

GLYCOSIDES

- Compounds that yield one/more sugar and a non-sugar molecule (aglycone) upon hydrolysis.
- Glycoside ---- dil.acid /enzyme----hydrolysis--- AGLYCONE+ SUGAR/
SUGARS
- **AGLYCONE: Aliphatic/ Aromatic**
- **SUGAR:**
 - a) Hexose (glycose, fructose, mannose, galactose)
 - b) Pentose (arabinose, xylose)
 - c) Methyl pentose (rhamnose)
 - d) Desoxymethylpentose (digitoxose)

GLYCOSIDES

- Mainly of plant origin
- Widespread in Angiosperms (Angiospermae)
- Occur in every organ of the plants, dissolved in cellular juice.
- Since the sugars link from the reductor moiety, their solutions become reductor after they hydrolysed
- They can well be crystallized; generally white, have bitter taste.

GLYCOSIDES

- Some of them are colorful
- Yellow-----FLAVONOIDS
- Red/Blue/Purple-----ANTOSYANINS
- Orange-----ANTRAQUINONE
- The first isolated glycoside → SALICIN

GLYCOSIDES

- Sugar → furanose/pyranose ring systems which may have α or β isomers. Glycoside consist of an α or β glycosidal bond.
- Natural glycosides mostly tend to have β -linkage → (β -glycosides)

- The configuration of the anomeric carbon is α when the hemiacetal hydroxyl group is in the same orientation as the secondary hydroxyl group. In the opposite case the configuration is β .
- Physical properties and enzyme reaction of these isomers differ from each other.

GLYCOSIDES

- One or more sugars can be linked/ attached either from the same location as a **holoside** chain or different locations.

GLYCOSIDES

- Optically active; generally in l, rarely d form
- Soluble in water, MeOH, EtOH, Acetone, Ethylacetate, Pyridine
- Not soluble in O₂ free solvents
- Best soluble in 70⁰-90⁰ EtOH
- More sugar units in the structure, less soluble in organic solvents

GLYCOSIDES

- Some glycosides are distributed in different families (cynogenetic / flavonoids / saponins) whilst some of them restricted to particular families, such as:
 - Anthracenes ----- Rhamnaceae
 - Glucosinolates ----- Brassicaceae (Cruciferae)
 - Cardiac glycosides ----- Apocynaceae/Asclepiadaceae/
Scrophulariaceae

GLYCOSIDES

- Classification on the basis of the linkage between sugar and aglycone part:
- O-glycosides: Glycosidic bond between reducing group of the sugar with an Oxygen atom of –OH (phenol/alcohol) group of the aglycone (e.g. salicin)
 - $R-OH + HO-sugar \rightarrow R-O-sugar + H_2O$
- S-glycosides: Bond between reducing group of the sugar and Sulphur atom of –SH (thiol) group of the aglycone (e.g. sinigrin)
 - $R-SH + HO-sugar \rightarrow R-S-sugar + H_2O$

GLYCOSIDES

- N-glycosides: Glycosidic bond between reducing group of the sugar and Nitrogen atom of $-NH_2$ (amin) group of the aglycone (e.g. nucleosides DNA and RNA)
 - $R=NH + HO\text{-sugar} \rightarrow R=N\text{-sugar} + H_2O$
- C-glycosides: Sugar is linked directly to the carbon atom of aglycone (C-C linkage between the sugar and aglycone) (eg. aloin). C-glycosides are non-hydrolysable by acids or enzymes (e.g. aloin)
 - $RCH + HO\text{-sugar} \rightarrow RC\text{-sugar} + H_2O$

GLYCOSIDES

- Primary glycoside → Glycosides that originally found in the plant; e.g. Purpurea Glycoside A
- Secondary glycoside → Glycosides which lose one/terminal sugar from the primary glycoside; secondary glycosides occur while drying the plant by enzyme effect; e.g. Digitoxin

GLYCOSIDES

- Stabilization is necessary to prevent the conversion of the primary glycoside to secondary glycoside by losing one unit sugar.
- **STABILIZATION:** Deactivation of the enzymes responsible for the hydrolysis of the glycosides. It prevents glycoside decomposition.

GLYCOSIDES

STABILIZATION METHODS

1- BOURQUELOT METHOD:

Taze bitki+EtOH (%80)----kaynatılır—enzim denatüre (inaktif) olur. Aynı zamanda da bitkide bulunan etken maddeler alkole geçer

2- STABILIZATION WITH WATER OR ALCOHOL VAPOR UNDER PRESSURE (PERROT-GORIS METHOD):

Bu amaçla Alkol (EtOH)/ EtOH+CH₃COOH/ MeOH/Trikloroetilen kullanılır.

GLYCOSIDES

3- STABILIZATION WITH DRY AIR:

Plant material treated in 80-110⁰C air flow.

4- INDUSTRIAL STABILIZATION:

Drying for one minute in 300-800 ⁰C air flow.

UV lights

Stabilization with high frequency electric current (not economic)

GLYCOSIDES

HYDROLYSIS

1) ACID HYDROLYSIS: Acid hydrolysis is done by heating with dilute acid (%1,%5,%10 HCl/H₂SO₄)

- Hydrolysis period is short
- Half an hour on boiling waterbath. At the end of this period, aglycons will precipitate since they are not soluble in water; sugars will remain in the solution.

GLYCOSIDES

2- ENZYME HYDROLYSIS:

Optimum conditions (pH, temperature) should be provided

It will take long time (48 hours or more).

Relatively specific and selective

Decomposition of the sugar or aglycone is not occurring

GLYCOSIDES

The most used enzymes are:

A) INVERTASE ----- can hydrolyze α -glycosidic bonds

B) EMULSIN ---- can hydrolyze β glycosidic bonds

C) MYROSIN ----- can hydrolyze S-glycosidic bonds

D) RHAMNO-DIASTASE ----- breaking the bond between the aglycone and oligosaccharide; not effective on the bond between the sugars

GLYCOSIDES

- It is possible to separate the sugars in the glycosides that contain sugar chain stepwise starting from the terminal sugar.
- For example;
 - ❖ γ -k-strophanthoside $\xrightarrow{\text{acid}}$ k-Strophanthidin + simarose + β -gl + α -gl.
 - i. γ -k-strophanthoside $\xrightarrow{\text{invertase}}$ β -k-strophanthoside + α -gl.
 - ii. β -k-strophanthoside $\xrightarrow{\beta\text{-glycosidase}}$ α -k-strophanthoside + β -gl
 - iii. α -k-strophanthoside $\xrightarrow{\text{acid}}$ k-Strophanthidin + simarose

GLYCOSIDES

IDENTIFICATION

- If a plant/drug contain glycoside or not is tested by BOURQUELOT method:
 - i. Plant sample+EtOH ---enzyme inactivation+CaCO₃---preventing acid hydrolysis---stabilize---filter----EtOH solution
 - ii. Residue (posa)+ extract with EtOH
 - iii. Combine EtOH extracts----evaporate the solvent----Crude extract+mix with boiling water----chlorophyll will precipitate----filter----WATER SOLUTION (glycosides)---dilute to a constant volume----GLYCOSIDE SOLUTION is obtained

GLYCOSIDES

- This glycoside solution is tested for the quantification of reductor sugars.
- Optical rotation is determined using this solution.

GLYCOSIDES

- Gradual enzyme hydrolysis will be done using this Glycoside solution by using Invertase, Emulsin, Rhamno-diastase enzymes and Acid; reductor sugar quantification and optical rotation are determined in every step.

GLYCOSIDES

- According to the test results;
- If optical rotation is levogyrous before hydrolysis and change to right after the hydrolysis; additionally detection of increase on reductor sugar after hydrolysis show us the presence of glycosides

GLYCOSIDES

EKSTRAKSİYONU

- Heterozitlerin aglikonları farklı yapılarda oldukları için değişik solvanlarda farklı derecelerde çözündüklerinden ortak bir ekstraksiyon yöntemi yoktur.
- Ama genel bir ekstraksiyon yapılacak olursa; teşhiste uygulanan stabilizasyon burada da uygulanır.
- Yine burada da stabilizasyondan sonra aynı şekilde klorofil çöktürülerek heterozitleri içeren sulu çözelti elde edilir.

GLYCOSIDES

- Glycosides can be obtained from water extract by:
- Crystallization
- GLYCOSIDE + alkaline Pb acetate----foreign matters are precipitated-----Filtrate (glycosides)
- In the meantime Phenolic Glycosides are precipitated with Pb salts. In this case Pb acetate can be used to precipitate Phenolic glycosides

GLYCOSIDES

ENZYMOLYTIC INDEX OF REDUCTION

- For a given glycoside, in well-defined conditions, after the enzyme hydrolysis the rate of the increase on the reductor sugar to the change on the optical rotation is called Enzymolytic Index of Reduction. This value is constant for each glycoside.

GLYCOSIDES

Medicinal Importance – Uses and Effects

- ❖ Most of the glycosides are toxic compounds with strong physiological effects.
- Cardioactive glycosides with cardiotonic properties
- Laxative-purgative effect (Anthraquinone glycosides)
- Reducing the capillary fragility; vascular wall strengthener effect (Flavonoids)
- Antipyretic, analgesic, antispasmodic

GLYCOSIDES

Medicinal Importance – Uses and Effects

- There are some glycosides used for aromatic purposes
- They are effective at even mg levels
- For example;
- Rutin----- 20 mg
- Digitoxin----- 1-1.5 mg, continue dosage 0.1-0.2 mg
- Sennoside A----- 20 mg

GLYCOSIDES

1) ALKOL HET.----- Radix Gentianae

2) FENOL HET.----Fr. *Vanillae*

3) FLAVONOİTLER-----Fr. *Petroselinii*

4) KAH-----F. *Digitalis*

5) ANTRASENOZİTLER---Rh. *Rhei*

6) SENEVOL HET.-----S. *Sinapis nigrae*

7) SİYANOGENETİK HET.--- S. *Amygdalae amarae*

GLYCOSIDES

8) SAPONOSİTLER----R.Saponaria albae

9) İRİDOİT HET.-----Flos Verbasci

10) KUMARİN HET.-----C. Fraxini

CARDIAC GLYCOSIDES (CG)

- These compounds are characterized by the effects on the myocardial muscle
- They increase both tonus and contractility of myocardial muscle
- They increase the contraction power of the myocardium without increasing oxygen consumption. As a result, they increase the cardiac yield
- The therapeutic effects of glycosides depend on the structure of the aglycones and the type and number of the sugars.

CARDIAC GLYCOSIDES

- Since they are present in glycosidic form, it increases the binding of these compounds to the myocardial muscle.
- Cardiac glycosides show + inotropic effect

CARDIAC GLYCOSIDES

- Scrophulariaceae-----*Digitalis* sp.
- Apocynaceae-----*Strophanthus*, *Nerium*
- Asclepiadaceae-----*Periploca*
- Ranunculaceae---*Adonis*, *Helleborus*
- Liliaceae-----*Scilla maritima*

CARDIAC GLYCOSIDES

CARDIOACTIVE GLYCOSIDES

- The main structure is Steroid → their structure consist of cyclopentanoperhydrophenanthrene ring. A lactone ring is bonded to this system.
- Cardiac glycosides carrying 5 membered unsaturated lactone ring are called CARDENOLIDE (C23)
- Cardiac glycosides carrying 6 membered unsaturated lactone ring are called BUFADIENOLIDE (C24)
- Cardiac glycosides in 14,21-EPOXY-CARDENOLIDE structure has also been found.

CARDIAC GLYCOSIDES

- Cardiac glycosides contain 2 or more sugars
- Glucose is the most common one
- Among sugars, 2-desoxymethylpentoses are specific to cardiac glycosides:
 - Digitoxose
 - Oleandrose
 - Cymarose
 - Diginose

Digitoxose

CARDIAC GLYCOSIDES

- They also can contain methylpentose such as:
- Rhamnose
- Tevetose
- Digitalose

CARDIAC GLYCOSIDES STRUCTURE-ACTIVITY RELATIONSHIP

In order to show cardioactive effect the main structure must;

- At C17 ---- an unsaturated lactone
- At C10 ----- $-\text{CH}_3$, $-\text{CH}_2\text{OH}$, $-\text{CHO}$
- At least 2 $-\text{OH}$ groups at -----C3 and C14
- A/B rings----- must be in cis- position

CARDIAC GLYCOSIDES

IDENTIFICATION

1) AGLYCONES

a) Liebermann Reaction:

- It is not a specific reaction
- Steroid structures give (+) result
- Bufadienolides ve cardenolides give (+) result with this reaction
- CHCl_3 solution + anhydrous acetic acid + $\text{H}_2\text{SO}_4 \rightarrow$ blue-green colour

CARDIAC GLYCOSIDES

b) Cardenolide structure;

-Legal Test:

KAH+Sodium nitroprusside ----medium with pyridine +NaOH---- RED colour

-Kedde Reaksiyonu:

CHCl₃ solution+3,5 dinitrobenzoic acid----alkaline medium ----purple-red colour

CARDIAC GLYCOSIDES

-Baljet Reaction:

CHCl_3 solution+picric acid+NaOH----orange colour

-Reduction with $\text{AgNO}_3/\text{NH}_3$

2- for DESOXYOSES;

Keller-Kiliani Test:

CHCl_3 solution residue+ glacial acetic acid + FeCl_3 ---- layering with $\text{H}_2\text{SO}_4 \rightarrow$ dark ring between two layers

CARDIAC GLYCOSIDES

3- TLC (Thin Layer Chromatography)

SVENDSEN-JENSEN Method: used for identification of 16-hydroxy-cardenolide teşhisinde kullanılır.

CARDIAC GLYCOSIDES

QUANTITATIVE ANALYSIS

1) BIOLOGICAL ASSAY: Recorded in some Pharmacopoeias

a) Pigeon test → recorded in TF

b) Frog test → recorded in TF

c) Cat and guinea pig test

CARDIAC GLYCOSIDES

- Results are compared to international reference numbers in IU type and activity is calculated.
- A qualified drug's activity must be at least 10 IU.
- 1 IU= 76 mg (0.076 g) -----Activity of International reference sample

CARDIAC GLYCOSIDES

- International Reference: Folia Digitalis powder samples collected from different locations, moisture degree arranged to 3%, dried in 55-60°C.
- Stored in “ National Institute for Medical Research” in London (stored in coloured ampuls)

CARDIAC GLYCOSIDES

- Quantification via biological methods have some inconveniences:
 - - Toxicity is calculated, not activity.
 - - Healthy animals are used
 - - Drugs are given parenterally to animals; orally of drug may differ.

CARDIAC GLYCOSIDES

2- COLORIMETRIC ASSAY (Ph.I):

3- CHROMATOGRAPHIC ASSAY

4- CHROMATOGRAPHIC+BIOLOGICAL ASSAY

CARDIAC GLYCOSIDES

Findings detected by comparing the results obtained:

- 1) Primary glycosides are more effective than secondary glycosides.
- 2) Formyl substituents have significant effect on cardiotoxicity.
- 3) There is not a correlation between cardiotoxicity and total glycoside amount. Samples with the same glycoside amount but with different types of glycosides have different effects.