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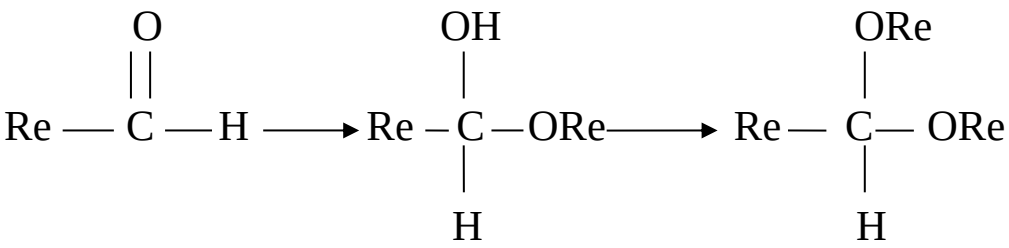
Glycosides

Glycosides are compounds that yield on hydrolysis, one or more sugar part and another non-sugar part. The sugar part is known as **glycone** , and the non-sugar part is the **aglycone.** In general there are two *basic classes* of glycosides: *C- glycosides,* in which the sugar is attached to the aglycone through C-C bond, and the *O- glycosides* in which the sugar is connected to the aglycone through oxygen –carbon bond.

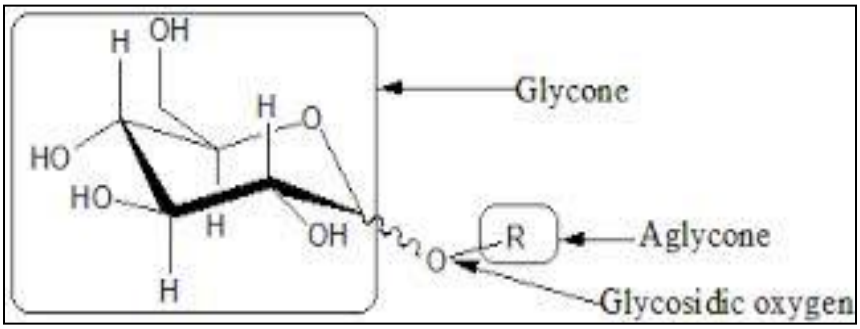
Chemically the glycosides are acetals in which the hydroxyl group (OH) of the glycone is condensed with the hydroxyl group of aglycone. More simply the glycosides may be considered as sugar ether. Two forms of glycosides are present, the α-form and the β-form, but the β-form is the one that occur in plants, even the hydrolytic enzymes act on this type.



Hemiacetal Acetal

Inside the body the glycosides will be cleaved to glycone and aglycone parts, the glycone part confers on the molecule solubility properties, thus is important in the absorption and distribution in the body, while the aglycone part is responsible for the pharmacological activity.

Generally all glycosides are hydrolyzed by boiling with mineral acids , on the other hand the presence of specific enzyme in the plant tissue, but in different cells from those that contain the glycosides, are able to hydrolyzed the glycosides, such as the emulsin enzyme which is present in the almond kernel, and the myrosin enzyme which is found in the black mustard seeds.



(Fig.1) General Structure of Glycosides

Generally in the extraction of glycosides we have to consider the following points:

1. Apolar solvent, which is mostly alcohol, but not water, since water may induce fermentation, in addition water need high temperature due to its high boiling point.
2. Neutralization of the extract with base, since the presence of acid lead to hydrolysis of the glycoside.
3. Use of heat is to inhibit the activity of hydrolytic enzymes that present in
   the plant cell.

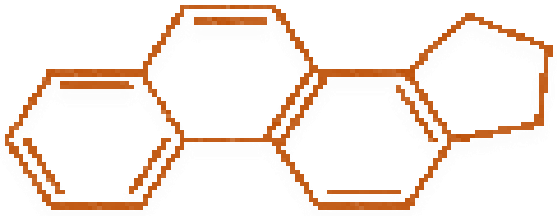
The glycosides are classified according to the chemical structure of the aglycone to:

1. Cardioactive glycosides.
2. Anthraquinone glycosides.
3. Saponin glycosides.
4. Cyanophore glycosides.
5. Isothiocyanate glycosides.
6. Flavonoid glycosides.
7. Alcohol glycosides.
8. Aldehyde glycosides.
9. Lactone glycosides.
10. Phenol glycosides.
11. Miscellaneous glycosides.

Exp. No.1

[Lab.1] Cardioactive Glycosides

They are named so, due to their action on the heart muscle. The aglycone part here is steroid, which is chemically cyclopentaphenanthrene .



cyclopentaphenanthrene nucleus

The steroidal aglycones are of two types:

1) Cardinolides(α-β unsaturated 5 – member lactone ring).
2) Bufadienolides (doubly unsaturated 6-member lactone ring).

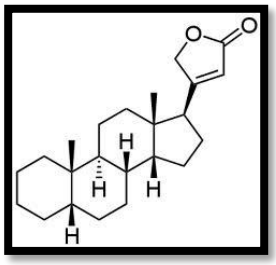
The more prevalent in nature is cardinolides type.

For maximum activity of cardioactivce glycosides the following points are important:

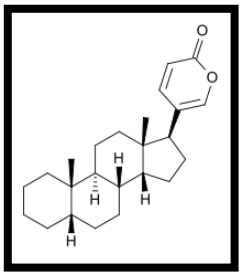
1) 17 -β –lactone ring (cardinolide or bufadinolide).
2) 3 -β - OH.
3) 14 -β-OH.
4) CATSC (C= ***cis*** between two rings (A&B). A= ***Anti*** in one ring (5&19).

   T=***Trans*** between two rings (B&C). S= ***Syn*** in one ring (8&18).

   As represented bellow:



Cardenolide



Bufadienolide

Plants Containing CardioactiveGlycosides:

1) Digitalis (digitalis or foxglove) **Digitalis purpurea** of the family Scrophulariaceae.

   The name digitalis is from Latin *digitus* which means finger refers to finger – shaped, while *purprea* refers to *purple* color of their flower. This plant contains anumber of glycosides as digitoxin , gitoxin and getaloxine.
2) **Digitalis lanata** of the same family, from which the digoxin is obtained.
3) The plant used in our laboratory is **Nerium oleander** of the family **Apocyanaceae**. The main glycoside of which is oleandrin.

Nerium oleander

Isolation and Identification of the Cardioactive Glycosides:

1. Extraction:

Aim: To isolate the cardioactive glycosides.

Equipments:

- *Large beaker & two medium size beakers.*
- *Two conical flasks.*
- *Centrifuge & Centrifuge tubes.*
- *Separatory funnel.*
- *Water bath.*

Reagents:

- *70% ethanol.*
- *Lead sub acetate.*
- *10% sodium phosphate solution.*

- *Chloroform: Ethanol (3:1 v/v).*
- *Anhydrous sodium sulphate.*
- *4N HCl acid.*
- *Chloroform.*

Procedure:

***Method of extraction*:** Maceration.

Plant used: Nerium oleander.

Part used: dry leaves.

Maceration ***10 gm*** of the powdered leaf in ***100 ml*** of *70%**ethanol*** for **24 hrs. (**Prepared previously)

Take ***10 ml*** of alc. Extract in conical flask

↓ Add

10 ml of ***lead sub acetate*** solution
(Mixing& standing for **5 mins.)**

↓ Centrifuge
(5 mins.)

Decant and take the supernatant (upper layer)

↓ Add

10 ml of *10%**sodium phosphate*** solution

↓

Centrifuge
(***5 mins.***)

Take supernatant and divide in to ***two*** divisions

Fraction A

Take one division and put in a separatory funnel

↓ Add

[***10 ml*** of ***Chloroform: Ethanol*** *(3:1 v/v)*] two times

↓ (Shake& stand)

Combine the organic lower layer and put it in the conical flask

↓ Add

Small quantity of ***Anhydrous sod. Sulphate*** & allow standing for few minutes until get a clear solution, decant the Chloroform-ethanol extract and reduce the volume on water bath to get:

Fraction A

Fraction B

Place the other division of the extract in the conical flask

↓ *Add*

3 ml of ***4N HCl***

↓ *Boiling in water bath*

*(**15 min**$_s$)*

Cool &transfer to a separatory funnel

↓ *Add*

[***10 ml*** of ***Chloroform***] tow times

Combine the chloroform extracts (lower layers)

↓ *Add*

Small quantity of ***Anhydrous sod. Sulphate*** & allow standing for few minutes until get a clear solution then decant the chloroform layer and concentrated on water bath to about 1ml. and we get:

Fraction B

Results:

Fraction A : Contain the whole glycosides.

Fraction B : Contain the aglycone (genin) part only.

[Lab.2] The Chemical Tests

1. Baljet's Test:

Aim: The identification of the cardio active glycosides in ***general***.

Equipments& Reagents:

- ✓ Test tube.
- ✓ Picric Acid.
- ✓ Sodium hydroxide solution.

Procedure :

Take ***1ml*** of fraction A, add ***2 drops*** of ***Picric acid*** then make it alkaline with Sod. Hydroxide solution.(litmus paper).

Results:

Turbid , **yellow** to **orange** in color.

2. Keller- Killian's Test

Aim: The identification of the cardio active glycosides in ***general***.

Equipments& Reagents:

- ✓ Test tube.
- ✓ Glacial acetic acid
- ✓ 0.1 % of ferric chloride solution.
- ✓ Conc. H_2SO_4.

Procedure:

Take***1ml*** of fraction A, and ***2ml*** of ***glacial acetic acid***, add ***1 drop*** of ***0.1 %*** of ***ferric chloride solution.***Take ***1ml*** of conc***.*** H_2SO_4 and add to the above mixture in drops so as to make two layers.

Results:

Two layers are formed; the upper one has ***light bright green*** color. The lower layer has transparent clear color (H_2SO_4 layer).The junction appears as a ***reddish –brown*** ring.

Other Chemical Tests for the Identification of Sterol Glycosides:

1. Raymond's Reaction:

Aim: To identify the ***sterol*** nucleus.

Equipmentsand Reagents:

- ✓ Test tube.
- ✓ 10% sodium hydroxide solution.
- ✓ 1% m-dinitrobenzene.

Procedure:

To 1ml of fraction A add ***1-2 drops*** of ***10% sodium hydroxide*** and few drops of an alcoholic solution of ***1%m-dinitrobenzene.***

Result:

Pink color appears.

2. Kedde's Reaction :

Aim : To identify the ***sterol*** nucleus.

Equipments and Reagents:

- ✓ Test tube.
- ✓ 1% 3,5-dinitrobenzoic acid.
- ✓ 0.5 N aqueous methanolic KOH (50 %).

Procedure:

To a solution of glycoside add a solution of ***1% 3, 5-dinitrobenzoic acid*** in 0.5N aqueous ***methanolic KOH (50%)***.Report the color.

3. Lieberman's Sterol Reaction:

Aim: To identify the ***sterol*** nucleus.

Equipments and Reagents:

- ✓ Test tube.
- ✓ Porcelain dish.
- ✓ Anhydrous acetic acid.
- ✓ Conc.H_2SO_4.

Procedure:

Take ***1ml*** of fraction A in a test tube then add ***5ml*** of ***anhydrous acetic acid*** and shake well. Take ***4 drops*** of the above mixture and place in a porcelain dish, and then add ***one drop*** of ***conc.H_2SO_4.***

Result:

A change of color from **rose**, through **red**,**violet** and **blue** to **green**. The colors are slightly different from compound to compound.

Discussion:

This reaction is due to the steroidal part of the molecule and it is characteristic of the ***aglycone*** of the scillarenin type (unsaturated steroidal part).

4. Legal's Reaction:

Few ml_s of the glycoside or the purified extract of the crude drug is dissolved in pyridine. When sodium hydroxide and sodium nitroprusside are added alternatively, a transient blood-red color develops. This is atest for the unsaturated lactone ring of the genin.

The Identification of Cardio active Glycosides By Chromatography:

By the use of thin layer chromatography **(T.L.C)**

- The stationary phase = *Silica gel G.*
- The mobile phase =***Chloroform: Ethanol: Water (7:3:1)***

  Or ***Ethyl acetate: Methanol: Water (75:10:5).***

- The standard compound = ***Oleandrin.***
- The spray reagent = Lieberman's ***reagent.***
- Mechanism of separation =*Adsorption*.
- Developing = *Ascending.*
- ***Other mobile phases :***

  Butanone: Xylene: Formamide (50:5:4)

  Chloroform: tetrahydrofuran: Formamide (50:50:6).

Procedure:

1) Prepare ***100ml*** of mobile phase, and place it in the glass tank.
2) Cover the tank with glass lid and allow standing for ***45 minutes*** before use.
3) Apply the sample spots (fraction A & fraction B), and the standard spot on the silica gel plates, on the base line.

4) Put the silica gel plate in the glass tank and allow the mobile phase to rise to about *two-third* the plate.
5) Remove the plate from the tank, and allow drying, and then detecting the spots by the use of the spray reagent and heat the plates at ***105 -110 ^{0}C*** for ***5-10 min$_{s}$*** in the oven.
6) Note the spots, and calculate the Rf value for each spot.

Note/ the Rf value should be ***less than 1***, because if the Rf value = **1**, this means that there is no separation, and the sample moved with the solvent.