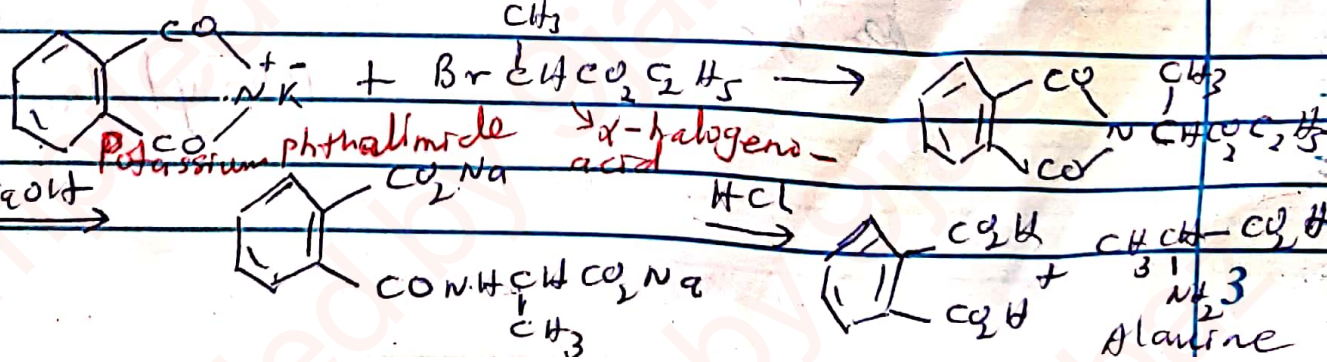
	Name	Abbr.	Formula
4	Aspartic acid (D)	Asp	$HOOCCH_2-\underset{+NH_3}{CH}COO^-$
5	Cysteine (C)	Cys	$HSCH_2-\underset{NH_3}{CH}COO^-$
6	Cystine	Cys – Cys	$OOC\underset{+NH_3}{CH}CH_2S-SCH_2-\underset{+NH_3}{CH}COO^-$
7	Histidine (H)	His	
8	Lysine (K)	Lys	$H_3\overset{+}{N}-(CH_2)_4-\underset{+NH_3}{CH}COO^-$
9	Methionine (M)	Met	$CH_3SCH_2CH_2-\underset{+NH_3}{CH}COO^-$
10	Proline (P)	Pro	
11	Tryptophan (W)	Try	

Preparation of Amino Acids

(1) Gabriel's Phthalimide Synthesis



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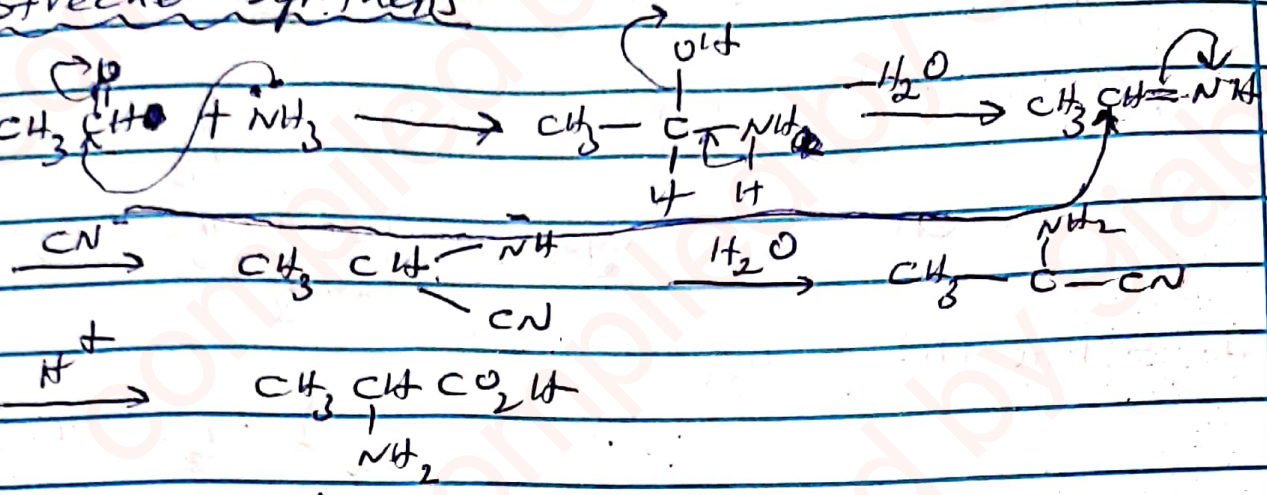
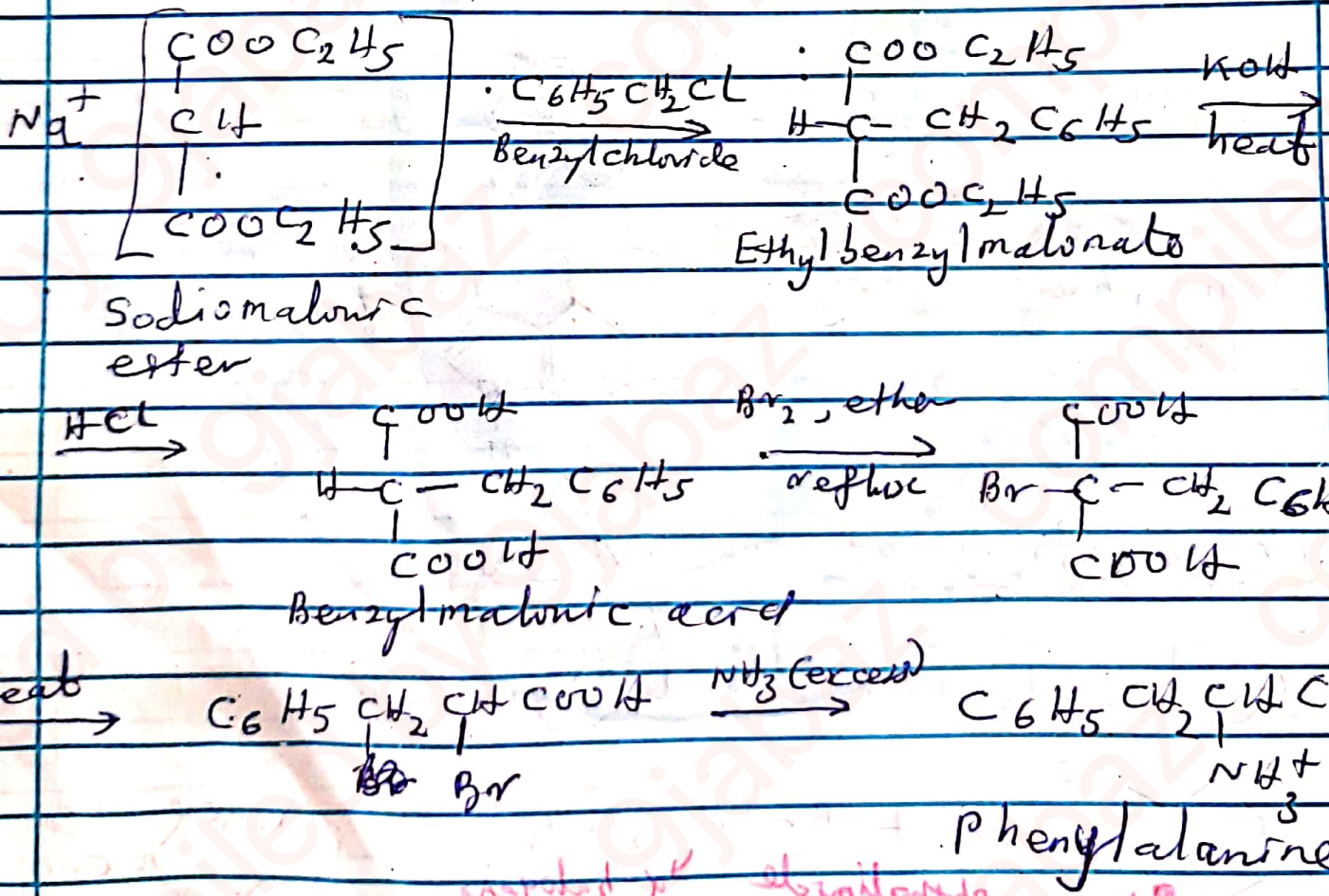
(ii) Strecker Synthesis



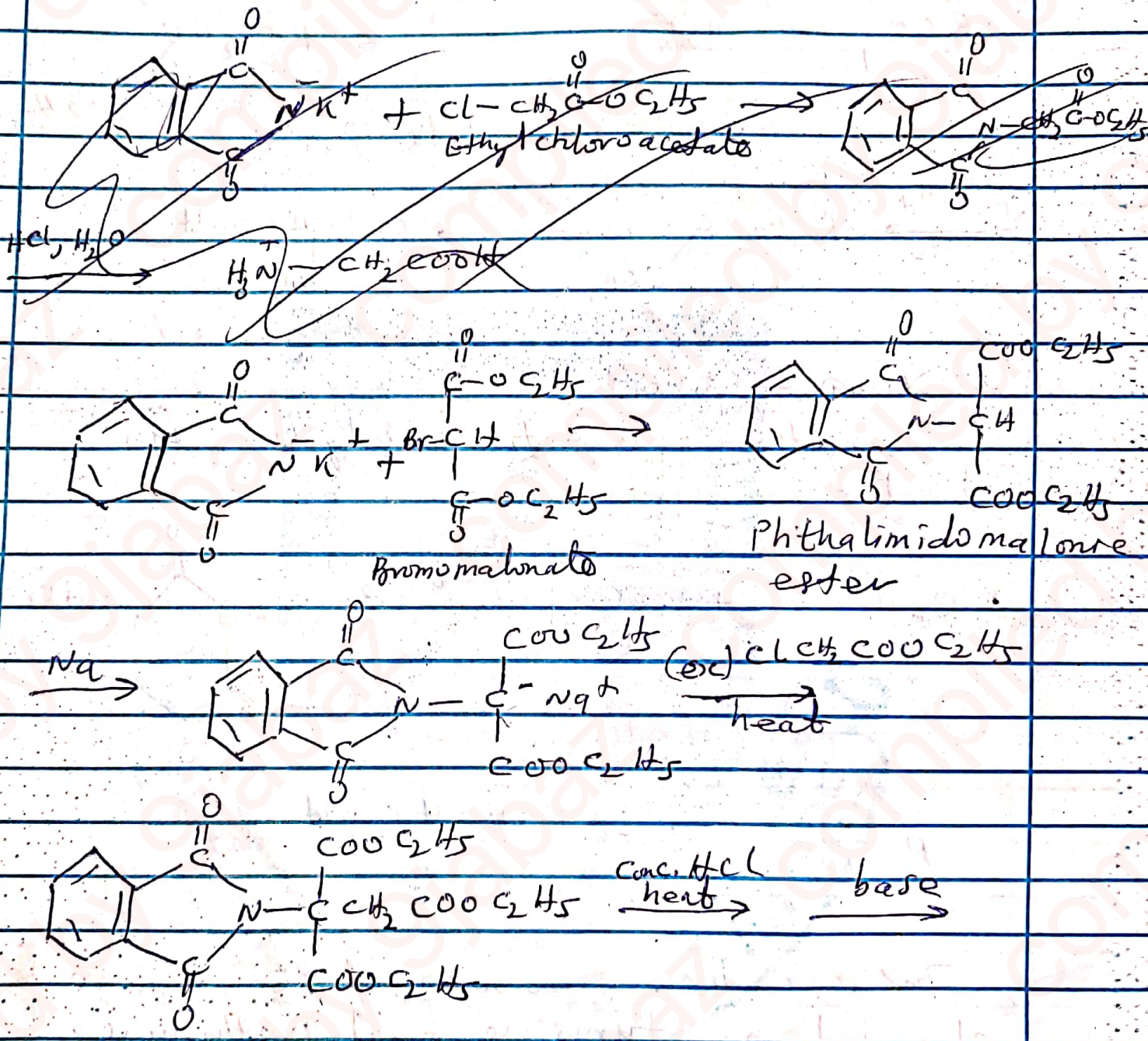
Alanine

(iii) Malonic ester Synthesis of Amino Acids



Phenylalanine

(IV) Synthesis of Aspartic acid (Gabriel phthalimide synthesis)



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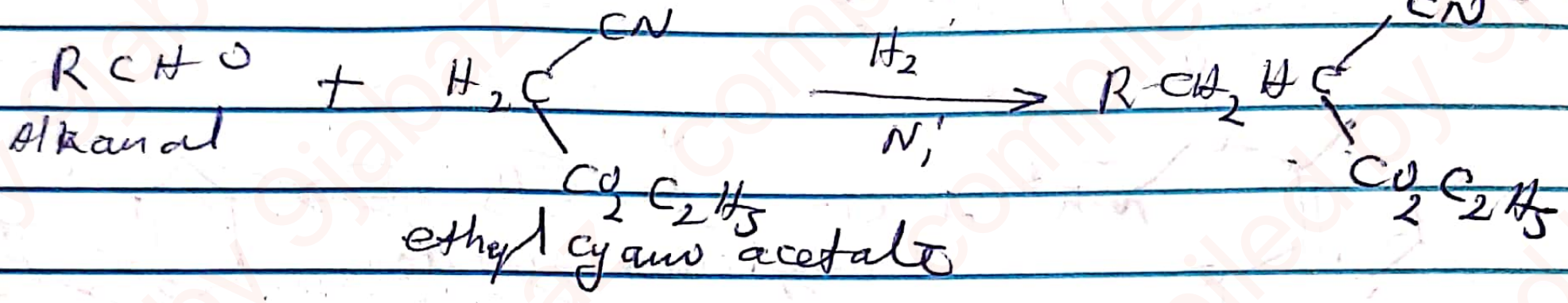
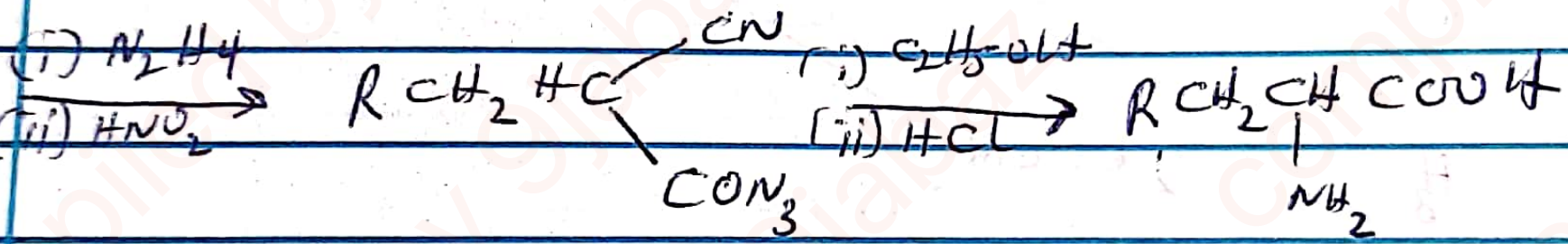
(v) Darapsky Synthesis





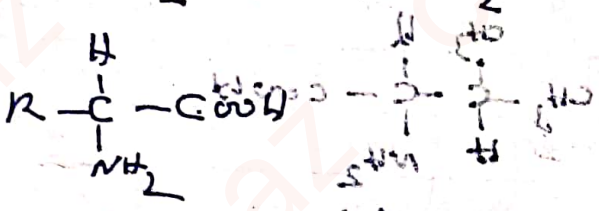
(vi) Synthesis of Aromatic

Synthesis of amino acids

(a) Amination of α-Halo acid

The halo acid is made by

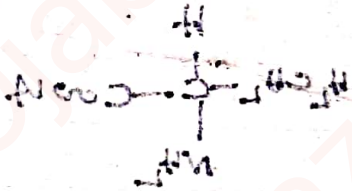
$$RCH_2COOH + Br_2 \xrightarrow{PBr_3} RCHBrCOOH \xrightarrow[(b)\ dil.\ acid]{(a)\ NH_3\ (excess)}$$



Bromination can also be accomplished using Malonic acid.

$$\left(EtO-\overset{O}{\overset{\|}{C}}\right)_2\bar{C}H\ Na^+ \xrightarrow[(S_N2)]{RBr} \left(EtO-\overset{O}{\overset{\|}{C}}\right)_2CHR \xrightarrow[heat]{KOH/H_2O} \xrightarrow{H^+}$$

$$\left(HO-\overset{O}{\overset{\|}{C}}\right)_2CHR \xrightarrow[Br_2/THF]{heat} \left(HO-\overset{O}{\overset{\|}{C}}\right)_2CBrR \xrightarrow[heat]{-CO_2} HO-\overset{O}{\overset{\|}{C}}-\underset{Br}{\overset{H}{\underset{|}{\overset{|}{C}}}}-R \xrightarrow[(b)\ dil.\ acid]{(a)\ NH_3\ (ex)}$$



$$HO-\overset{O}{\overset{\|}{C}}-\underset{NH_2}{\overset{H}{\underset{|}{\overset{|}{C}}}}-R$$

(b) Gabriel Synthesis

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Peptides

When amino acids polymerized they form polyamide. The polyamides derived from α-amino acids are called peptides or polypeptides.

$$n\ H_2N{-}CH_2{-}COOH \xrightarrow{-(n-1)H_2O} H_2N{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}\left(NH{-}CH_2{-}\overset{O}{\overset{\|}{C}}\right)_{n-2}{-}NH{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OH$$

Polyglycine.

The amido linkage in the polymer is called peptide bond or peptide linkages.

In a heteropolyamide, the amino acid at the end of the polymer bearing a free NH_2- group is called the "N-terminal amino acid". The amino acid with free carboxyl group is called "C-terminal amino acid". Amino acid residue in a peptide are numbered starting from the N-terminal amino acid.

Polypeptides are named as derivatives of C-terminal amino acid, the N-terminal amino acid being listed first and the C-terminal last.

Example is glycylalanine

$$H_2N{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OH + H{-}\overset{H}{\overset{|}{N}}{-}\underset{CH_3}{\underset{|}{CH}}{-}\overset{O}{\overset{\|}{C}}{-}OH \xrightarrow{-H_2O}$$

$$H_2N{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}NH{-}\underset{CH_3}{\underset{|}{CH}}{-}CO_2H$$

N-terminal Glycylalanine C-terminal

Question.....

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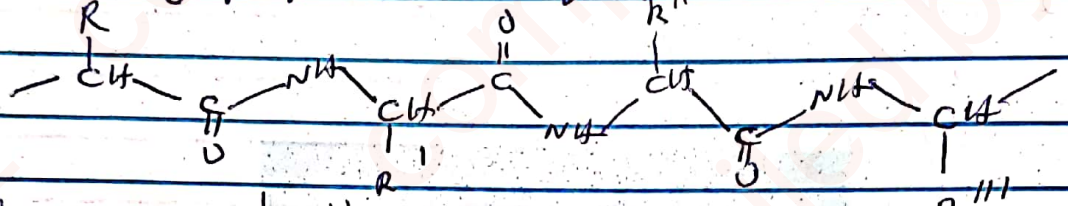
PROTEINS

Proteins are the naturally occuring condensation polymers of α- amino acids. Hydrolysis of proteins with acids, bases or enzymes produces a mixture of α- amino acids. Amino acids units are linked by peptide linkages ($-\overset{O}{\overset{\|}{C}}-NH-$) in proteins.



Where R, R', R'', etc can be either by Hydrogen or an organic group.

Structures of Proteins

Simple proteins can be divided into fibrous and globular proteins. Proteins with high intermolecular hydrogen bonding are fibrous and those with intramolecular hydrogen bonding are globular. The globular proteins became insoluble or denatured on heating. Examples are egg albumin, white globulins & glutelins examples of fibrous proteins are silk, wool, hides and hooves.

glutelins

Structures of Proteins

(i) Primary structure

This describes the sequence of α- amino acids in a poly peptide chain.

Question.....

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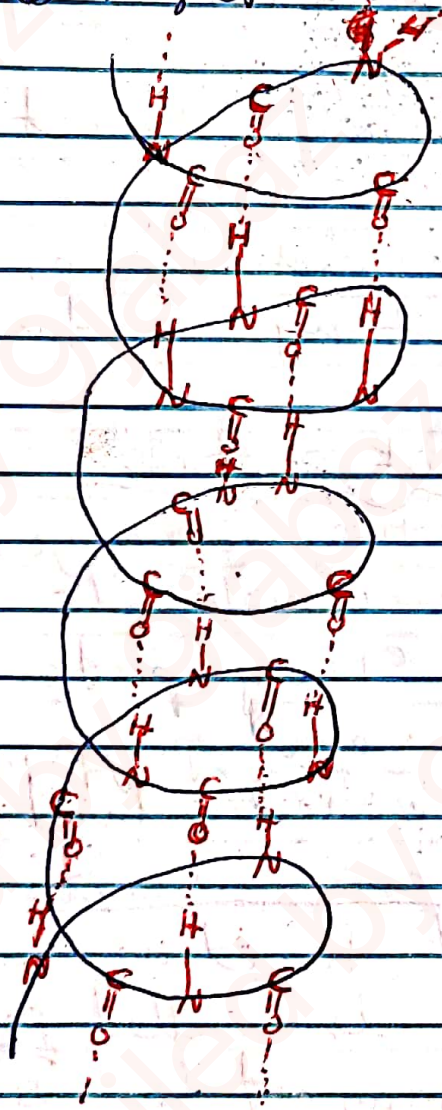
(iii) Secondary structure

It describes its conformation or molecular shape. It has been proved that the polypeptide chains have helical conformations in their crystal lattices. The helices are stabilized by intramolecular hydrogen bonds involving $-NH-C-$ groups. The α-helix is right handed.



α-Helix

Question.....

(iii) Tertiary structure

This term is used to describe the shape or folding resulting from the presence of Sulphur - sulphur cross-links between the polymer chains.

It is the three dimentional shape of a protein. The tertiary structure will have a single polypeptide chain backbone with one or more protein secondary structures, the protein domains.

Amino acid side chains may interact and bond in a number of ways. The interactions and bonds of side chains within a particular protein determine its tertiary structure.

(iv) Quaternary structure of protein

Quaternary structure is the arrangement of more than one protein molecule in a multi-subunit complex. Many proteins are acutally comprised of several polypeptide chains. In this case, the individual peptide chains are called protein subunits, and each unit ~~can not~~ cannot function on its own. These sub-units are also called protomers. These subunits may or may not be identical, and when they are held together by hydrogen bonds, they may be separated by e.g dissolving

Question.....

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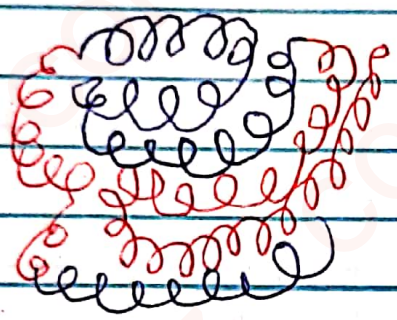
in water containing urea.

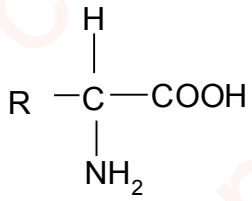
An example

The figure below is a sketch sketch of the structure of quaternary protein.

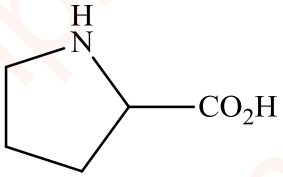
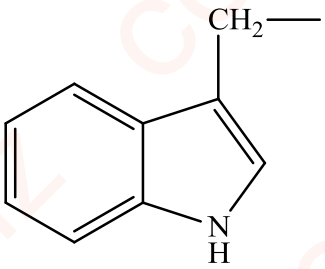


An example of a protein with quaternary structure is hemoglobin. In hemoglobin one protein binds to oxygen while another binds Carbon dioxide. This is how one protein can serve two functions. Another example is holoenzymes, proteins with several subunits. Each subunit of a holoenzyme has a different function. For example, one protein subunit may be the regulatory subunit. Another unit may be the catalytic subunit.

AMINO ACIDS AND PROTEIN



General structure of Amino Acid

Name	*Abbreviation*	*R*
Glycine	(gly)	H
Alanine	(ala)	CH_3
Proline	(Pro)	
Phenylalanine	(Phe)	$C_6H_5CH_2$-
Trytophan	(try)	
Methionine	(Met)	$CH_3S - CH_2CH_2 -$
Aspartic acid	(asp)	$HO_2CCH_2 -$
Asparagine	(asp (NH_2) or asn)	$H_2NC(O)CH_2 -$
Glutamine	(glu (NH_2) or gln)	$H_2NC(O)CH_2\,CH_2-$
Cysteine	(CySH)	$HSCH_2 -$
Cystine	(Cys – Scy)	$- CH_2S - SCH_2 -$

Essential amino acids

Essential amino acids are those that cannot be synthesized from substances ordinarily present in the diets by an organism at a rate equal to physiological requirements. The essential ones believed to be the following: isoleucine, leusine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. This is based on healthy individuals.

20 natural amino acid notation		
Amino Acid	**3-Letter**[4]	**1-Letter**[4]
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G

20 natural amino acid notation		
Amino Acid	**3-Letter**[4]	**1-Letter**[4]
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W

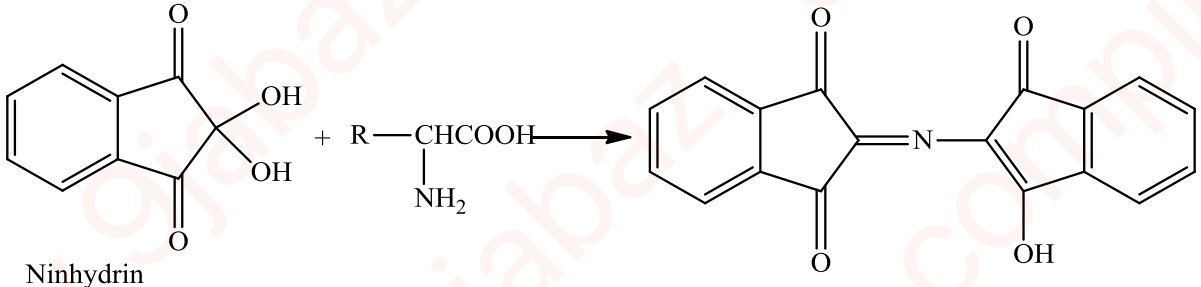
20 natural amino acid notation		
Amino Acid	**3-Letter**[4]	**1-Letter**[4]
Tyrosine	Tyr	Y
Valine	Val	V

Chemical reactions and interactions of Amino acids and proteins

Reactions of various functional groups of amino acids and proteins are used for the chemical estimations.

(i) Reaction with ninhydrin(2,2,-D, hydroxyl-13-indasdione

The reaction leads to the formation of coloured complex which is used for quantitative determination of amino acids.

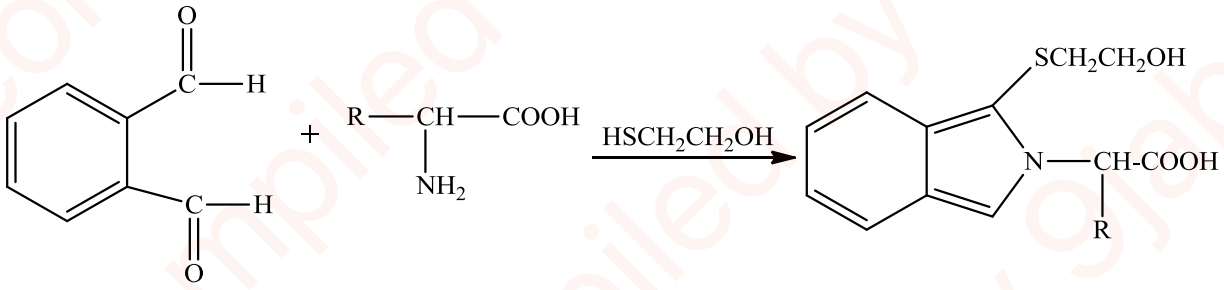


Ninhydrin

Ruhemmann's purple

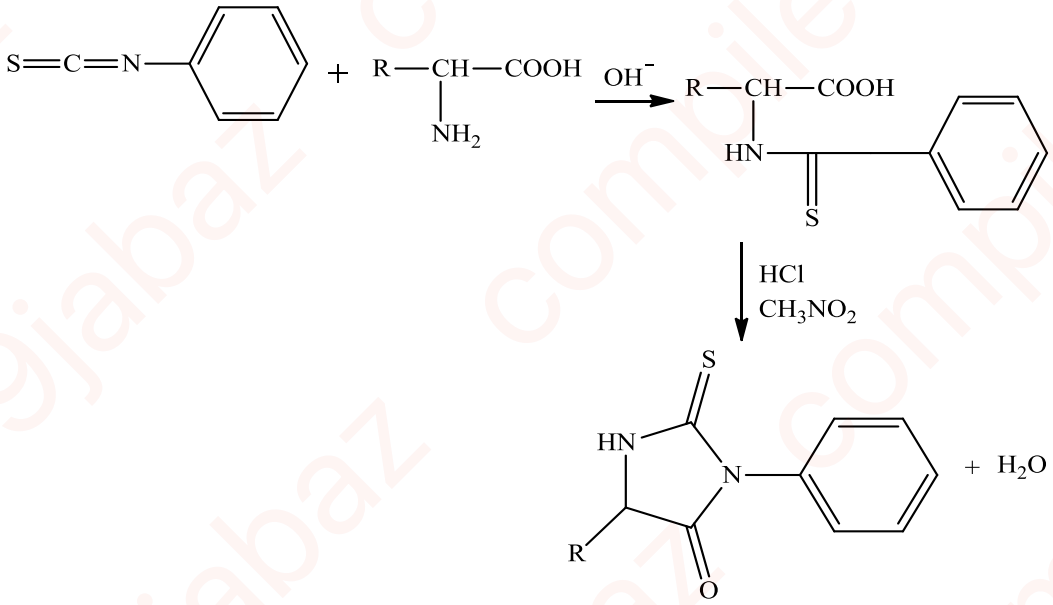
$+ \quad R\text{-}CHO + CO_2 + 3H_2O$

(ii) Reaction with 1,2- Benzene dicarbonal: 1,2- Benzene dicarbonal reacts with amino acids to give highly fluorescent isoindole derivatives.

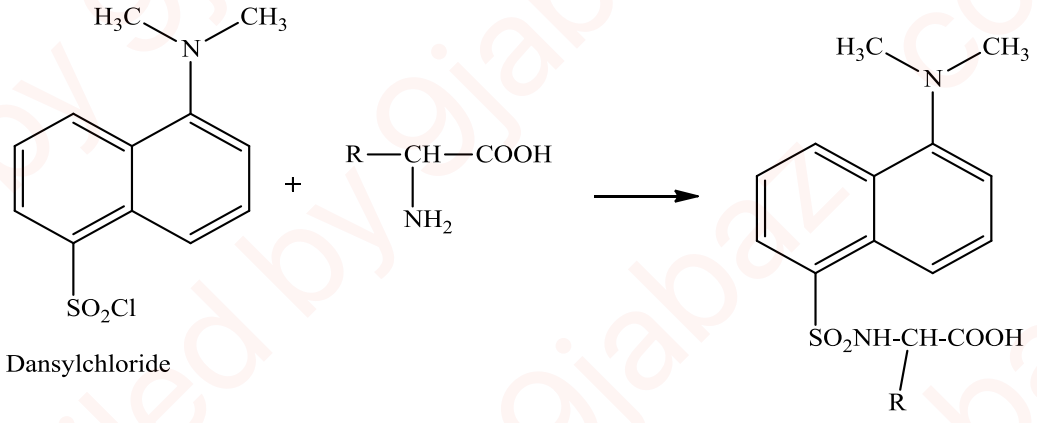


1, 2 - Benzenedicarbonal or O-phtaldialdehyde

(iii) Reaction with phenylisothiocyanate



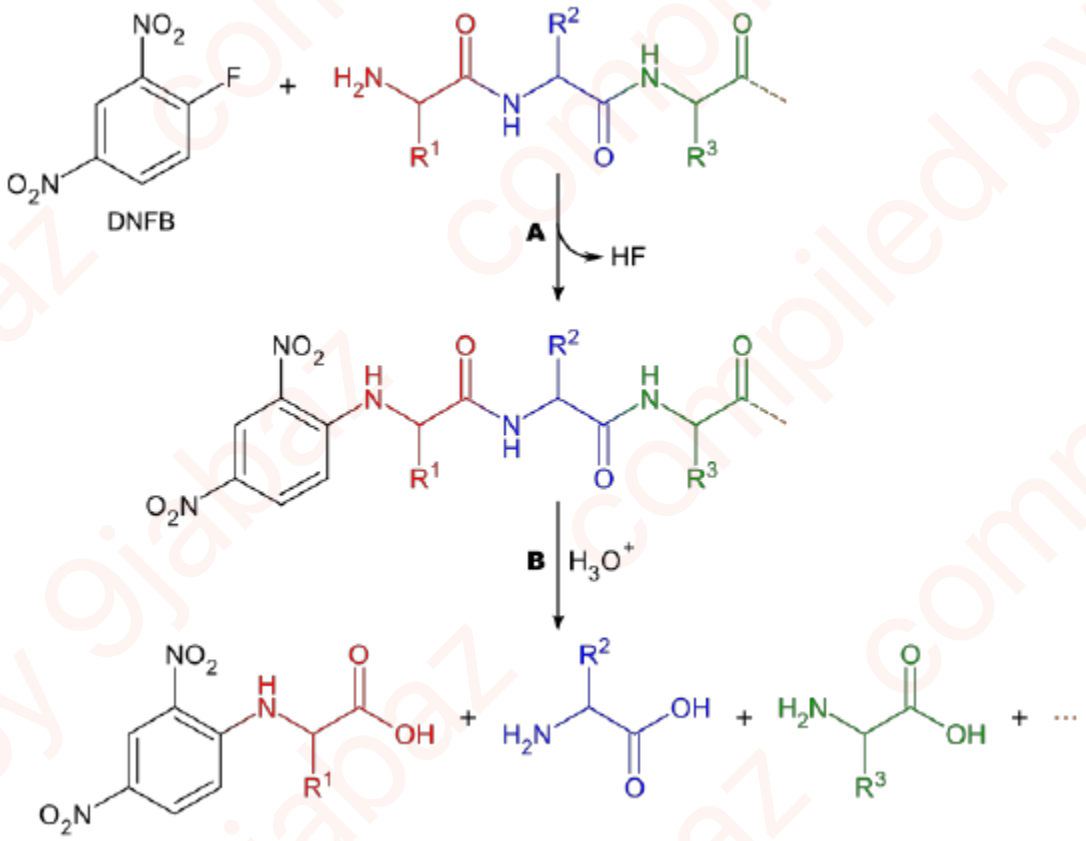
(iv) Reaction with Dansyl chloride (1, 1-Dimethy-aminonaphthalene-5-sulfonyl chloride)



Dansylchloride

The following reactions are used for the N-terminal analysis of proteins:

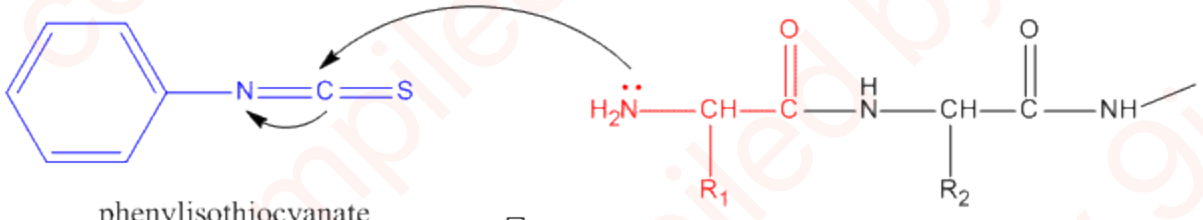
A. Sanger's reagent, FDNB (fluorodinitrobenzene), modifies N-terminus for determination via amino acid analysis.



B. **Amino Acid Sequence - Edman Degradation**

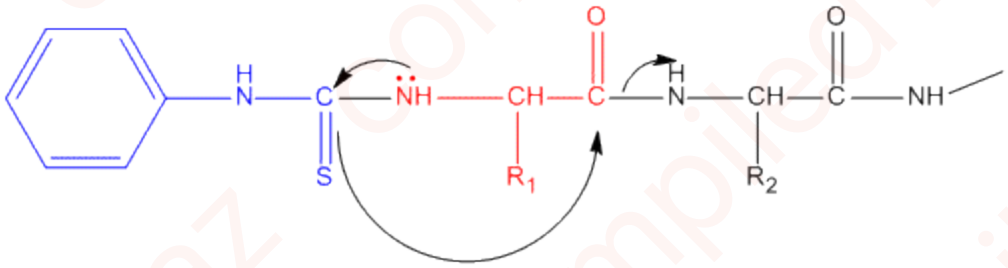
Two methods exist to determine the entire sequence of a protein. In one, the protein is sequenced; in the other, the DNA encoding the protein is sequenced, from which the amino acid sequence can be derived. The actually protein can be sequenced by automated, sequential Edman Degradation.

EDMAN DEGRADATION - AMINO ACID SEQUENCING

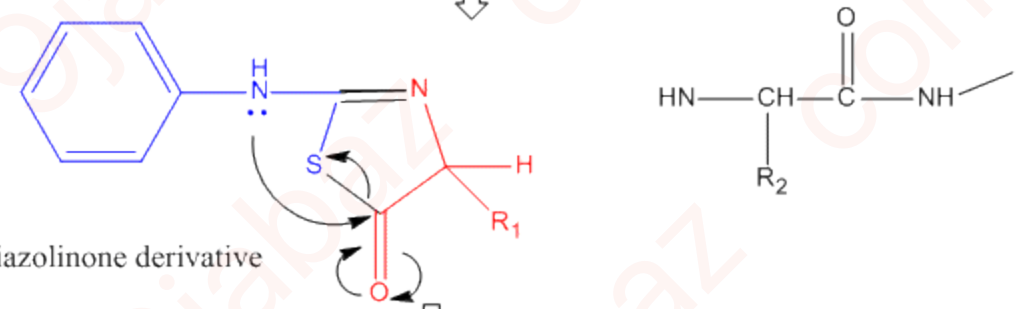


phenylisothiocyanate

mild alkaline conditions, pH 9

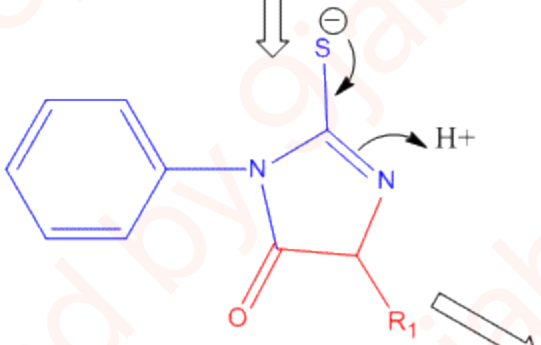


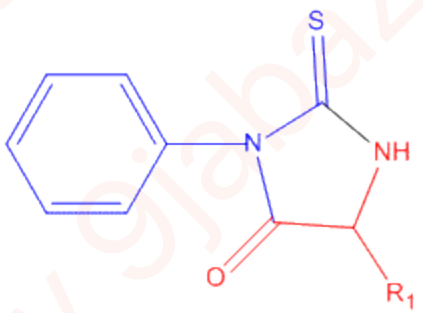
anhydrous HF



thiazolinone derivative

aqueous H+





PTH-Amino Acid
phenylthiohydantoin-AA

In this technique, a protein adsorbed to a solid phase reacts with phenylisothiocyanate. An intramolecular cyclization and cleavage of the N-terminal amino acid results, which can be washed from the adsorbed protein and detected by HPLC analysis. The yields in this technique are close to 100%. However, with time, more chains accumulate in which an N-terminal amino acid has not been removed. If it is removed on the next step, two amino acids will elute, creating increasing "noise" in the elution step - i.e. more than 1 amino acid derivative will be detected. Hence the maximal length of the peptide which can be sequenced is about 50 amino acids. Most proteins are larger than that. Hence, before the protein can be sequenced, it must be cleaved with specific enzymes called endoproteases which cleave proteins after specific side chains. For example, trypsin cleaves proteins within a chain after Lys and Arg, while chymotrypsin cleaves after aromatic amino acids, like Trp, Tyr, and Phe. Chemical cleavage by small molecules can be used as well. Cyanogen bromide, CNBr, cleaves proteins after methionine side chains. The individual proteins must be cleaved using two different methods, and each peptide fragment isolated and sequenced. Then the order of the cleaved peptides with known sequence can be pieced together by comparing the peptide sequences obtained using different cleavage methods. Many proteins also have disulfide bonds connecting Cys side chains distal to each other in the polypeptide chain. Proteolytic or chemical cleavage of the protein would lead to the formation of a fragment containing two peptides linked by disulfides. Edman degradation would release two amino acids from such fragments. To avoid this problem, the protein is oxidized with performic acid, which irreversibly oxidizes free Cys, or Cys-Cys disulfides to cysteic acid residues. A summary of the steps involved in protein sequencing are shown below:

PROTEIN SEQUENCING STRATEGY - 8 STEPS

1. If the protein contains more than one polypeptide chain, the chains are separated and purified. If disulfide bonds connect two different chains, the S-S bond must be cleaved (as described in step 2) and each peptide independently purified.
2. Intrachain S-S bonds between Cys side chains are cleaved with performic acid.
3. The amino acid composition of each chain is determined
4. The N-terminal and C-terminal residues are identified.

5. Each polypeptide chain is cleaved into smaller fragments, and the amino acid composition and sequence of each fragment is determined.
6. Step 5 is repeated, using a different cleavage procedure to generate a different and overlapping set of peptide fragments.
7. The overall amino acid sequence of the protein is reconstructed from the sequences in overlapping fragments.
8. The position of the S-S is located.

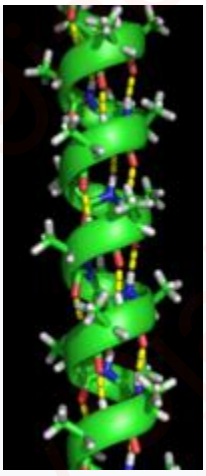
PROTEIN STRUCTURES

Protein primary structure is the linear sequence of amino acids in a peptide or protein. By convention, the primary structure of a protein is reported starting from the amino-terminal (N) end to the carboxyl-terminal (C) end. Protein biosynthesis is most commonly performed by ribosomes in cells. Peptides can also be synthesized in the laboratory.

Isomerisation

The chiral centers of a polypeptide chain can undergo racemization. Although it does not change the sequence, it does affect the chemical properties of the sequence. In particular, the L-amino acids normally found in proteins can spontaneously isomerize at the atom to form D-amino acids, which cannot be cleaved by most proteases. Additionally, proline can form stable trans-isomers at the peptide bond.

Secondary structure

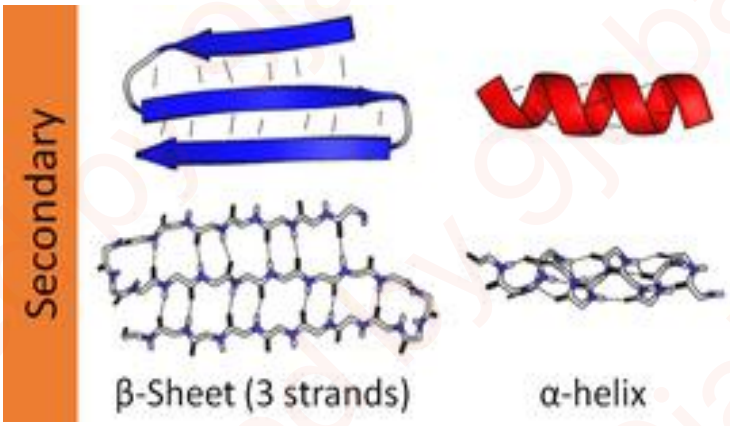


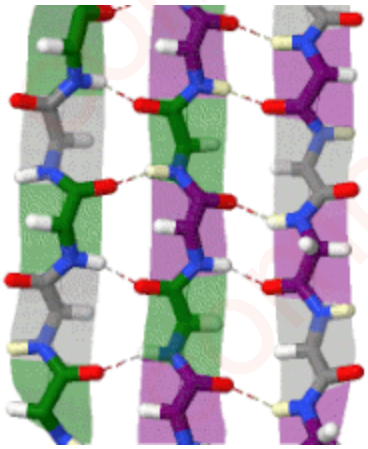
An α-helix with hydrogen bonds (yellow dots)

Secondary structure refers to highly regular local sub-structures on the actual polypeptide backbone chain. Two main types of secondary structure, the α-helix and the β-strand or β-sheets, were suggested in 1951 by Linus Pauling.[5] These secondary structures are defined by patterns of hydrogen bonds between the main-chain peptide groups.

Secondary structure of protein refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone.

- The proteins do not exist in just simple chains of polypeptides.
- These polypeptide chains usually fold due to the interaction between the amine and carboxyl group of the peptide link.
- The structure refers to the shape in which a long polypeptide chain can exist.
- They are found to exist in two different types of structures α – helix and β – pleated sheet structures.
- This structure arises due to the regular folding of the backbone of the polypeptide chain due to hydrogen bonding between -CO group and -NH groups of the peptide bond.
- However, segments of the protein chain may acquire their own local fold, which is much simpler and usually takes the shape of a spiral an extended shape or a loop. These local folds are termed secondary elements and form the proteins secondary structure.





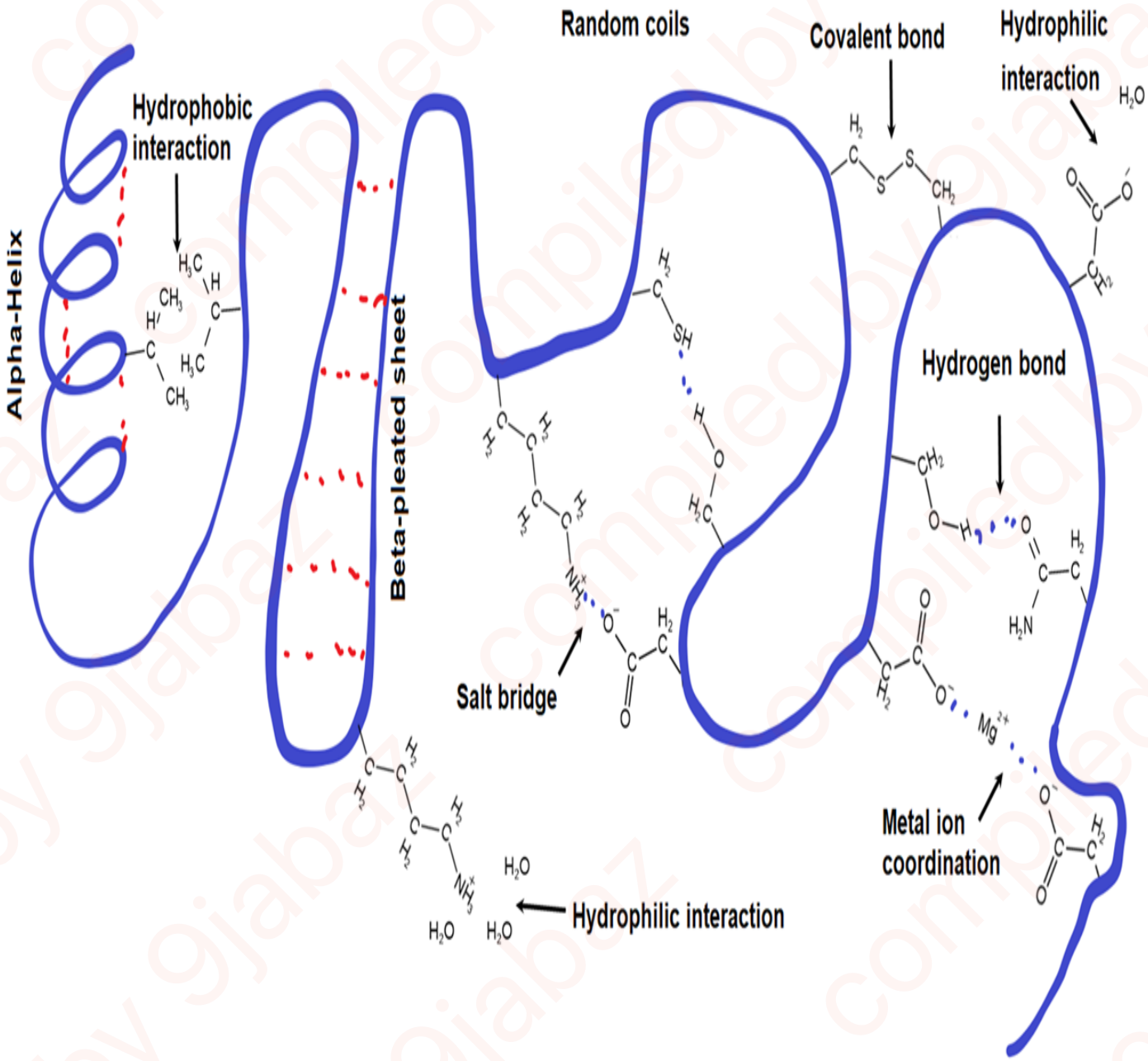
Three-dimensional structure of parts of a beta sheet in green fluorescent protein

Tertiary Structure of Protein

- This structure arises from further folding of the secondary structure of the protein.
- H-bonds, electrostatic forces, disulphide linkages, and Vander Waals forces stabilize this structure.
- The tertiary structure of proteins represents overall folding of the polypeptide chains, further folding of the secondary structure.
- It gives rise to two major molecular shapes called fibrous and globular.
- The main forces which stabilize the secondary and tertiary structures of proteins are hydrogen bonds, disulphide linkages, van der Waals and electrostatic forces of attraction.

The three-dimensional arrangement of all the atoms of a single polypeptide chain in space, held together by stabilizing interactions between groups on the side chains and between the side chain groups and the backbone groups is called the **tertiary structure of proteins**.

The stabilizing interactions involved in stabilizing the tertiary structure include disulfide linkage, salt bridge, coordinate bonds with metal ions, hydrogen bonding, and hydrophobic interaction, as shown in Fig. and explained below.



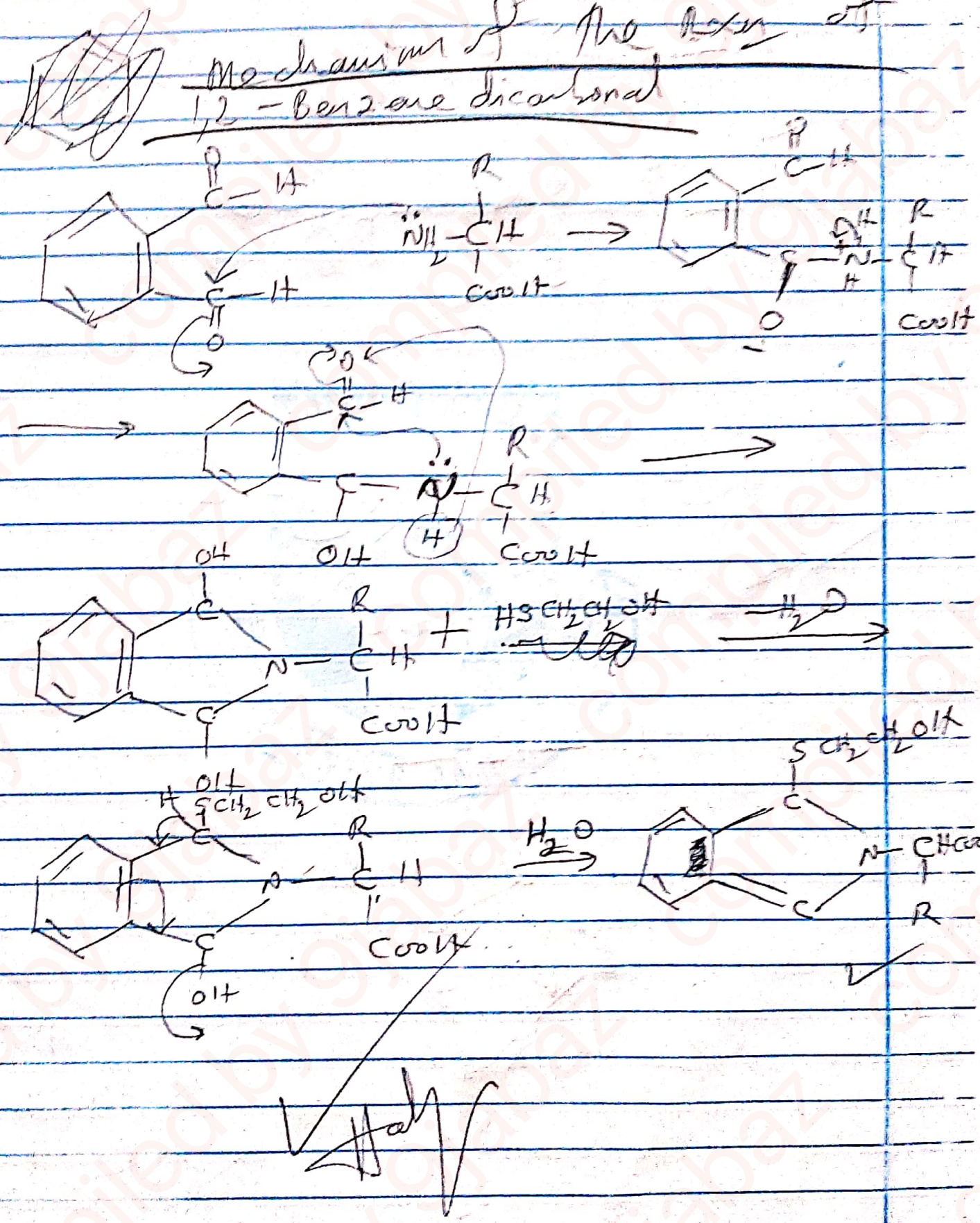
This figure illustration of disulfide linkage, salt bridge, coordinate bonds with metal ions, hydrogen bonding, and hydrophobic interaction that stabilize the tertiary structure of proteins.

QUATERNARY STRUCTURE OF PROTEIN

See the scanned note on proteins.

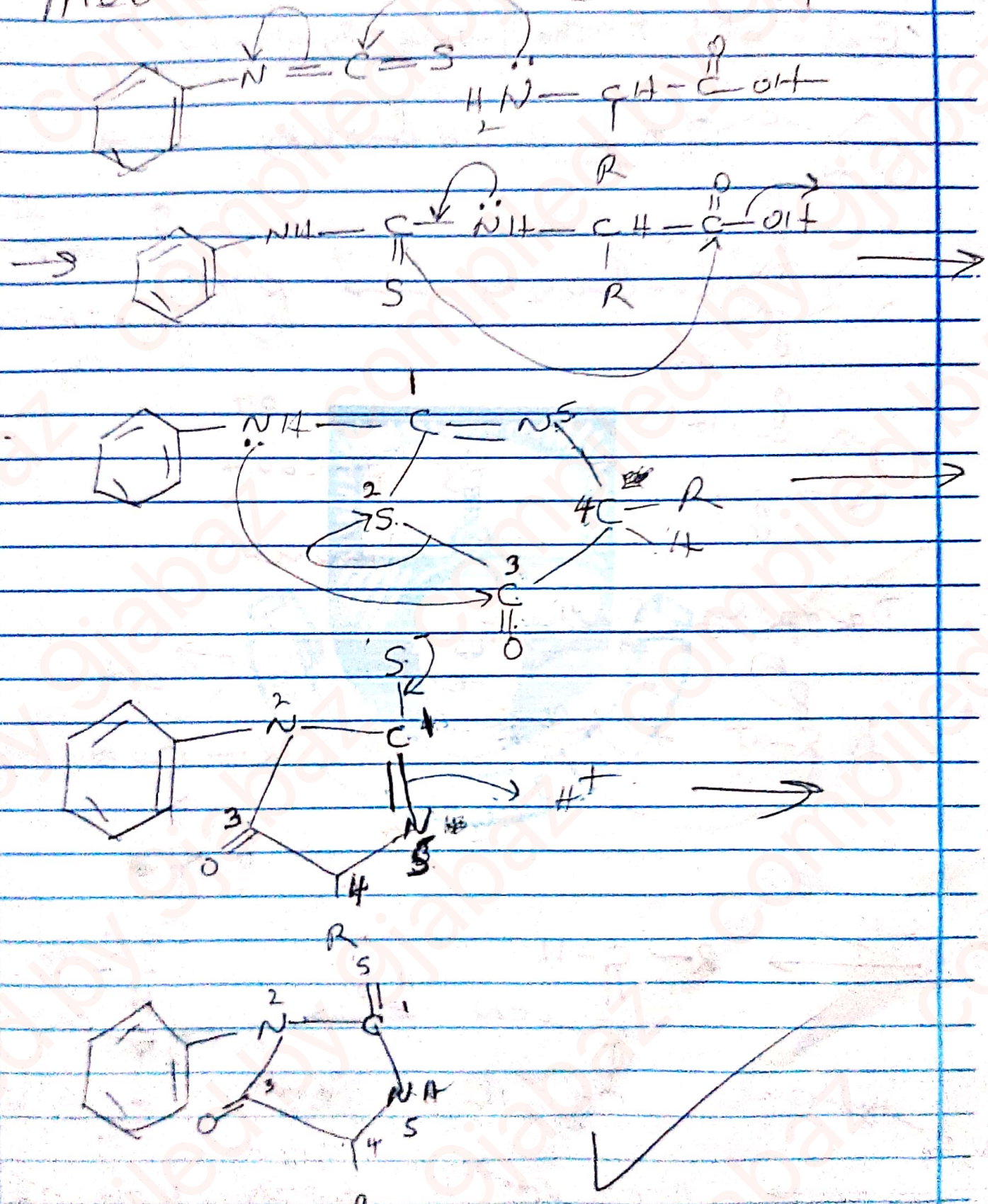
Question.....

Mechanism of the reaction of 1,2-benzenedicarbonal



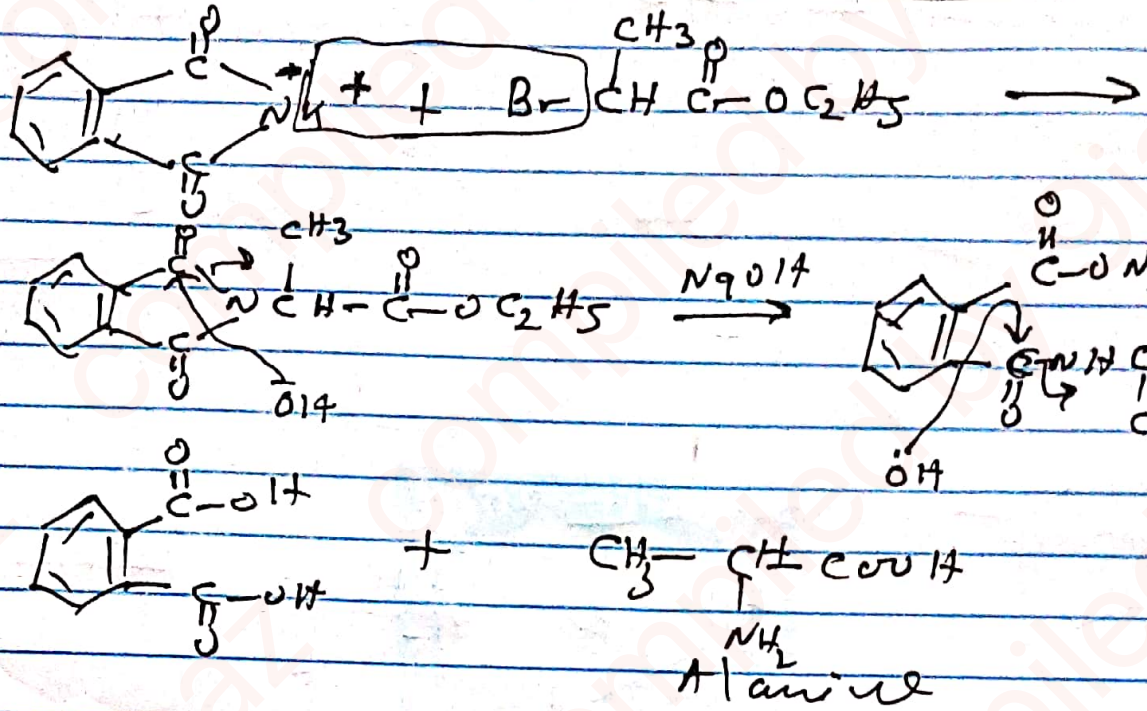
Question

Mechanism of phenyl isothiocyanate reaction with aa.

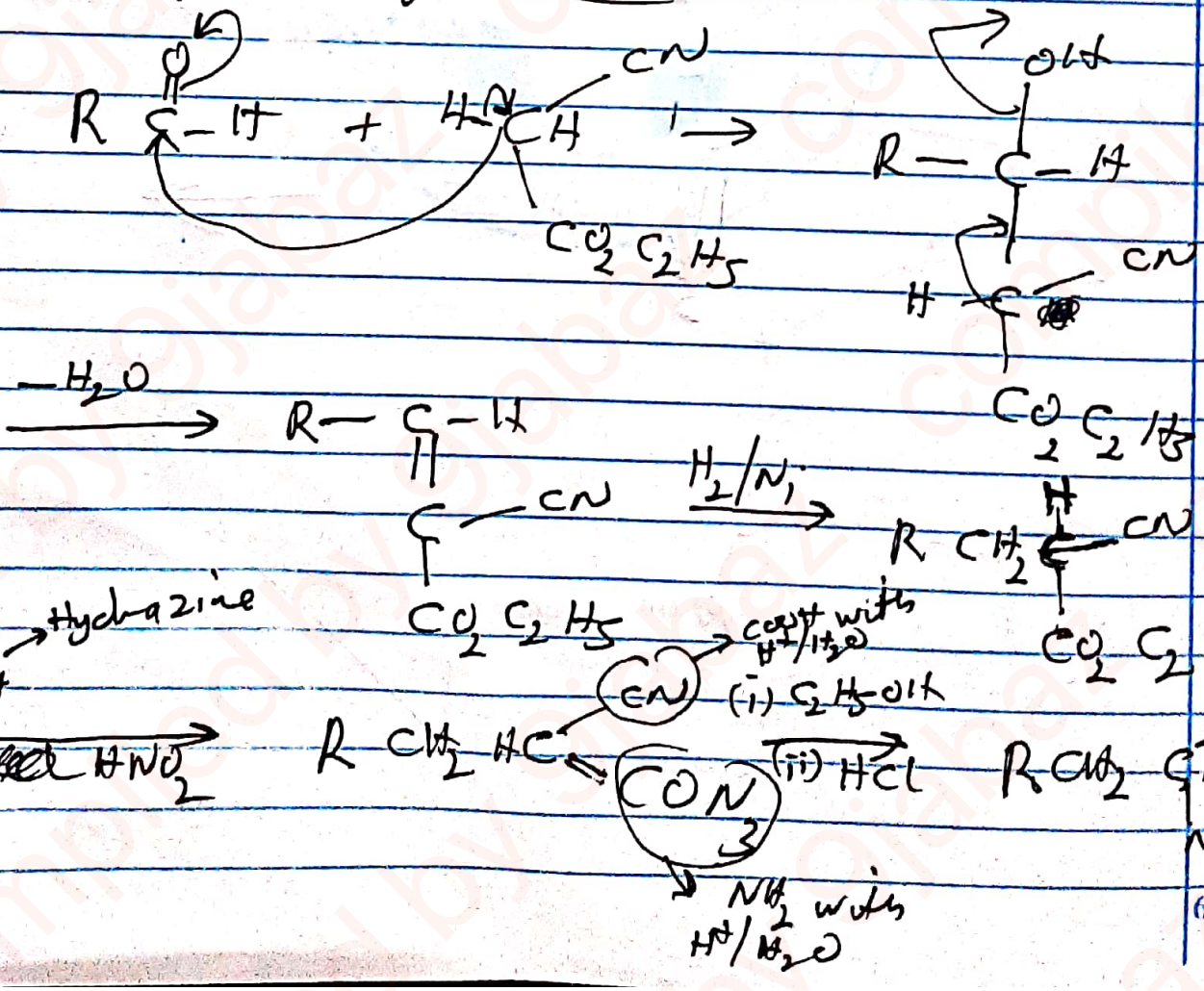


Question.....

Gabriel's phthalimide Synthesis



Darapsky Synthesis



Lignin and Its Properties

Introduction

Lignin was first mentioned in 1813 by the Swiss botanist A. P. de Candolle, who described it as a fibrous, tasteless material, insoluble in water and alcohol but soluble in weak alkaline solutions, and which can be precipitated from solution using acid. He named the substance "lignine", which is derived from the Latin word *lignum*, meaning wood. It is one of the most abundant organic polymers on Earth, exceeded only by cellulose and chitin. Lignin constitutes 30% of terrestrial non-fossil organic carbon on Earth, and 20 to 35% of the dry mass of wood. Lignin is a class of complex organic polymers found in the structural tissues of plants. There are variations in the properties of lignin depending on its origin, such as polydispersity molar masses and hyperbranched structures.

Lignin (Fgure 1.0) is a group of polyphenolic, organic polymers present in plants. It is a macromolecular compound with an average molecular weight of ~20,000. Lignin is derived from the word *lignum*, which means "wood" in Latin. It is found in the cell wall of plants, where it is one of the main constituents together with cellulose microfibrils and hemicellulose formed by oxidative coupling of free lignin monomers secreted directly into the plant cell wall. Lignocellulose is a structure for all three constituents (cellulose, lignin and hemicellulose)

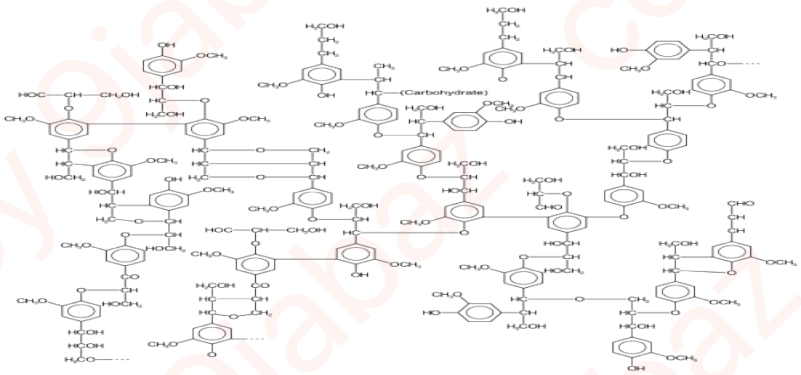


Figure 1.0: Idealized structure of lignin from a softwood

Lignin in plants mainly provides rigidity by strengthening the structure of cell walls, especially in larger roots and stems. It provides additional tensile strength of 25–74 MPa and a Young's modulus of 2.5–3.7 GPa . Lignin regulates water and other liquid transportation through the plant. It also protects against biological stresses by inhibiting enzymatic degradation of other components. Lignin provides protection for the plant against microorganisms and contains antioxidants that defend plants against oxidation of their macromolecules. In specialized plant cell types, lignin also waterproofs the cell walls.

Lignin is the second most abundant biological material, with cellulose being the first. During the papermaking pulping and bleaching process, cellulose is extracted to make paper while the lignin is separated and is a waste material during this process. The abundance of untapped

"waste" lignin and the low cost of lignin make it attractive to be explored for further applications given its good properties.

This structure shows that much of the carbon is present in aromatic rings that are bonded to oxygen-containing groups, and lignin is the only major plant biopolymer that is largely aromatic. Because of this characteristic, lignin is of considerable interest as a source of aromatic compounds including phenolic compounds, which have the−OH group bonded to aromatic rings or even aromatic hydrocarbons. The abundance of hydroxyl (−OH), methoxyl ($-OCH_3$), and carbonyl (C=O) groups in lignin also suggests potential chemical uses for the substance.

Composition and structure

The composition of lignin varies from species to species. An example of composition from an aspen sample is 63.4% carbon, 5.9% hydrogen, 0.7% ash (mineral components), and 30% oxygen (by difference), corresponding approximately to the formula $(C_{31}H_{34}O_{11})_n$.

Lignin is a collection of highly heterogeneous polymers derived from a handful of precursor ***lignols***. Heterogeneity arises from the diversity and degree of crosslinking between these lignols. The lignols that crosslink are of three main types, all derived from phenylpropane: coniferyl alcohol (3-methoxy-4-hydroxyphenylpropane; its radical, G, is sometimes called guaiacyl), sinapyl alcohol (3,5-dimethoxy-4-hydroxyphenylpropane; its radical, S, is sometimes called syringyl), and paracoumaryl alcohol (4-hydroxyphenylpropane; its radical, H, is sometimes called 4-hydroxyphenyl) (Figure 2.0).

The relative amounts of the precursor "monomers" (lignols or monolignols) vary according to the plant source. Lignins are typically classified according to their syringyl/guaiacyl (S/G) ratio. Lignin from gymnosperms is derived from the coniferyl alcohol, which gives rise to G upon pyrolysis. In angiosperms some of the coniferyl alcohol is converted to S. Thus, lignin in angiosperms has both G and S components.

Lignin's molecular masses exceed 10,000 u. It is hydrophobic as it is rich in aromatic subunits. The degree of polymerisation is difficult to measure, since the material is heterogeneous.

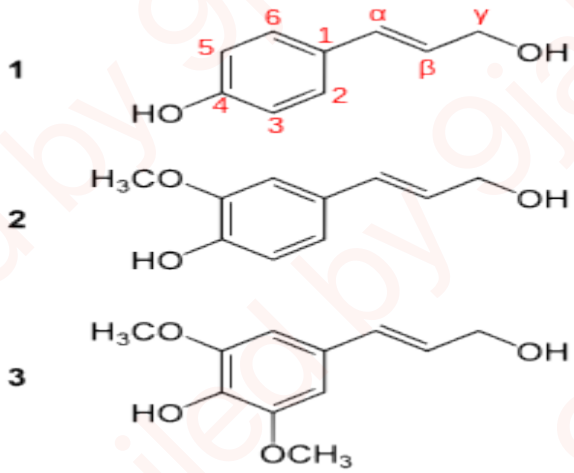


Figure: 2.0: The three common monolignols: paracoumaryl alcohol, H coniferyl alcohol, G sinapyl alcohol, S

Biological function

Lignin fills the spaces in the cell wall between cellulose, hemicellulose, and pectin components, especially in vascular and support tissues: xylem tracheids, vessel elements and sclereid cells.

Lignin plays a crucial part in conducting water and aqueous nutrients in plant stems. The polysaccharide components of plant cell walls are highly hydrophilic and thus permeable to water, whereas lignin is more hydrophobic. The crosslinking of polysaccharides by lignin is an obstacle for water absorption to the cell wall. Thus, lignin makes it possible for the plant's vascular tissue to conduct water efficiently. Lignin is present in all vascular plants, but not in bryophytes, supporting the idea that the original function of lignin was restricted to water transport.

It is covalently linked to hemicellulose and therefore cross-links different plant polysaccharides, conferring mechanical strength to the cell wall and by extension the plant as a whole. Its most commonly noted function is the support through strengthening of wood (mainly composed of xylem cells and lignified sclerenchyma fibres) in vascular plants. Finally, lignin also confers disease resistance by accumulating at the site of pathogen infiltration, making the plant cell less accessible to cell wall degradation.

Economic significance

In pulp mills, using the kraft or the sulfite process, lignin is removed from lignocellulose to yield pulp for papermaking.

Global commercial production of lignin is a consequence of papermaking. In 1988, more than 220 million tons of paper was produced worldwide. Much of this paper was delignified; lignin comprises about 1/3 of the mass of lignocellulose, the precursor to paper. Lignin is an impediment to papermaking as it is colored, it yellows in air, and its presence weakens the paper. Once separated from the cellulose, it is burned as fuel. Only a fraction is used in a wide range of low volume applications where the form but not the quality is important.

Mechanical, or high-yield pulp: This is used to make newsprint, still contains most of the lignin originally present in the wood. This lignin is responsible for newsprint's yellowing with age. High quality paper requires the removal of lignin from the pulp. These delignification processes are core technologies of the papermaking industry as well as the source of significant environmental concerns.

Sulfite pulping: In sulfite pulping, lignin is removed from wood pulp as lignosulfonates, for which many applications have been proposed. They are used as dispersants, humectants, emulsion stabilizers, and sequestrants (water treatment). Lignosulfonate was also the first family of water reducers or superplasticizers to be added in the 1930s as admixture to fresh concrete in order to decrease the water-to-cement (*w/c*) ratio, the main parameter controlling the concrete porosity, and thus its mechanical strength, its diffusivity and its hydraulic conductivity, all parameters essential for its durability. It has application in environmentally sustainable dust suppression agent for roads. Also, lignin can be used in making biodegradable plastic along with cellulose as an alternative to hydrocarbon-made

plastics if lignin extraction is achieved through a more environmentally viable process than generic plastic manufacturing.

kraft process: This is another method of converting wood into wood pulp. Lignin removed by the kraft process is usually burned for its fuel value, providing energy to power the paper mill. Two commercial processes exist to remove lignin from black liquor (The spent liquor from Kraft process) for higher value uses: LignoBoost (Sweden) and LignoForce (Canada). Higher quality lignin presents the potential to become a renewable source of aromatic compounds for the chemical industry, with an addressable market of more than $130bn.

Given that it is the most prevalent biopolymer after cellulose, lignin has been investigated as a feedstock for biofuel production and can become a crucial plant extract in the development of a new class of biofuels.

Thermal method

A high temperature utilization is also applied at commercial scale that's disintegrate the plant biomass and direct conversion of biomasses into polysaccharides and carbon materials. The thermal treatment scale depends on the plant biomass materials and conversion into target molecules. Low thermal temperature ranges from 120 to 180°C are uses for proteins, carbohydrates and other sugars molecules solubilization. Whereas, high thermal treatment are applied for production of volatile organics, carbons and gases from biomass. Thermal heating in autoclave uses steam heat under high pressure on lignocellulosic biomass results in a considerable break in lignin linkages in biomass. Electromagnetic radiation from microwaves with a frequency range of 250–300 MHz energy induced molecular vibration and is showed more efficient and less time consuming pretreatment method compared to conventional heating. The microwave heating combine with acid, alkali and organic solvents has high impact than thermal treatment method alone. So thermal treatment with any technology can be used to disintegrate the plant biomass into its components.

Chemical method

Chemical method with thermal heating is commonly used for fractionation of biomass into lignin cellulose, and hemicellulose. The function of acids in low concentration break the outer surface and release bulk of extratives and hemicellulose of the biomass. Whereas, the concentrated acids at high thermal treatment disassociate the firm structure of the biomass. This treatment released both lignin and hemicellulose sugars of the biomass simultaniously. Sulfuric acid, hydrocholric acid, and nitric acid with (1–10% w/v) are mostly reported in the acid chemcial treatment method. The alkaline solutions particularly of sodium hydroxide, potassium hydroxide, and calcium hydroxide specifically target linkages bonds in lignin and have very less penetration power for the cellulose of biomass. The alkaline solution with thermal heating pretreatment makes the biomass digestible for enzymes saccharification into sugars allowing maximum entrance to hemicellulose and cellulose molecules. It is hard to classify the most appropriate pre-treatment process for different kinds of biomasses. But, the right choice pre-treatment process needs to enhance porosity of the biomass and reduce the inhibitors generation. Ideally, low acids and alkali concentration has revealed optimmum separation of lignin, hemicellulose and cellulose of the biomass without addition of inhibitors. Further, dilute acids and alkali pre-treatment can are economical for bioenergy and biochemicals production from biomass

polysaccharides at large scale. Potassium hydroxide, calcium hydroxide and sodium hydroxide are the highly tested alkali for lignocellulose degradation, however, sodium hydroxide has a greater potential reaction rate compare to other alakli. Whereas, in cost perspective, calcium hydroxide has less cost per kg than the others and could prove to the inexpensive pretreatment of biomass. Therefore, for industrial scale process, it is better to select the inexpensive acids and alkali with optimum thermal process for yield of polysaccharides and biofuels production from pretreated biomass through anaerobic fermentation.

Organosolv method

Organosolv extraction method include, an organic solvent dissolved in water at high hydrothermal condition for biomass hydrolysis to remove a pure sulfur-free lignin. Common tested solvents have very low boiling point and can be simply recycle back after the pretreatment process. The subsequent lignin structure has small changes related to the native lignin. The organosolv treatments in fact chop favorably the bonds between carbohydrate-lignin molecules separating a partially modified lignin. Due to this characteristic, lignin obtained with organosolv treatments is a best monomer for making polymers, such as bio-based polymers, polyesters, polyurethanes, and phenolformaldehyde resins. The most universal organic solvents are acetone, ethanol, methanol, or a mixture thereof. The purified lignin is recuperated after the precipitation process using water.

Biotreatment

Biotreatment is basically biological pretreatment method applying either bacteria, fungi or yeast cells in single culture of mix microbial communities composition. Additionally, specific enzymes in a single entity or mixed enzymes solution have also been used in biotreatment system. The nature and function of enzymes and microbial culture could be different but the overall target is to fractionate the biomass into separate polysaccahrides molecules. A number of enzymes such as lignin peroxidases, laccases, xylanases, endoglucanases, peptidases, esterase and hydrolases have been applied in biomass saccharification process. Beside enzymes, a number of fungal strains *Phanerochaete chrysosporium, Trametes versicolor, Pleurotus ostreatus, Ceriporiopsis subvermispora,* and bacterial species particularly *Clostridum sp. Pseudomonas sp.* LD002, actinomycetes, *Streptomyces viridosporus* T7A, *Rhodococcus jostii* RHA 1 and *Pseudomonas putida* , *B*acillus sp. strains*,* and thermophile *anaerocellum thermophilum* are reported for oxidative hydrolysis of lignin from biomass. Fungi like white and brown-rot fungi have the ability to hydrolyze both lignin and cellulose. Such strains has the enzymes machinery of lignin peroxidase, manganese peroxidase, versatile peroxidase, laccase and cellulases [49]. The eficiency and percentage of lignin degradation depends on the enzymes and growth of the microorgansims like *Stereum hirsutum (*white-rot fungi) has 14.5%, degradation of the lignin from the wood biomass [49]. Wherase, *Coniochaet ligniaria* fungus reported upto 75% of lignin degradation from pepper plant, likwise, *Pleurotus Florida* with 45–50% degradation of lignin from corn straw. The importance of biological treatment method is consumption of low energy, safe for environment and has no involvement of toxic chemcials. However, the problems that needs to be adjusted is sluggish growth, optimum conditions of enzymes, products inhibition, pretreatment time, enzymes purification, economical approach, feasible for the degradation of diverse agriculture biomasses. However, all of these studies

proposed that biological treatment is economical pre-treatment method among the all for inexpensive biofuels productions.

Biodegradation

In contrast to other bio-polymers (e.g. proteins, DNA, and even cellulose), lignin resists degradation. It is immune to both acid- and base-catalyzed hydrolysis. The degradability varies with species and plant tissue type. For example, syringyl (S) lignin is more susceptible to degradation by fungal decay as it has fewer aryl-aryl bonds and a lower redox potential than guaiacyl units. Because it is cross-linked with the other cell wall components, lignin minimizes the accessibility of cellulose and hemicellulose to microbial enzymes, leading to a reduced digestibility of biomass.

Some ligninolytic enzymes include heme peroxidases such as lignin peroxidases, manganese peroxidases, versatile peroxidases, and dye-decolourizing peroxidases as well as copper-based laccases. Lignin peroxidases oxidize non-phenolic lignin, whereas manganese peroxidases only oxidize the phenolic structures. Dye-decolorizing peroxidases, or DyPs, exhibit catalytic activity on a wide range of lignin model compounds, but their *in vivo* substrate is unknown. In general, laccases oxidize phenolic substrates but some fungal laccases have been shown to oxidize non-phenolic substrates in the presence of synthetic redox mediators.

These degradations are brought about by some classes of fungi (bacteria as discussed above) and by pyrolysis.

Pyrolysis

Pyrolysis of lignin during the combustion of wood or charcoal production yields a range of products, of which the most characteristic ones are methoxy-substituted phenols. Of those, the most important are guaiacol and syringol and their derivatives. Their presence can be used to trace a smoke source to a wood fire. In cooking, lignin in the form of hardwood is an important source of these two compounds, which impart the characteristic aroma and taste to smoked foods such as barbecue. The main flavor compounds of smoked ham are guaiacol, and its 4-, 5-, and 6-methyl derivatives as well as 2,6-dimethylphenol. These compounds are produced by thermal breakdown of lignin in the wood used in the smokehouse.

Lignin applications

Isolated lignin through any pretreatment methods can be use directly as unmodified crude lignin or pure lignin with further modification reactions in the applied field. This section will briefly describe the main applications of both native lignin and modified lignin.

Native lignin

Lignin has many applications; one of them is direct combustion of lignin as black liquor as a fuel and power in paper and wood pulping industry (**see the attached you tube video**). Also the native lignin increase combustion heat to bio-fire made of propane-1,3-diol and cellulose. In view of its binding stuffs, it is commonly used as a stabilizer in coal briquettes in which it raises the boiling speed and strength in packing paper material. Regardless of the importance of lignin

for power generation, it has many other uses like as toluene or benzene, non-toxic binder in pelleted feeds, nonhazardous chelating agent, and transporter of nutrients as lignosulfonate for the plants to discharge them gradually in the soil. Lignin can also be used as a sequestrant of heavy metals (e.g. zinc, nickel, cadmium and mercury) for purification of polluted water due to chelating property.

Chemical analysis

The conventional method for lignin quantitation in the pulp industry is the Klason lignin and acid-soluble lignin test, which is standardized procedures. The cellulose is digested thermally in the presence of acid. The residue is termed Klason lignin. Acid-soluble lignin (ASL) is quantified by the intensity of its Ultraviolet spectroscopy. The carbohydrate composition may be also analyzed from the Klason liquors, although there may be sugar breakdown products (furfural and 5-hydroxymethylfurfural).

A solution of hydrochloric acid and phloroglucinol is used for the detection of lignin (Wiesner test). A brilliant red color develops, owing to the presence of coniferaldehyde groups in the lignin.

Thioglycolysis is an analytical technique for lignin quantitation. Lignin structure can also be studied by computational simulation.

Thermochemolysis (chemical break down of a substance under vacuum and at high temperature) with tetramethylammonium hydroxide (TMAH) or cupric oxide has also been used to characterize lignins. The ratio of syringyl lignol (S) to vanillyl lignol (V) and cinnamyl lignol (C) to vanillyl lignol (V) is variable based on plant type and can therefore be used to trace plant sources in aquatic systems (woody vs. non-woody and angiosperm vs. gymnosperm). Ratios of carboxylic acid (Ad) to aldehyde (Al) forms of the lignols (Ad/Al) reveal diagenetic information, with higher ratios indicating a more highly degraded material. Increases in the (Ad/Al) value indicate an oxidative cleavage reaction has occurred on the alkyl lignin side chain which has been shown to be a step in the decay of wood by many white-rot and some soft rot fungi.

Lignin and its models have been well examined by ^{1}H and ^{13}C NMR spectroscopy. Owing to the structural complexity of lignins, the spectra are poorly resolved and quantitation is challenging.

An overview of the general components of plant biomasses.

Substrates	Cellulose	Hemicellulose	Lignin
Softwoods	45–50	25–35	25–35
Bamboo	41–49	24–28	24–26
Hardwoods	40–55	24–40	18–25
Paper	40–55	25–35	15–20
Corn cob	40–50	20–35	15–17

Substrates	Cellulose	Hemicellulose	Lignin
Peanut	40–45	15–17	20–30
Corn straw	38–45	26–34	17–20
Para grass	35–45	25–30	15–20
Corn stalk	34–36	26–27	16–21
Wheat straw	30–40	30–50	15–16
Sweet sorghum	27–38	21–25	11–17
Grasses	25–40	35–50	10–30
Switch grass	45	31.4	12
Rice straw	38	19	13
Barley straw	37	24	16
Sugarcane bagasse	33	23	5
Arundo donax	31	30	21
Lawn grass	30	43	3–5

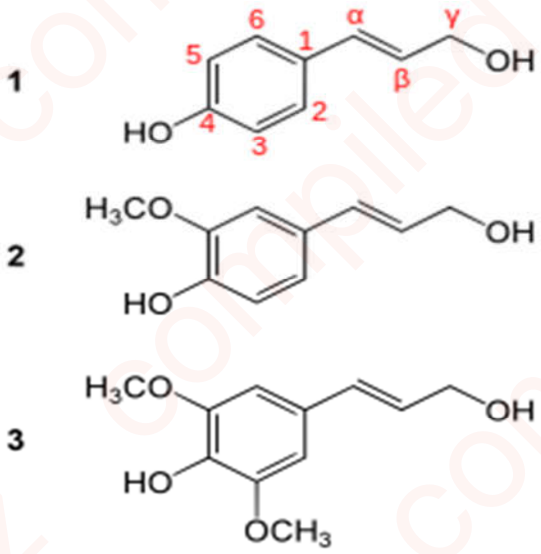


Figure: 2.0: The three common monolignols: paracoumaryl alcohol, H coniferyl alcohol, G sinapyl alcohol, S

BSc DEGREE EXAMINATION
2021/2022 Rain Semester Examinations

CHM 308: Natural and Synthetic Macromolecules

June, 2023 **Time Allowed: 2 hrs.**

Instruction: Answer sections A and B in separate booklets

SECTION A

1. (a) Using the technique of oxidation, methylation and hydrolysis in this order, prove that the structure of lactose is made up of two saccharides and indicate the carbons involved in its glycosidic bond formation.
Note: The IUPAC names of all the products formed in these reactions must be provided.
(b) D-fructose is a non-reducing sugar but was observed to give positive test with Tollens' reagent. With the aid of a suitable mechanism, account for this observation.
c. (i) Using D-arabinose, show with mechanism how a reducing sugar reacts with cyanide and provide the name of the reaction.
(ii) If the product of the reaction in question 1 c. (i) above is hydrolysed using dilute HCl, draw and provide the name(s) of the product(s) formed.
d. (i) Draw the structures of the following polysaccharides and provide the structure(s) and IUPAC name(s) of the disaccharide(s) formed when they are partially hydrolysed with dilute HCl:
(i) Cellulose (ii) Amylose and (iii) Amylopectin.

2. (a) (i) A DNA was observed to have primary structure represented with alphabets as given below, draw the actual primary structure of the DNA:
pGpApCpT.
(b) List all the four bases found in DNA and draw the structures of how they are paired.
(c) 2 – amino pyridine is one of the important bases found in nucleic acid, explain with chemical equation how this compound can be synthesized from guanidine.

3. (a) Draw the structures of the following amino acids:
(i) Aspartic acid (ii) Cysteine and (iii) Lysine.
(b) With the aid of chemical equation only, explain how phenylalanine is obtained from Sodiomalonic ester using malonic ester synthesis.
(c) Sanger reagent (FDNB) is a useful reagent for the N-terminal analysis of proteins. With the aid of chemical equation, explain how this reagent function in this analysis.

Total mark is 30

4. a. Define an epimer.

b. Classify the following sugars as either reducing or non-reducing sugar and give reasons for your classification: (i) Sucrose (ii) maltose (iii) Gentiobiose and (iv) Fructose.

(c) In not more than one sentence, give reason why β - anomer of D-glucose is 64% while α - anomer of D-glucose is 36%.

(d) In one sentence, give reason why DNA is more stable than RNA and list the names of all the bases found in RNA.

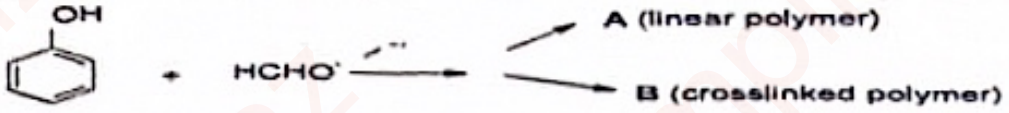
(e) Arrange in ascending order based on strength of protein structures given below and give reasons for your answer:

Secondary, Primary, Tertiary and Quaternary structures of protein.

Total mark is 10

SECTION B

5. With the aid of suitable chemical equations, explain the following transformations and provide the names of all the polymers formed that are represented with alphabets (A to F).

(a) 

(b) $H_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2 + HCHO \longrightarrow C$

(c) $H_2N\text{-}(CH_2)_6\text{-}NH_2 + HO\text{-}\overset{\overset{O}{\|}}{C}\text{-}(CH_2)_4\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH \longrightarrow D$

(d) $HO\text{-}CH_2\text{-}CH_2\text{-}OH + HO\text{-}\overset{\overset{O}{\|}}{C}\text{-}C_6H_4\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH \longrightarrow E$

(e) $[C_6H_5\text{-}CH{=}CH_2]_n \longrightarrow F$

6. (i) Write the polymer chain of natural rubber showing how the three isoprene units are joined together.

(ii) Write down all the properties of natural rubber which make it unsuitable for commercial Purposes.

(iii) Discuss how the properties mentioned in 6(ii) above can be improved.

(iv) What do you understand by biodegradable polymers?

(v) State all the differences between chain growth (addition) polymerization and step growth (condensation) polymerization.

(vi) Discuss the Chemistry of resins taken into consideration their production, structures, and uses.

Total mark is 30

2021/2022 Mid Semester Test (Rain)

CHM 308: Natural and Synthetic Macromolecules

June, 2024 **Time Allowed: 45 Min.**

SECTION A

1. (a) In term of structure, define a nucleotide.
2. (b) Draw the structure of ATP and give its IUPAC name. If the ATP is completely hydrolyzed by NaOH, provide the names and structures of the compounds obtained.
3. c. Translate the polynucleotide given below to line notation using letters and lines: pGpGpApTpCpA.
4. d. Use Chemical equations to explain the following transformations:
   (i) D - glucose ⟶ D – arabinose
5. What is the name of the reaction in question (1 d i) above?
6. (ii) Methyl – α – D – glucopyranose ⟶ Methyl – α – 2, 3,4,6 – tetra – o – methyl – D – glucopyranoside.
7. (iii) Use the structure of cellulose to explain how its monomer(s) combined. What is or are the IUPAC name(s) of its monomer(s)?

Total mark is 15 marks

SECTION B

2 a. Write short notes on: (i) Thermoplastics (ii) Thermosets (iii) Elastomers and (iv) Ring opening polymerization.

b. List the different applications of Low Density Polyethylene (LDPE) that you have studied.

3 a. (i) With the aid of balanced equations only, describe the various routes for the preparation of acrylonitrile.

(ii)Write down the balanced equation stating all the conditions for the preparation of Poly(acrylonitrile).

(iii) What is the molecule weight (molar mass) of polystyrene with a degree of polymerization 1000.

b. In a tabular form only, give the corresponding monomer and repeat units for the following polymers: (i) Polystyrene (ii) polyethylene and (iii) Polycaprolactam.

Total mark is 15 marks