№ 41. CARBOHYDRATES – CHARACTERISTICS, CLASSIFICATION. MONOSACCHARIDES – STRUCTURE, ISOMERISM, PHYSICAL PROPERTIES, CHEMICAL PROPERTIES. REPRESENTATIVES.

I. Characteristics. Carbohydrates are the most abundant class of organic compounds in Nature. It has been estimated that more than 50% of the dry weight of the earth's biomass - all plants and animals - consists of glucose polymers. They occur in every living organism. The sugar and starch in food, and the cellulose in wood, paper and cotton, are nearly pure carbohydrate. Modified carbohydrates form part of the coating around living cells, other carbohydrates are found in the DNA that carries genetic information, and some others are used as medicines. Carbohydrates are very familiar class of compounds; usually used group name for them is sugars (or saccharides). They comprise substantial portion of our food and provide most of the energy necessary for our life; storage depot for energy. In most living organisms, glucose (a simple carbohydrate) is oxidized to CO_2 and H_2O to provide energy needed by the cell. Plants store energy by converting glucose to starch, whereas animals store energy by converting glucose to glycogen which is another form of starch. In the form of cellulose that is constituent of cell walls, carbohydrates are the structural framework of plants. Other saccharides' derivatives include many other important biomolecules that play key roles in the immune system, fertilization, pathogenesis, blood clotting, and development.

Along with nucleic acids and proteins, carbohydrates are the third class of biopolymers. In contrast to the first two classes, carbohydrates are the most diverse class of compounds, including complex structures from intermolecular interactions with proteins and nucleic acids.

Many materials used in everyday life are based on carbohydrates – paper, textile (cotton), rayon fibers (via the viscose process), cellulose acetate, celluloid, nitrocellulose, and many more. Almost all of these materials are based on the fundamental biochemical chain of reactions occurring in green plants - the photosynthesis. In this complex process sunlight provides energy to convert CO_2 into glucose. Many molecules of glucose are then chemically linked by the plant to form either cellulose or starch. When eaten and metabolized, carbohydrates provide the major source of energy required by organisms. Thus, carbohydrates are the chemical intermediates by which solar energy is stored and at later time used to support life on our planet.

The word *saccharide* comes from the Greek word *σάκχαρον* (*sákcharon*), meaning "sugar" (in Latin "*saccharum*"). The term "saccharide" is the basis of classification system of carbohydrates. Historically carbohydrates were considered "hydrates of carbon" with general formula $C_n(H_2O)_m$. Thus, in many languages the corresponding term for saccharide means "hydrate of carbon". There are examples of organic compounds that have the same molecular formula, such as formaldehyde, acetic acid, lactic acid but do not have the typical properties of a carbohydrate and do not belong to the class of carbohydrates. On the other side, deoxyribose and galactosamine are carbohydrates that differ from the general formula. The current definition is – **a carbohydrate is a polyhydroxy aldehyde or polyhydroxyketone, or a compound that hydrolyzes to them**.

II. Classification Depending on the number of elementary units, carbohydrates are divided into four chemical types: monosaccharide, disaccharide, oligosaccharide, and polysaccharide.

- A **monosaccharide** or "simple sugars" is a carbohydrate that on attempted hydrolysis is not cleaved to smaller carbohydrates. Monosaccharides are the simplest carbohydrates. They are aldehydes or ketones with two or more hydroxyl groups. The general chemical formula of an unmodified monosaccharide is $(C{\bullet}H_2O)_n$, literally a "carbon hydrate". Monosaccharides are important fuel molecules as well as building blocks for more complex carbohydrates. The smallest monosaccharides, for which the number of carbon atoms n = 3, are dihydroxyacetone and D- and L-glyceraldehyde.
- A **disaccharide** on hydrolysis is cleaved to two monosaccharide units, which may be the same or may be different. For instance, **sucrose** – common table sugar – is a disaccharide that yields one molecule of glucose and one of fructose on hydrolysis.

- An **oligosaccharide** (*oligos* is a Greek word meaning "few") gives 3 to 10 monosaccharide units on hydrolysis. A general property of monosaccharides, disaccharides and most of oligosaccharides is their high solubility in water and usual, characteristic sweet taste.

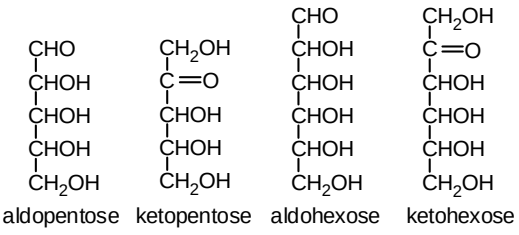
- A **polysaccharide** is hydrolyzed to more than 10 monosaccharide units. For instance, cellulose is a homopolysaccharide that gives thousands of identical glucose molecules when completely hydrolyzed. The natural biopolymers belong to the polysaccharide type. Polysaccharides (and oligosaccharides) are called also complex carbohydrates. They can be subdivided into two groups, depending on the nature of their monomeric units (which are the monosaccharides building the polymer):
- **homopolysaccharides** contain as building block identical monosaccharide units;
- **heteropolysaccharides** contain more than one type of monosaccharide. Even more complex are: glycoproteins – proteins that contain covalently attached oligosaccharide (glycans) chains, and glycolipids – lipids with a carbohydrate attached

More than 200 different monosaccharides are known. Most of them have trivial, common names such as glucose, fructose, galactose, and mannose. The characteristic ending –ose indicates to a certain degree that the compound belongs to carbohydrate family but does not show any other structural feature. Therefore, more precise classification of monosaccharides is available according to three criteria:

- whether the monosaccharide contains an aldehyde or a keto group;
- the number of carbon atoms in their chain;
- The stereochemical configuration of the chiral carbon atom that is farthest from the carbonyl group.

Monosaccharides that are **polyhydroxyaldehydes** are called **aldoses**; those that are **polyhydroxy ketones** are **ketoses**. Aldoses and ketoses are further classified according to the number of carbon atoms in the main chain. For instance, a triose contains three carbon atoms, a tetrose - four. The Table shows the classification based on combination of these two criteria.

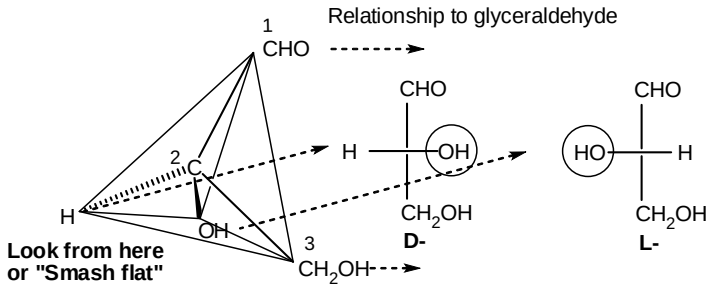
Number of carbon atoms	Aldose	Ketose
Four	Aldotetrose	Ketotetrose
Five	Aldopentose	Ketopentose
Six	Aldohexose	Ketohexose
Seven	Aldoheptose	Ketoheptose



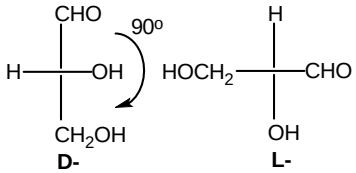
Examples of monosaccharides with 5 and 6 carbon atoms are shown. **Notice**: the most oxidized carbon is written at the top of a monosaccharide structure! These formulas, however, **do not** indicate which stereoisomer is depicted. The shown aldopentose has three chiral carbon atoms. Consequently, there are eight possible isomers that differ by the spatial location of H and OH on the stereogenic centers, according to the formula number of stereoisomers = 2^n, where n is the number of chiral centers. In order to distinguish between these stereoisomers, the D- / L- notation is used. It describes the configuration of the farthest carbon from the carbonyl carbon.

III. Monosaccharides. III.1. Structure and isomerism.

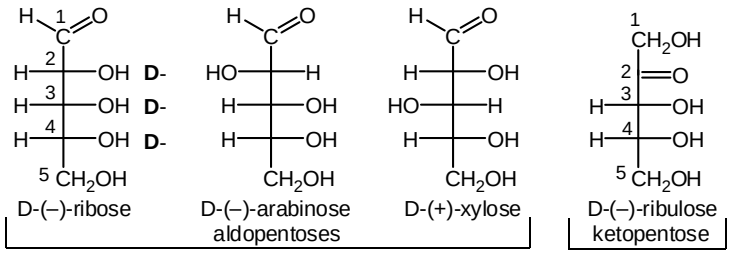
Stereochemistry is the key to understanding carbohydrate structure, a fact that clearly appreciated by the German chemist Emil Fischer. He received Nobel Prize in 1902 for his proof of glucose structure. In order to draw and compare easily sugar structures Fischer introduced projection formulas that represent clearly stereochemical relationships and corresponding D- / L- notation system. The principle of these projections was described also for amino acids and for lactic acid. Since the fundamental molecule in carbohydrate stereochemistry is **glyceraldehyde** (an aldotriose), the application of Fischer projections is shown. The glyceraldehydes molecule is oriented in such way that the most oxidized carbon is at the top and points away from an observer. The observer looks towards a virtual line connecting the groups that are closer (H and OH). A projection of the bonds onto the page is a cross. The chiral carbon atom lies at the center of the cross but is not explicitly written. In the glyceraldehydes shown with tetrahedron, the hydroxyl group projects on the **right**, and the configuration is designated as **D-**. If the hydroxyl group projects to the **left** – the configuration is **L-**.



Two groups in a Fischer projection cannot switch places because the result is enantiomer, and projection formulas cannot be rotated at 90° (as shown), 270°, etc. but can be rotated at 180°. The descriptors D- and L- not only define the spatial arrangement of groups in an enantiomer but also relate the enantiomer with its optical rotation. The D-glyceraldehyde rotates the plane of polarized light to the right and this correspondence is indicated in its complete name: D-(+)-glyceraldehyde. The levorotatory glyceraldehyde is L-(–)-glyceraldehyde. The configurational descriptors D- and L- **do not** indicate also the direction of the optical rotation. Some D- sugars are dextrorotatory (+) but some are levorotatory (–), for example D-ribose is (–), whereas D-glucose is (+).

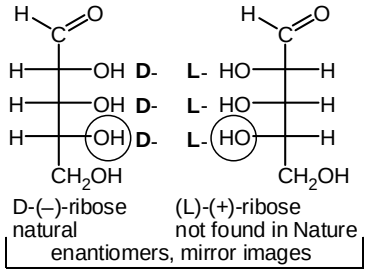


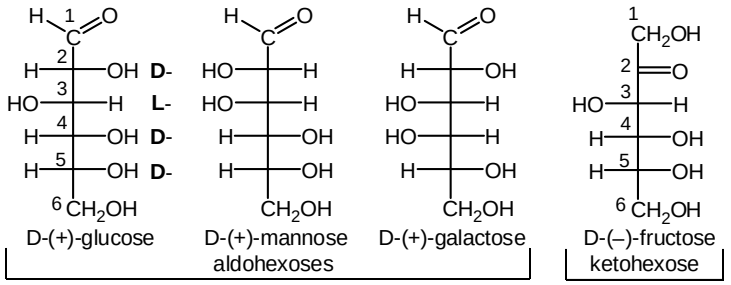
Carbohydrates that have a spatial arrangement of substituents analogous to those of D-(+)- and L-(–)-glyceraldehyde are said to have the D- and L- configuration, respectively. All natural monosaccharides are with unbranched carbon chains containing chiral (stereogenic) carbon atoms. **The configuration of the entire sugar molecule is that of the highest-numbered stereogenic center** (the farthest carbon from the carbonyl carbon). New nomenclature exists for designating configuration around chiral carbon atoms. It is somewhat inconvenient for carbohydrates and the old, trivial designation of configuration has retained. According to the configuration of the highest-numbered stereogenic center the carbohydrates belong to two series: the D-series and the L-series. The examples of several aldopentoses and a ketopentose that belong to the D-series are: the most abundant D-(–)-ribose, D-(–)-arabinose, D-(+)-xylose, and D-(–)-ribulose. **Notice**: These aldopentoses have 3 chiral centers each, but only one designator of configuration. The monosaccharide configuration is designated for one carbon only – C-4 in these cases. The configuration of C-2 and C-3 is understood from the name.



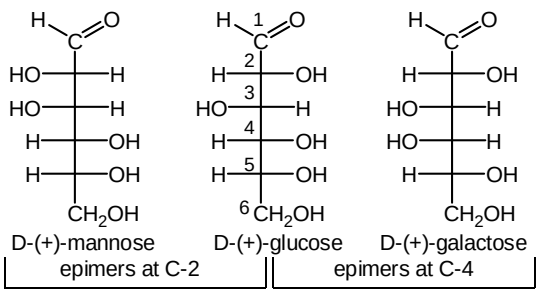
Epimers. Stereoisomers that have multiple stereogenic centers but differ in configuration at only one of them are referred to as epimers. Many of the common sugars are closely related to each other, differing only by the stereochemistry at a single carbon atom. For instance, ribose and arabinose differ only by the configuration of C-2. Ribose and arabinose are epimers at C-2. Similarly,the both carbohydrates D-arabinose and D-xylose are epimers of D-ribose.

Possible enantiomers (mirror image molecules) of the common monosaccharides are not natural products and are of limited interest, e.g. L-(+)-ribose is enantiomer of D-(–)-ribose and not found in Nature. The D-family sugars are abundant, natural sugars that are derived from D-glyceraldehydes, so the OH group of the last chiral atom is at right. L- Family sugars that have the OH group of the last chiral atom on the left are rear sugars and just found in the oligosaccharides present as antigenic moieties. They can not be metabolized and make energy.



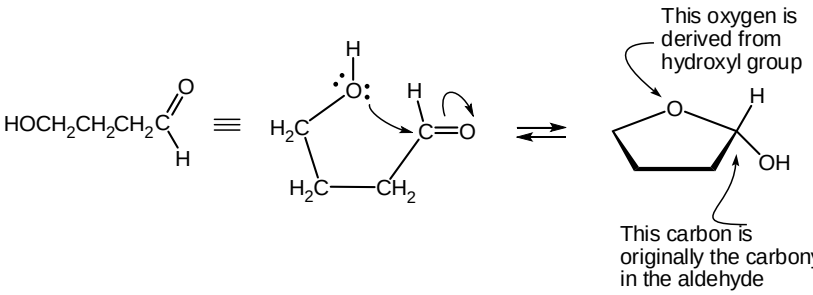


Part of the family of D-aldohexoses includes D-(+)-glucose, D-(+)-mannose, and D-(+)-galactose. The most abundant D-ketohexose is D-(–)-fructose. D-(+)-Mannose is epimer at C-2 of D-(+)-glucose. D-(+)-galactose is epimer at C-4 of D-(+)-glucose

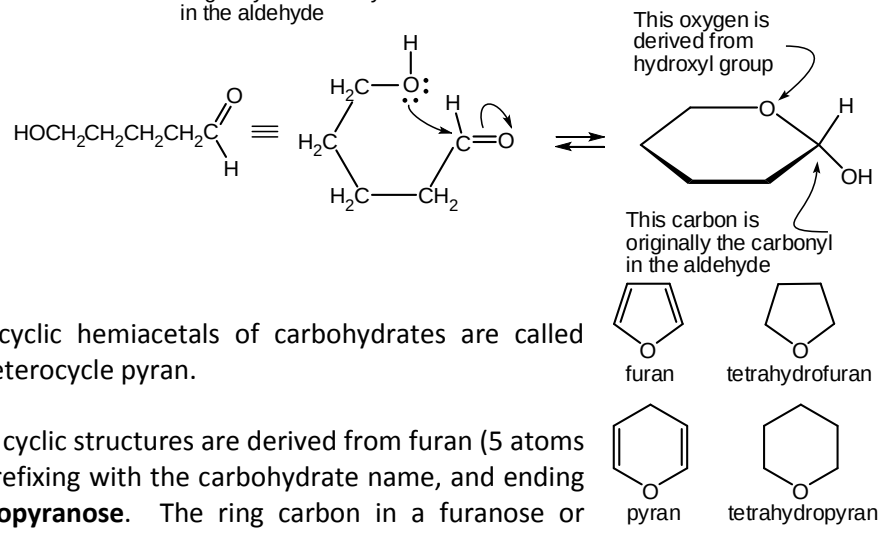


Cyclic forms of carbohydrates

Nucleophilic addition of an alcohol to an aldehyde leads to hemiacetal. When the hydroxyl and aldehyde functional groups are in the same molecule, a **cyclic hemiacetal** is formed by intramolecular nucleophilic addition.



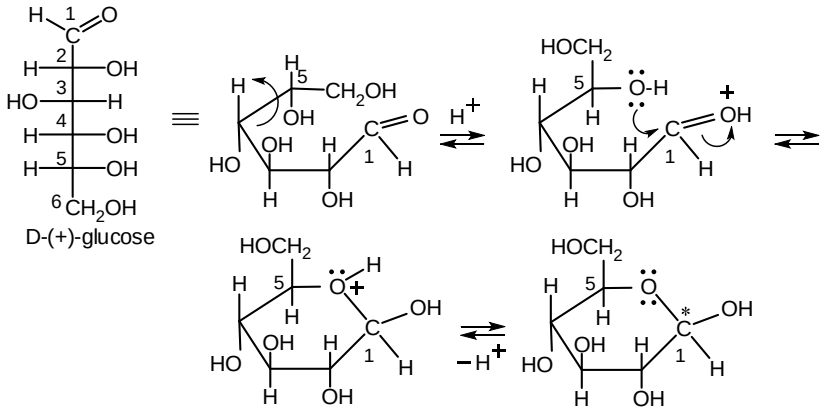
Such cyclic hemiacetals are particularly stable when the ring has five or six atoms. The relationship of a hydroxyaldehyde to a cyclic five- and six-membered hemiacetal is shown.

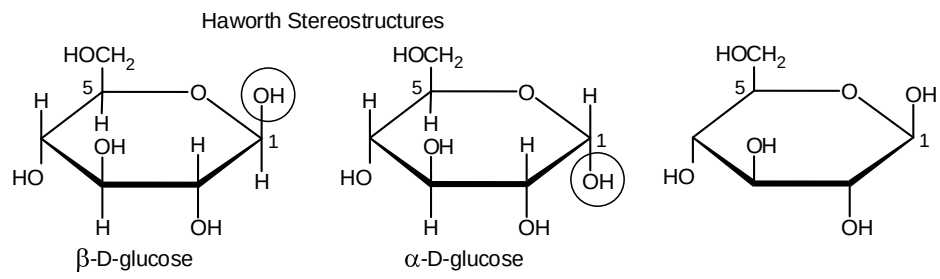


Cyclic hemiacetal formation is most favorable when the ring that results is five-membered or six-membered. Five-membered cyclic hemiacetals of carbohydrates are called **furanose forms**, from the name of heterocycle furan. Six-membered cyclic hemiacetals of carbohydrates are called **pyranose forms**, from the name of heterocycle pyran.

The carbohydrates' names of cyclic structures are derived from furan (5 atoms ring) and pyrane (6 atoms ring) by prefixing with the carbohydrate name, and ending –ose, e.g. **D-fructofuranose**, **D-glucopyranose**. The ring carbon in a furanose or pyranose that originates from the carbonyl group is called **anomeric carbon**. Formation of the the cyclic hemiacetal creates an additional chiral center giving two diastereomeric forms, designated α and β. These diastereomers are called **anomers**. The designation α indicates that the OH at the anomeric center is on the same side of the Fischer projection structure as the hydroxyl that designates whether the structure is D or L.

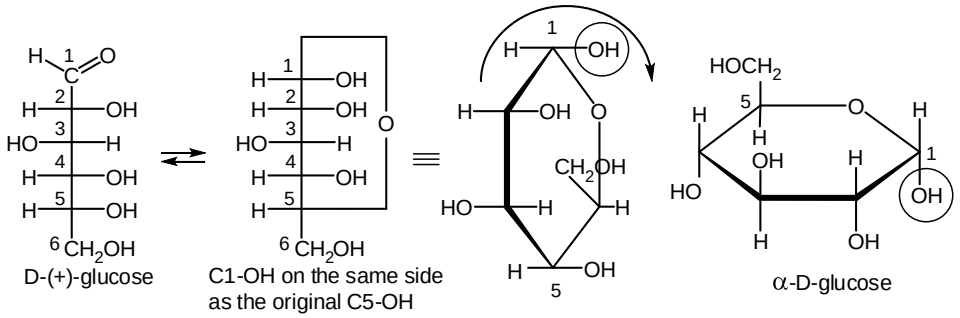
Cyclic hemiacetal formation in D-glucose. Aldohexoses, including glucose, contain an aldehyde and five OH groups and are capable of forming four cyclic forms – typically only the pyranoses are considered. Aldoses exist exclusively as their hemiacetals; very little of the open-chain form is present at equilibrium with the cyclic hemiacetal forms in solution. In order to understand the structures of glucose (and other pyranoses or furanoses) and its chemical reactions, the Fischer projection formulas should be translated into cyclic hemiacetal forms. Consider the D-(+)-glucose Fischer projection formula. The entire formula can be rotated (but not expressed anymore with projection) at 90° clockwise in such way that C-2−C-3 bond is closest to the observer. This places the C-5 hydroxyl group below the median plane. An internal rotation about C-4−C-5 bond brings the C-5 OH very close to the aldehyde carbon, so that a nucleophilic attack (shown with the arrows) takes place. Thus formed pyran ring contains new stereogenic carbon, indicated with * − the anomeric carbon. The graphics shows (although not very clear at this point) that the newly created OH group points up, in opposite orientation to the OH at C-5 in the first rotated open-chain structure. Therefore, the configuration of the anomeric center is β. Much better representation of the sugar cyclic hemiacetal forms is achieved by **Haworth projection** formulas. In these formulas, the pyran ring is oriented with its oxygen atom at the right upper corner, and



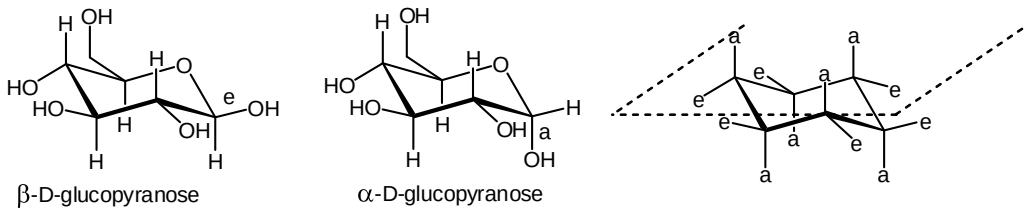


the furan ring with its oxygen atom at the middle, pointing away from the observer. The entire ring is imagined horizontal and perpendicular to the paper plane. The Haworth projections depict a median plane through the ring, almost horizontally. Substituents that are to the right in a Fischer projection are "down" in the corresponding Haworth formula, lying below this median plane. Sometimes, the hydrogen atoms can be omitted from Haworth formula, since they are understood. **Glucose exists in two pyranose forms**, depending on the configuration of C-1 – **α- and β-**. In the α-glucopyranose the C-1 OH points down, in the same direction as the "original" C-5 OH group.

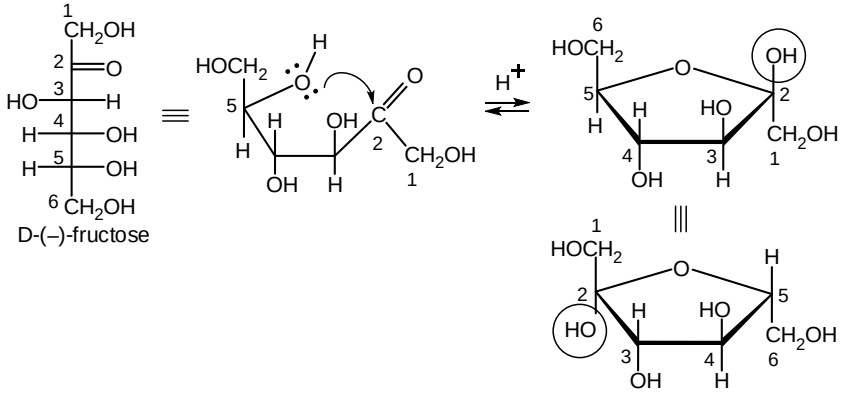
This relationship between Fischer and Haworth projections can be seen when older (not preferred now) cyclic structure is drawn as a rectangle.

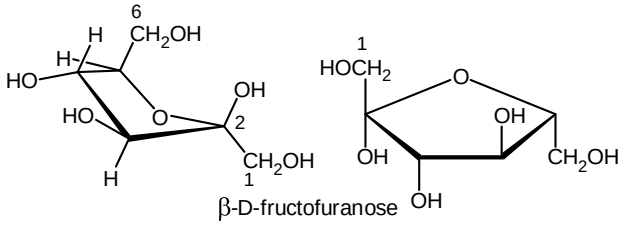


Haworth formulas are satisfactory for representing the configurational relationships but are not informative as to the actual conformation. The best representation of the actual locations of all groups is by conformational structures. X-ray crystallographic studies have revealed that the pyrane ring of glucose is in chair conformation and the more stable form is β-D-glucopyranose. It has all larger groups equatorial (e) where the steric hindrance (pressure) is minimal. The median plane used in Haworth formulas is shown with dashed lines on a cyclohexane ring, along with the definition of axial and equatorial positions.



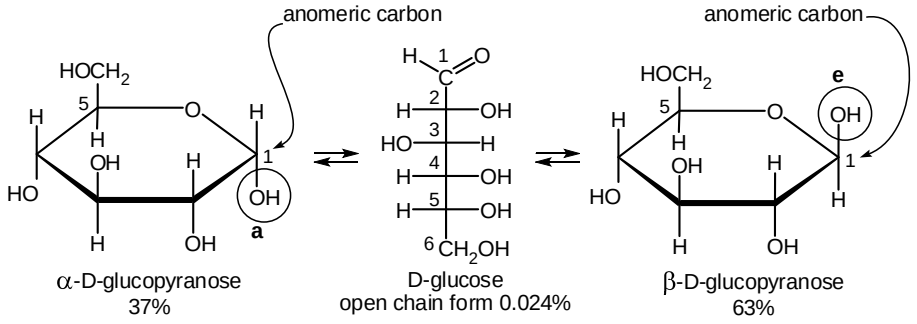
Cyclic hemiacetal form of D-fructose. Aldoses are more common than ketoses, and their role in biological processes has been more thoroughly studied. Nevertheless, a large number of ketoses are known, and several of them are very important intermediates in carbohydrate biosynthesis and metabolism. As an example of furan ring formation in a ketose, consider D-fructose. Free ketoses, like aldoses, exist mainly as cyclic hemiacetals. Although the six-membered pyranose ring is more stable, fructose participates in table sugar as a furanose. The furanose forms result from addition of the C-5 hydroxyl to the carbonyl group. As a difference from pyranoses, the anomeric carbon in furanoses bears both a hydroxyl group and a carbon substituent. In the case of 2-ketoses, this substituent is CH_2OH group. In contrast, the anomeric carbon in aldoses' cyclic hemiacetals is readily identifiable because it is bonded to two oxygens. Following the same approach as above for glucose, the resulting Haworth formula of D-(–)-fructose is drawn with the ring oxygen pointing back and the anomeric carbon (here at C-2) on the right. The numbering system runs clockwise, with C-1 on the right, pointing down. Often the entire ring should be rotated at 180°, as shown above, because in this orientation is easier to connect C-2 OH in disaccharides, such as sucrose. The





conformational representation of a furanose form is more difficult to draw, having in mind that the five-membered ring adopts a conformation like half-opened mail envelope.

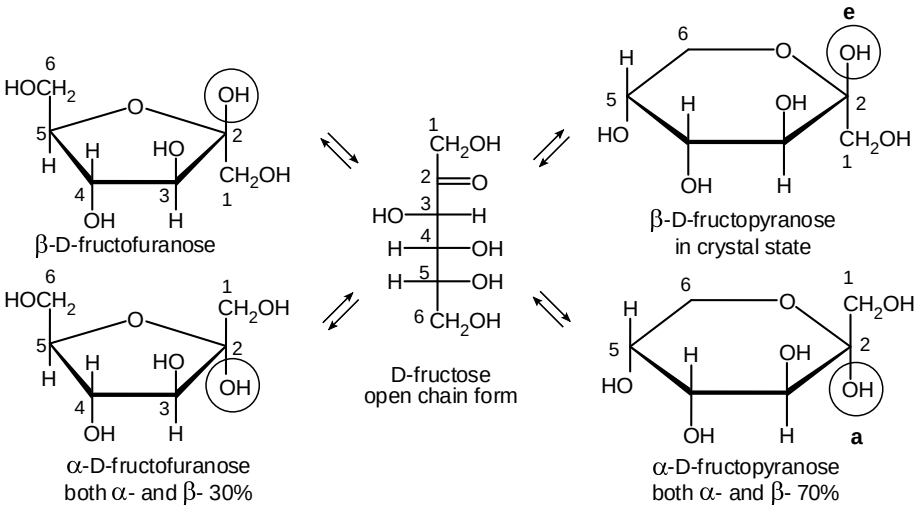
Anomers of carbohydrates. Anomeric carbon is the ring carbon atom derived from the carbonyl group of an open-chain carbohydrate. In aldoses' cyclic hemiacetal forms the anomeric carbon is the only carbon that bears two oxygen atoms. In ketoses' cyclic hemiacetal forms, the anomeric carbon has both a hydroxyl group and a carbon substituent. In carbohydrate chemistry, an anomer is a special type of epimer – a diastereomer of a cyclic saccharide that differs only in the configuration at the anomeric carbon. The possibility for two anomers arises from the ring closing reaction that converts a flat carbonyl group into a chiral carbon. Depending on which face of the carbonyl group (protonated, see glucose example) is attacked, the hemiacetal OH group can be directed in the one of two possible spatial orientations. These are depicted in Haworth formula either as "down" – α-form, or as "up" – β-form.



α-D-glucopyranose and β-D-glucopyranose are anomers. They differ only by the relative configuration of the anomeric center, that is – only by the location of C-1 OH group.

An easy way to remember the configuration is: the anomeric OH in any cyclic β-form is *syn*- (on the same side) to terminal CH_2OH (the highest numbered, in the above example C-6); in any α-form is *anti*- (on opposite sides) to terminal CH_2OH.

The situation with anomers of fructose is more complicated because the free monosaccharide exists in solid in single pyranose form but in solution an equilibrium is established between two anomers of pyranose form and two anomers of furanose form. In the table sugar (sucrose) opening and reforming the furan ring is not possible and fructose is in fixed furanose β-anomer.



Mutarotation. In spite of their easy interconversion in solution, α- and β-forms of carbohydrates can exist as independent compounds, and many have been isolated in pure form as crystalline solids. When crystallized from ethanol D-glucose yields α-D-glucopyranose, mp 146°C, $[a]_D$ +112.2. Crystallization from water/ethanol mixture produces β-D-glucopyranose, mp 148-155°C, $[a]_D$ +18.7. In the solid state the two forms do not interconvert and are stable indefinitely. Their structures have been unambiguously are confirmed by x-ray crystallography.

The optical rotations, cited above for each anomer are those measured immediately after each one is dissolved in water. On standing in water solution, the optical rotation of the solution containing the α- isomer decreases from +112.2 to $[\alpha]_D$ +52.5; the rotation of the solution of the β-isomer increases from +18.7 to the same $[\alpha]_D$ +52.5, an equilibrium value from which the individual amounts are calculated. This phenomenon is called mutarotation. Mutarotation is the term given to the change in the specific rotation of a cyclic monosaccharide as it reaches an equilibrium between its α- and β-anomeric forms through open-chain form. What is happening is that each solution, initially containing only one anomeric form, undergoes equilibration to the same mixture of α- and β-pyranose froms. The open chain form is an intermediate in the process. The distrubition between the α- and β-anomeric forms at equilibrium is readily calculated from the optical rotations of the pure isomers and the final optical rotation. The amount of β-anomeric glucopyranose is 63%,

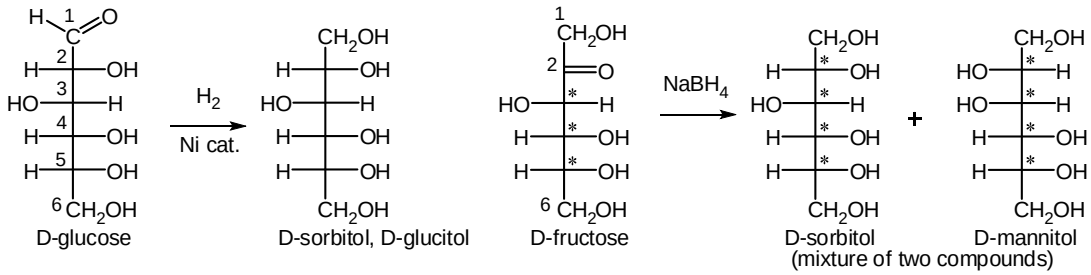
and that of α-anomeric glucopyranose – 37%, that means β- is more stable. These values are shown on the scheme above, that describes also the intermediate open-chain form with very little amount at equilibrium.

III.2. Physical properties. The monosaccharides are solid crystalline substances that are soluble in water. Their solubility in alcohol is limited, and they do not dissolve in organic solvents such as benzene and ether. Their taste is sweet. Stereoisomerism is profound feature of monosaccharides because of the presence of several chiral carbon atoms.

Structure determination. Present-day methods for structure determination in carbohydrate chemistry are substantially the same as those used for any other type of compound. These methods include full range of modern instrumental methods that apply spectroscopy in variety of electromagnetic wavelengths. If the unknown carbohydrate is crystalline, x-ray diffraction can provide precise structural information which is almost equivalent to taking a photograph of the molecule.

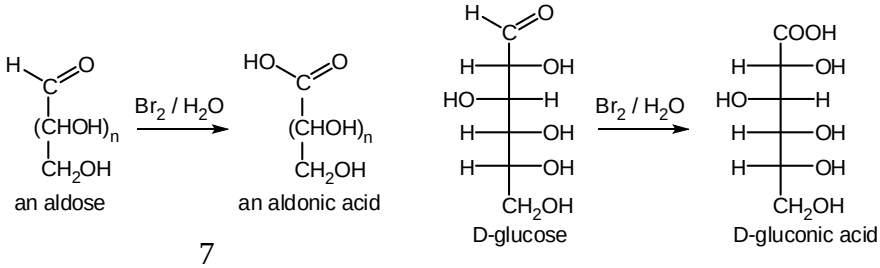
Before the widespread availability of instrumental methods, the major approach to structure determination relied on a set of chemical reactions and tests. The response of an unknown carbohydrate to various reagents and procedures can give body of data from which the structure can be deduced. This classical approach is best exemplified by the works of E. Fischer on the structure of D-glucose, using only chemical reactions most of them shown below. Carbohydrates are multifunctional compounds and may react in typical reactions with any of their functional groups – OH, CHO, CO. The important problem is "how these groups in limited number are connected?". To understand better the scope and limitations of these tests, a short survey of the chemical reactions of carbohydrates follows. In many cases these reactions are application of chemistry of already described classes of organic compounds, however certain transformations are unique to carbohydrates.

IV. Chemical properties. VI.1. Reduction of monosaccharides. While carbohydrates exist almost entirely as cyclic hemiacetals in aqueous solution, they are in rapid equilibrium with their open-chain forms, and most of the reagents that react with aldehydes and ketones react in an analogous way with the carbonyl functional group of carbohydrates. Therefore, the open-chain structures are depicted in most illustrative cases, although such structures are not major in solution. The carbonyl group of carbohydrates can be reduced to an alcohol function. Aldoses give primary alcohol, ketoses – secondary new alcohol group.



Typical procedures include catalytic hydrogenation and sodium borohydride ($NaBH_4$) reduction. More powerful modern reducing agents are not compatible with the solvent (water or alcohol). The products of carbohydrate reduction are called **alditols**. Since these alditols do not have a carbonyl group, they are incapable of forming cyclic hemiacetals and exist exclusively in noncyclic form. Notice that glucose gives on reduction only one product – D-glucitol, whereas fructose gives a mixture of two epimeric alditols – D-glucitol and D-mannitol because ot the two possible configurations at the newly generated chiral center. The alditols are named by adding the ending -itol to the root name of the sugar. Another name for glucitol is D-sorbitol. It is used as a sweetener, especially in diets that require low sugar.

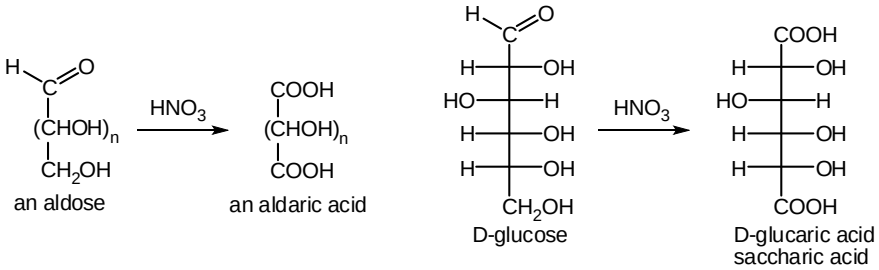
IV.2. Oxidation of monosaccharides. **1. Aldonic acids**. The aldehyde group of an aldose is easily oxidized to carboxylic group using bromine water. This reagent does not affect the alcohol groups of a sugar and does not oxidize the keto group in ketoses. **Bromine water does not oxidize ketoses**. That is why the reaction with bromine water is used as a distinguishing test between aldoses and ketoses. The



reagent is acidic and, therefore, does not cause epimerization or shift of a carbonyl group. The product of bromine water oxidation is an **aldonic acid (with one terminal COOH group)**. Their names are formed by replacing -ose ending of the aldose by -onic acid, e.g. glucose is oxidized to gluconic acid. Aldonic acids axist in equilibrium with their five- or six-membered lactones (cyclic esters).

2. Aldaric acids.

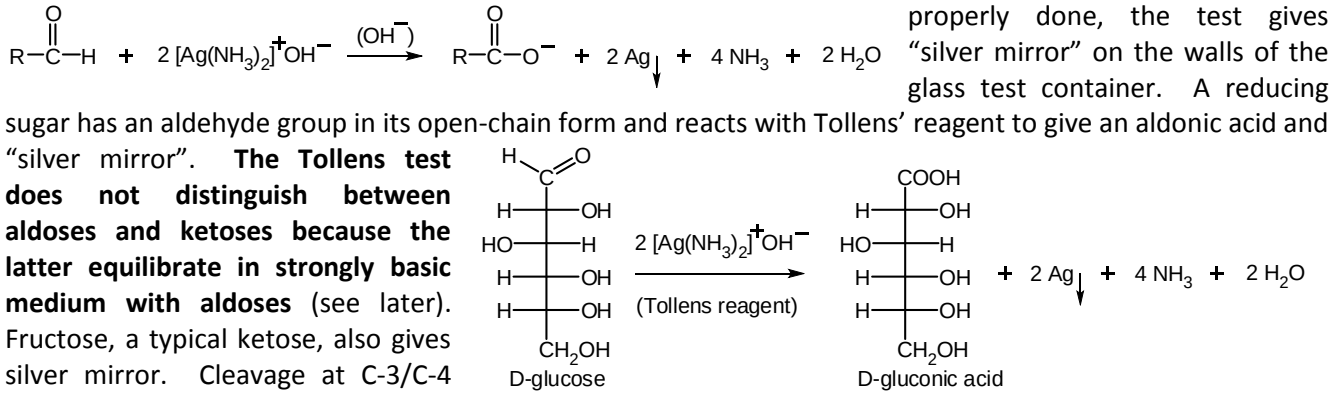
The reaction of aldoses with nitric acid leads to the formation of **aldaric acids**. Nitric acid is stronger oxidizing agent than bromine water, oxidizing both the aldehyde terminal C-1 CHO group and the other terminal C-n primary alcohol CH_2OH to carboxylic acid groups. These acids are also known as saccharic acids and are named by substituting –aric acid for the –ose ending in the carbohydrate name, e.g. nitric acid oxidizes glucose to glucaric acid.



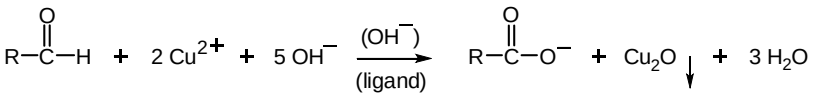
3. Oxidation with complexes containing Ag^+ and Cu^{2+} – Tollens, Benedict, Fehling tests for reducing sugars. A characteristic property of an aldehyde function is its sensitivity to oxidation. Even mild oxidizing agents such as metal cations, complexed with various ligands can oxidize the terminal CHO group in sugars. The group of sugars responding to such oxidizing agents are called **reducing sugars**. Reducing sugar is any sugar (mono-, disaccharides) that, in an alkaline solution, forms some aldehyde or ketone. This allows the sugar to act as a reducing agent, for example in the Benedict's reaction (Cu^{2+}, citrate), reaction with Fehling reagent (Cu^{2+}, tartrate) and Tollens' test (Ag^+). The reducing sugars are:

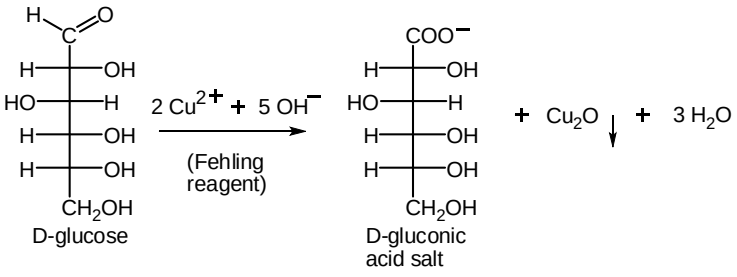
- Aldoses, of course, since they possess an aldehyde CHO function in their open-chain form;
- Ketoses because they can isomerize to aldoses (under the conditions of the test) by way of **enediol intermediate** and the aldoses are oxidized by the reagent;
- Any longer (di-, tri-, oligocaccharide) that contains free hemiacetal function is also a reducing sugar because the hemiacetal is in equilibrium with the open-chain form, an aldehyde containing form. Through it the sugar is susceptible to oxidation, for instance, maltose gives a positive test with Benedict reagent.

The Tollens test detects presence of an aldehyde group. An aldehyde reacts with the Tollens' reagent (diaminosilver cation in alkaline solution) to give a carboxylate ion and a precipitate of metallic silver. When properly done, the test gives "silver mirror" on the walls of the glass test container. A reducing sugar has an aldehyde group in its open-chain form and reacts with Tollens' reagent to give an aldonic acid and "silver mirror". **The Tollens test does not distinguish between aldoses and ketoses because the latter equilibrate in strongly basic medium with aldoses** (see later). Fructose, a typical ketose, also gives silver mirror. Cleavage at C-3/C-4 bond of fructose to glyceraldehyde – also with reducing capability, besides isomerization to glucose and mannose contributes to the positive Tollens test of fructose.

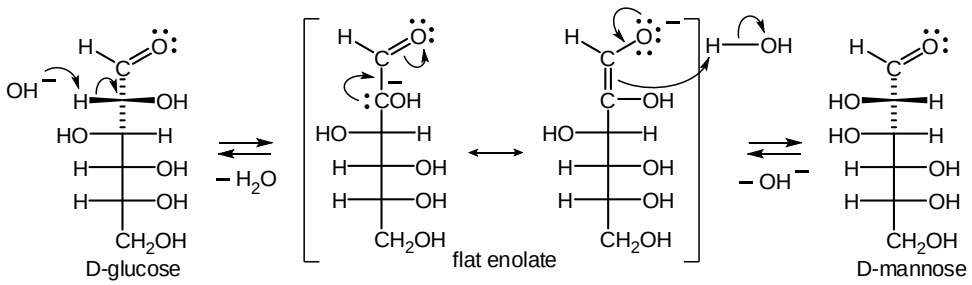


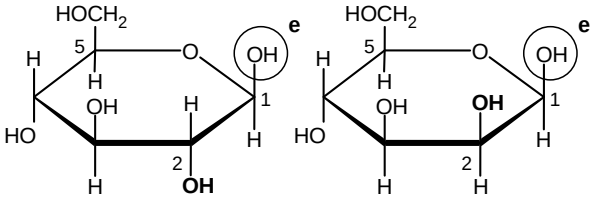
Benedict and Fehling tests for reducing sugars. An alkaline solution of copper (II) sulfate as its citrate complex (Benedict's reagent) or as tartrate complex (Fehling's reagent) is capable of oxidizing aliphatic aldehydes to the corresponding carboxylic acids. The formation of red precipitate of copper(I)oxide by reduction of Cu(II) is indication of a positive test for an aldehyde. The Fehling solution is prepared just before use by mixing two solutions: Fehling I – an aqueous solution of copper (II) sulfate and Fehling II – an alkaline solution of Seignette salt (sodium potassium tartrate). Reducing carbohydrates give positive Benedict and Fehling reactions. The Benedict test is positive also for ketoses which under the experimental conditions isomerize to aldoses that are oxidized by the reagent. The test is also positive for reducing disaccharides, e.g. maltose. Fehling test is used to screen for glucose in urine, thus detecting diabetes.



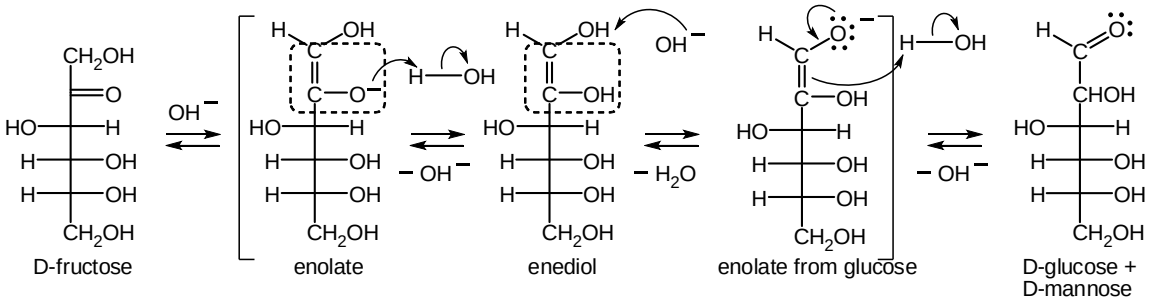


IV.3. Epimerization and isomerization with basic reagents. Carbohydrates undergo a number of isomerization and degradation reactions under both laboratory and physiological conditions. For example, a mixture of glucose, fructose, and mannose results when any one of the individual components is treated with aqueous base. The nature of this reaction can be understood by applying knowledge on the base-catalyzed enolization of glucose. The alpha-proton to a carbonyl group is more acidic. It can be reversibly removed by the base. This deprotonation leads to enolate ion that is planar and C-2 in it is no longer chiral. The configuration at C-2 is lost. The intermediate flat enolate can be reprotonated from either face of the enolate double bond. The result is an equilibrium mixture of the original sugar (glucose) and its epimer at C-2 – mannose. This stereochemical change is called **epimerization**. Glucose and mannose are epimers at C-2. Under these conditions epimerization occurs only at C-2 because it alone is at α-position to the carbonyl group.





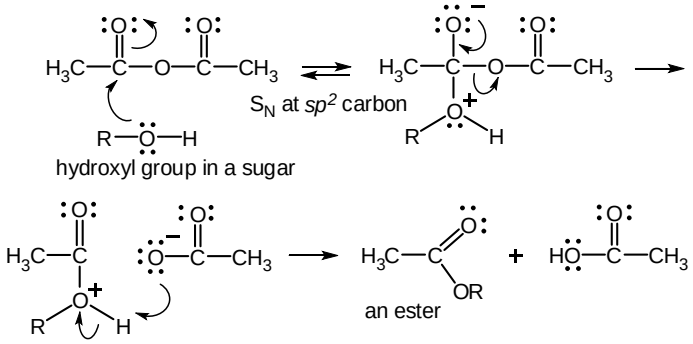
There is another reaction that can happen to the flat enolate ion from glucose. Proton transfer from water to the negatively charged oxygen at C-1 converts the intermediate ion to enediol which isomerizes to ketose. The reversed isomerization reaction is illustrated by the example where fructose (a ketose) is equilibrated with glucose and mannose (aldoses). The initially obtained enolate from fructose by removing a



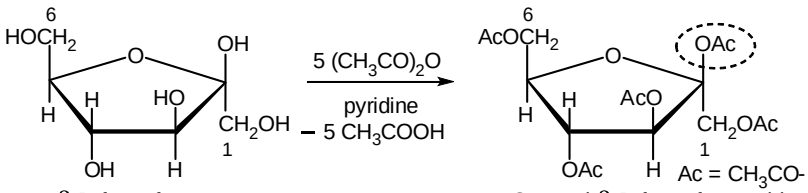
proton from C-1 by the action of hydroxide ion picks a proton to give enediol. Then the C-1 hydroxyl group loses a proton to form enolate corresponding to glucose. It is flat and can be protonated from either side, thus giving mixture of glucose and mannose.

The reaction by which ketoses isomerize, equilibrate with aldoses (and *vice versa*) in basic medium by way of enediol intermediate is called **enediol rearrangement**. Due to this isomerization reaction ketoses are reducing sugars as aldoses. They give positive Tollens test and react with Benedict and Fehling reagents (Cu^{2+} complexes with citrate and tartrate, resp.). The isomerization of D-glucose to D-fructose by way of an enediol intermediate is an important step in **glycolysis**, a complex, 11 steps, process by which an organism converts glucose to chemical energy in the absence of oxygen.

IV.4. Acylation and alkylation of carbohydrates. The alcohol groups of carbohydrates undergo chemical reactions typical of hydroxyl functional groups. They are converted to esters by reaction with carboxylic acid chlorides and anhydrides. The class reaction name is **acylation**. An esterification (acylation of alcoholic OH) proceeds via nucleophilic addition – elimination mechanism. The typical conditions are treatment with acetic anhydride in the presence of pyridine. During this reaction, all hydroxyl groups of a sugar are acetylated, including the one on the hemiacetal anomeric carbon.

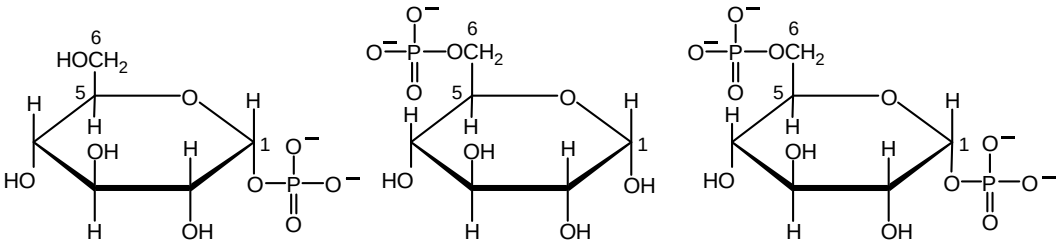


According to the general esterification mechanism, the anomeric bond R–O is not involved, therefore the configuration of an anomeric OH is preserved. Starting from pure α- or pure β-amoner, the product is the pure corresponding acetate anomer.



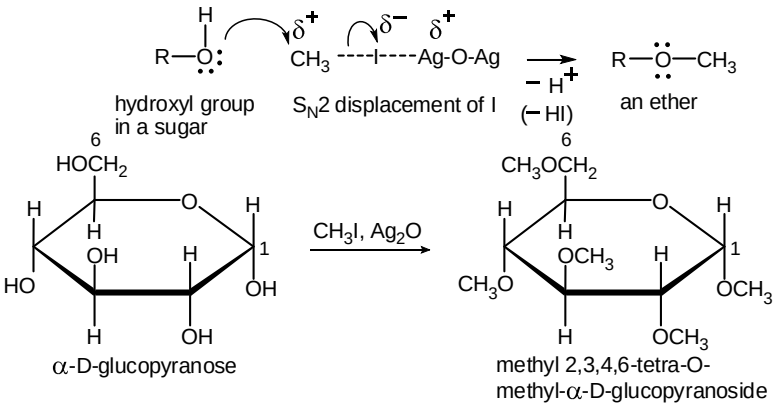
β-D-fructofuranose — penta-O-acetyl-β-D-fructofuranoside

In biochemical processes, for instance glycolysis, esters of carbohydrates with phosphoric acid are of great importance. Glucose-6-phosphate is the substrate for enzymatic isomerization of glucose to fructose and for glycogen synthesisi. Cyclic phosphate esters were already mentioned before.


glucose-1-phosphate — glucose-6-phosphate very common in cells — glucose-1,6-phosphate

Ether formation (alkylation) of sugars. Methyl ethers of carbohydrates are efficiently prepared by alkylation with methyl iodide in the presence of silver oxide. Silver oxide enhances the polarization of carbon–iodine bond, making the carbon more prone to nucleophilic attack by the sugar oxygen atoms of OH groups. The anomeric hydroxyl group is also converted to ether with retention of configuration.

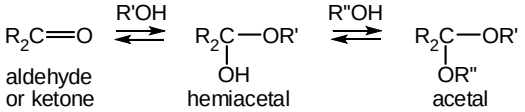
This reaction has been creatively used in determining the sugar ring size. Partial acid-catalyzed hydrolysis removes only the anomeric C-1 OCH_3 methyl group. After ring



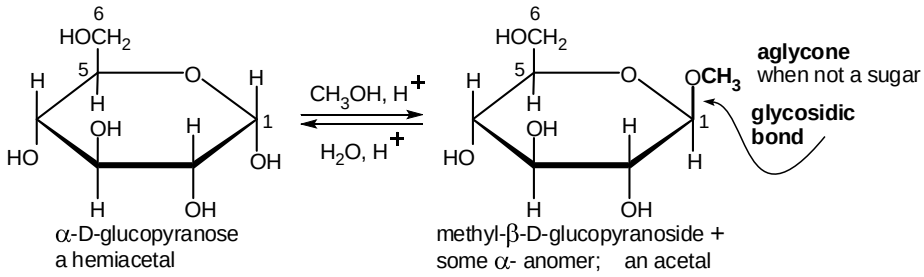
α-D-glucopyranose — methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside

opening, the C-5 OH is the only non methylated as ether group, thus revealing the ring size.

IV.5. Formation of glycosides. **Glycosides** are a large and very important class of carbohydrate derivatives characterized by the **replacement of the anomeric hydroxyl group by some other substituent**. Examples of glycosides are the already shown above pentaacetyl derivative of fructose and the pentamethyl ether of glucose where the anomeric OH has been replaced by an ester (-$OCOCH_3$) and an ether (-OCH_3) group, respectively. Glycosides are termed *O*-glycosides, *N*-glycosides, *S*-glycosides, according to the atom type attached to the anomeric carbon. Usually, the term glycoside without a prefix is taken to mean *O*-glycoside. Glycosides are classified also as α- or β- in the customary way, according to the configuration at the anomeric carbon. Structurally, *O*-glycosides are mixed acetals that involve the anomeric position of furanose or pyranose forms of carbohydrates. In general terms, the acetal formation occurs by addition of two molecules alcohol (same or different) to a carbonyl group:

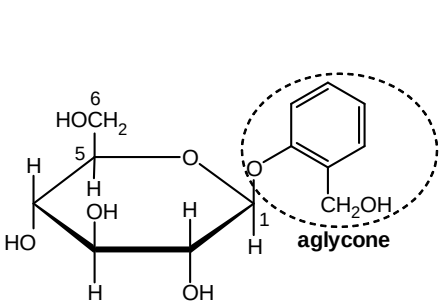


When this sequence is applied to carbohydrates, the first step takes place *intramolecularly* and spontaneously to yield a cyclic hemiacetal. The second step is *intermolecular*, requires an alcohol R"OH as a reagent, and proceeds readily only in the presence of an acid catalyst. The preparation of glycosides in the laboratory is carried out by simply allowing a carbohydrate to react with an alcohol in the presence of an acid catalyst. A point for emphasis about glycoside formation in this case is that, despite the presence of many hydroxyl groups in the carbohydrate, it is only the anomeric hydroxyl group that is replaced (compare with ether formation with CH_3I). Equilibration of the glycosides usually takes place under the reaction conditions. Regardless of the starting anomer used, an equilibrium mixture of both anomeric glycosides is obtained. Compare the reaction of acidified methanol and glucose with its reaction with CH_3I. The β-methyl glycoside predominates because it is more stable having the larger -OCH_3 group in equatorial position. Under neutral or basic conditions glycosides are configurationally stable. Unlike the free sugars from which they are derived, they do not exhibit mutarotation.

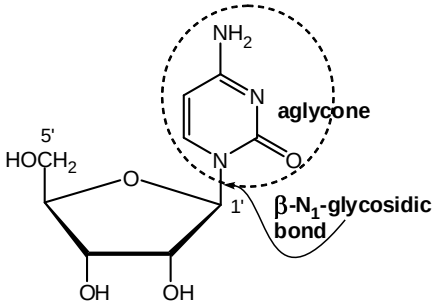


The usual covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate, is called a **glycosidic bond**. A glycosidic bond is formed between the hemiacetal group of a saccharide (or a molecule derived from a saccharide) and a group of some other organic compound such as an alcohol. If the group attached to the carbohydrate residue is not another saccharide it is referred to as an **aglycone** (CH_3OH in the above example). An aglycone is the non-sugar compound remaining after replacement of the glycosyl group from a glycoside by a hydrogen atom. Various aglycons have been introduced when defining nucleosides. Notice: all such sugar derivative names end on -side; the term glycoside is general whereas glucosides are glycosides derived from glucose.

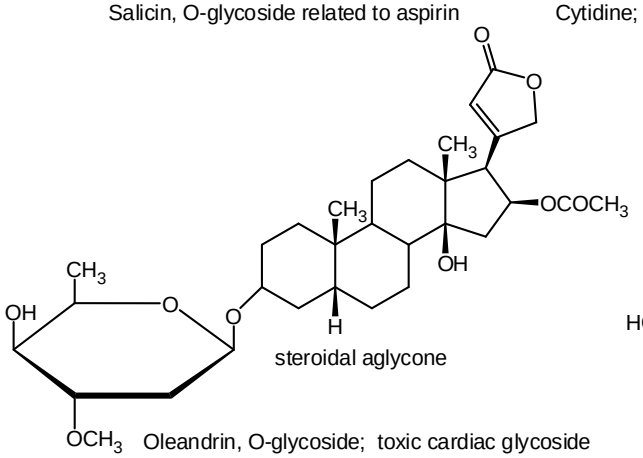
Examples of *O*-glycosides, *N*-glycosides, *S*-glycosides include Salicin, Cytidine, Oleandrine, and Siningrin.



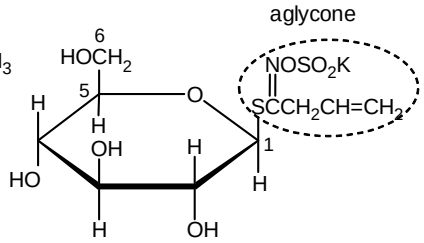
Salicin, O-glycoside related to aspirin



Cytidine; N-glycoside; a nucleoside



Oleandrin, O-glycoside; toxic cardiac glycoside

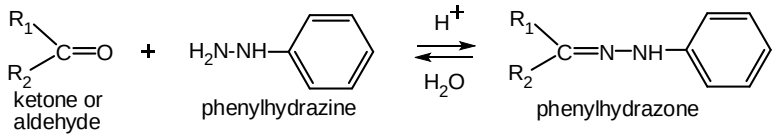


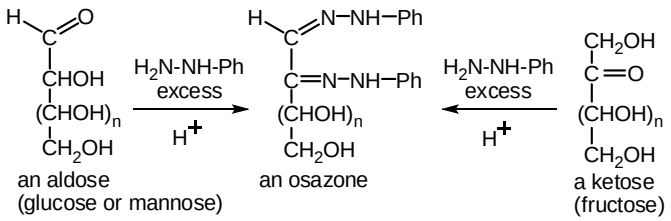
Sinigrin, S-glycoside gives the flavor of mustard and horseradish

Cytidine has been already shown as a nucleoside in DNA. Salicin has an aglycone related to aspirin, whereas another *O*-glycoside – Oleandrine, has its aglycone with much more complex steroidal structure. A rare glucoside – the *S*-glycoside Sinigrin gives the unmistakable flavor of mustard.

The disaccharides and polysaccharides are *O*-glycosides in which the alcohol forming the glycosidic bond is another monosaccharide.

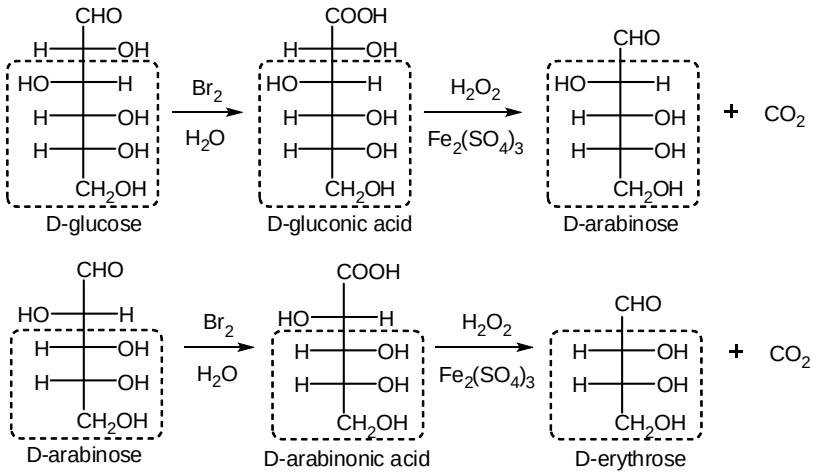
IV.6. Osazone formation. Prior to the introduction of modern spectroscopic methods, research in carbohydrate chemistry was hampered by the physical nature of many sugars. Their water solubility and their tendency to form noncrystallizable syrups made isolation and purification very difficult. An early approach to the problem was to convert the carbohydrate to a crystalline derivative with the customary reagents that react with a carbonyl group. Such reagent giving crystalline derivatives is phenylhydrazine converting carbonyl compounds into phenylhydrazones.





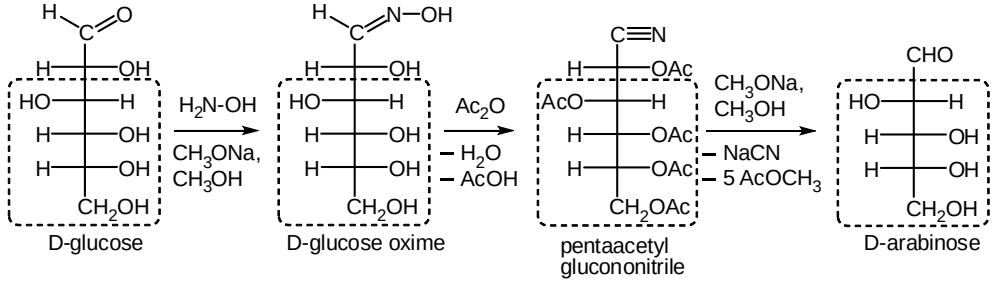
Long ago Emil Fischer discovered that while one equivalent of phenylhydrazine gives from an aldose or ketose the expected phenylhydrazone, excess of the reagent converts aldoses and ketoses into compounds containing *two adjacent* phenylhydrazone units. Such compounds are called **osazones**. Not only is the aldehyde function converted to a phenylhydrazone, but so also is the alcohol function on the adjacent carbon. The reaction is general for both aldoses and ketoses. It proceeds no further than the osazone no matter how much excess phenylhydrazine is present. Phenylosazones are yellow crystalline solids and are insoluble in water. Carbohydrates are classically characterized on the basis of the melting point of their osazone derivative. These derivatives were very helpful to Fischer for interrelating the configuration of various sugars. For example, his observation that (+)-glucose, (+)-mannose, and (–)-fructose all gave the same osazone established that all three had the same configuration at C-3, C-4, and C-5 (the unaffected portion by the reaction is the same).

IV.7. Chain shortening: Ruff and Wohl degradation. Much early activity in carbohydrate chemistry was devoted to unraveling the various stereochemical relationships among monosaccharides. The most important methods used were those that allowed for chain shortening and chain lengthening. The former are known as degradation methods that remove the aldehyde carbon and sequentially the following carbons in a Fischer projection formula until a known carbohydrate is obtained. The carbohydrates that gave (+)-glyceraldehyde were assigned to belong to the D- series; those leading to (–)-glyceraldehyde – to the L- series.

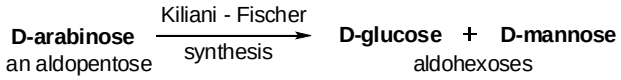


The **Ruff degradation** includes two steps: (1) oxidation to aldonic acid; (2) oxidative decarboxylation. The result is an aldose with one carbon less than the original aldose. The examples show conversion of D-glucose to D-arabinose and its further chain shortening to D-erythrose. These conversions prove that the configuration of the last two, or three chiral carbon atoms are the same in these three sugars.

The **Wohl degradation** also leads to chain shortening. It is achieved by converting the aldose aldehyde carbonyl group into a nitrile (CN). The resulting cyanohydrin loses HCN under basic conditions which is a reversed reaction to nucleophilic addition reaction. This loss of one carbon atom contracts the chain. The classic example is the conversion of glucose to arabinose as shown. The reaction does not affect the rest of chain.

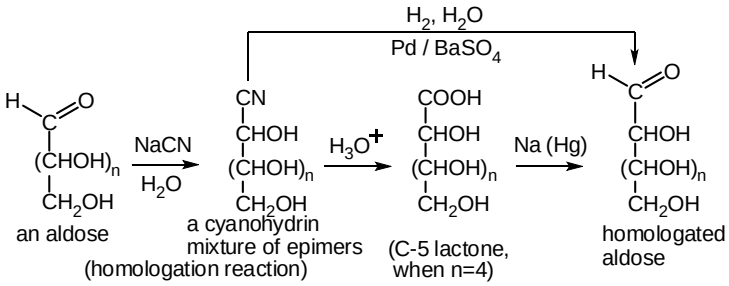


IV.8. Chain lengthening: Kiliani-Fischer synthesis. One of the most important methods used to interrelate the stereochemistry of carbohydrates is Kiliani-Fischer synthesis. It results in the lengthening of the sugar chain by one carbon atom. The presence of an aldehyde function in their open-chain forms makes aldoses reactive toward addition of hydrogen cyanide. Addition yields a mixture of epimeric cyanohydrins. Thus, two new aldoses differing only in their stereochemistry at C-2 result from Kiliani-Fischer synthesis. Chain extension of D-arabinose, for example, yields a mixture of D-glucose and D-mannose.



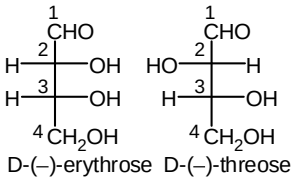
The carbon atom of the cyanide is the one that elongates the chain. The C-1 aldehyde group of the starting sugar becomes C-2 of the chain-lengthened sugar.

There are several variations and improvements of Kiliani-Fischer synthesis, mostly concerned with the conversion of the cyano group into aldehyde group. The Kiliani-Fischer synthesis is invaluable for production of sugars that are difficult or impossible to obtain from natural sources.



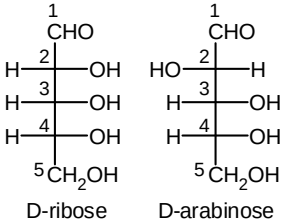
V. Representatives

Tetroses. Glyceraldehyde is considered to be the simplest chiral carbohydrate. It is an aldotriose and, since it contains one stereogenic center, exists in two stereoismeric forms, the D- and L- enantiomers. Adding one more chiral carbon gives next **aldotetroses**. They are **erythrose** and **threose**. Higher natural sugars are related to these two tetroses in the D-series.

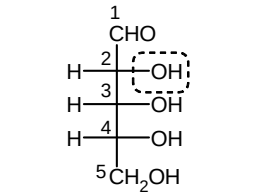


Pentoses. Insertion of one more CH_2OH in erythrose between its CHO and C-2 leads to the epimeric pentoses D-ribose and D-arabinose.

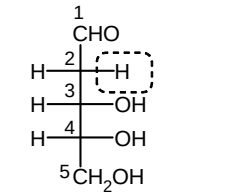
Hexoses. The structures, names, and cyclic forms of the most abundant hexoses have been already presented.



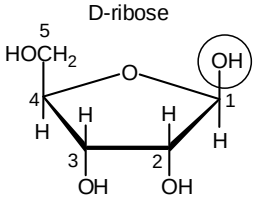
Deoxy sugars. A commonplace variation on the general pattern seen in carbohydrate structure is the replacement of one or more the hydroxyl substituents by some other atom or group. In **deoxy sugars** the hydroxyl group is replaced by hydrogen. An example is 2-deoxy-D-ribose. The hydroxyl at C-2 in D-ribose is absent in 2-deoxy-D-ribose. The derivatives of this carbohydrate, called deoxyribonucleotides are the fundamental building blocks of **deoxyribonucleic acid** (**DNA**), the molecules responsible for storing genetic information. The DNA chain is a polymer obtained by linking 2-deoxyribofuranose units between C-3' and C-5' with phosphate ester groups.



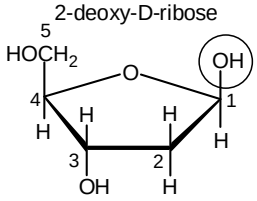
D-ribose



2-deoxy-D-ribose

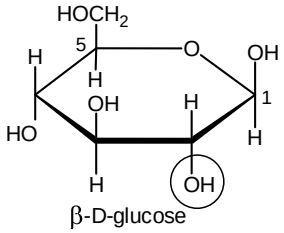


β-D-ribofuranose

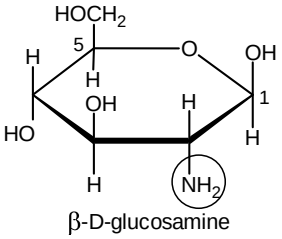


β-2-deoxy-D-ribofuranose
(in H_2O preferred is 2-deoxy-ribopyranose)

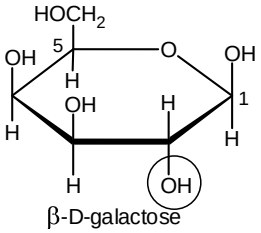
Amino sugars. Another structural variation is the replacement of a hydroxyl group in a carbohydrate by an amino group. Compounds with such structure are called amino sugars. Many amino sugars are known at present, some of them isolated and identified only recently as components in complex biologically active substances, e.g. antibiotics. The most common amino sugars are β-D-glucosamine (and its acetyl derivative) and β-D-galactosamine. N-Acetyl-D-glucosamine is the principle component of the polysaccharide chitin that forms outer skeleton of spiders, crabs, insects.



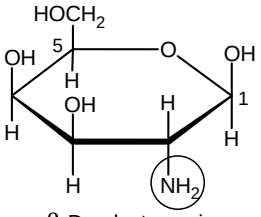
β-D-glucose



β-D-glucosamine



β-D-galactose
(C-4 epimer of glucose)



β-D-galactosamine

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