

Al-Nahrain University
College of pharmacy

Practical pharmacognosy

3rd stage 1st term

Pharmacist Ayah AlQrimli

Lecture 1

Objectives of pharmacognosy lab

- Students must understand **glycoside chemistry**
- Students will be able **extract** different medicinal plants
- Students will be able to **identify** the different glycosides by TLC or chemical test

Glycosides

Glycosides are organic compounds that upon hydrolysis give one or more sugar parts and another non sugar part.

The sugar part is known as **glycon** ,while the non sugar part is the **aglycon**(genin).

Glycosides

Glycoside → Sugar Glycon + Non-sugar part Aglycon (genin)

- Absorption
- Circulation in the blood
- Distribution
- Kinetics

- Effect
- Pharmaceutical importance and activity
- Toxicity

Sugar + aglycon=glycoside

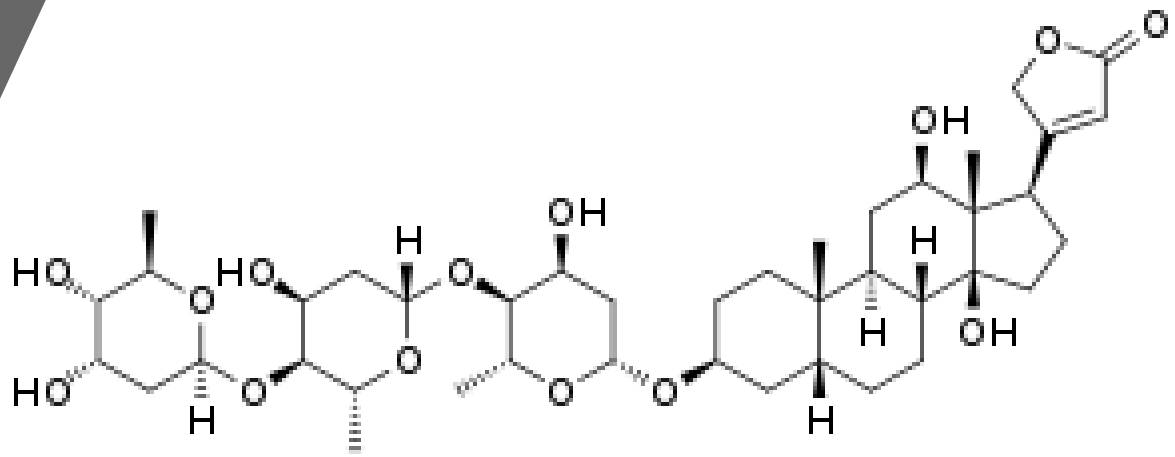
Inside the body the glycosides will be cleaved to aglycon and glycon parts , the glycon sugar part enhances the solubility in water , absorption transport and bioavailbility of the aglycon in the plant or in the body.

It could be any type of sugar :glucose , fructose, galactose etc

Aglycon could be any natural coumpound : sterol, terpene ,flavonoid etc

Pharmacist Ayah AlQrimli

Glycoside structure example

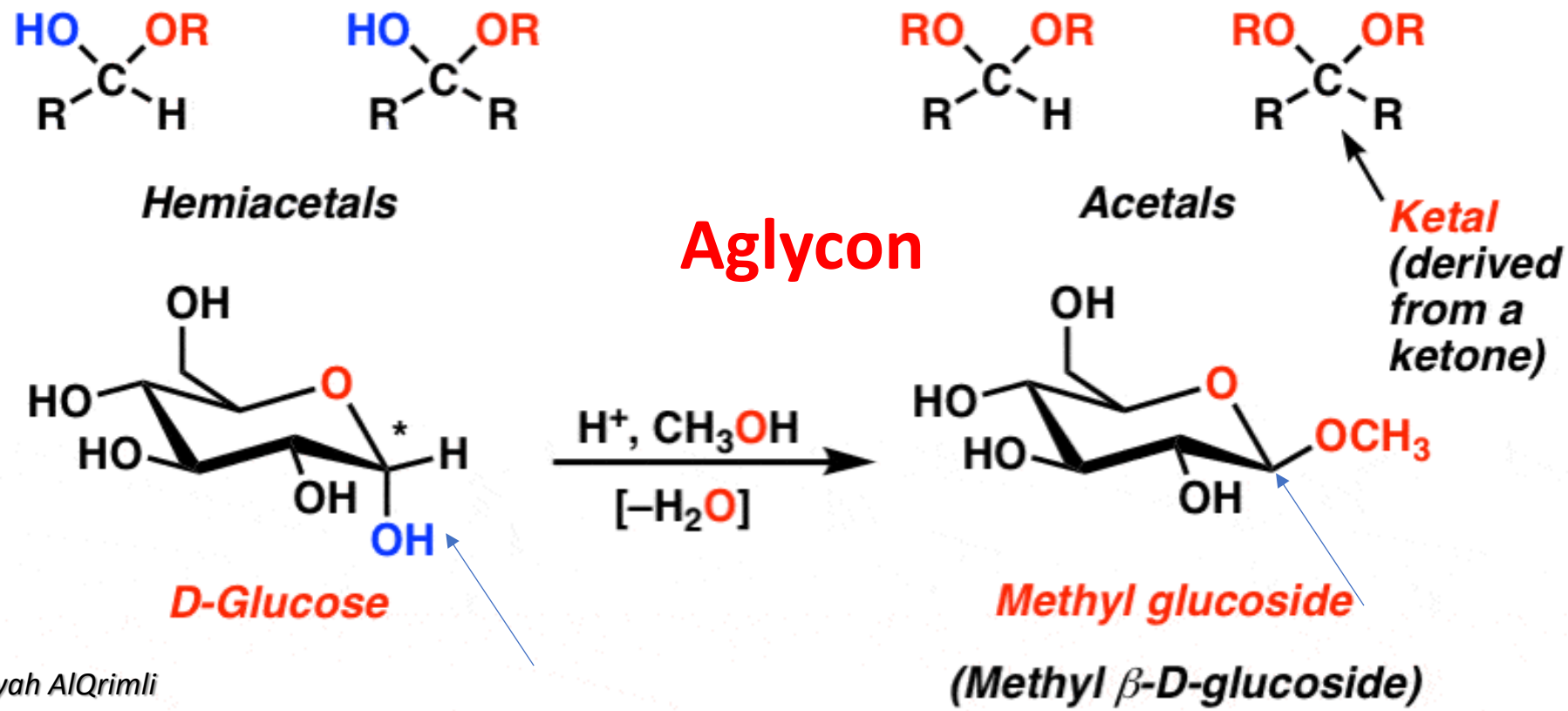




Pharmacist Ayah AlQrimli

Chemically they are acetals

- Chemically glycosides are acetals where the hydroxyl group (OH) of the sugar (glycon)is condensed with the hydroxyl group of aglycon



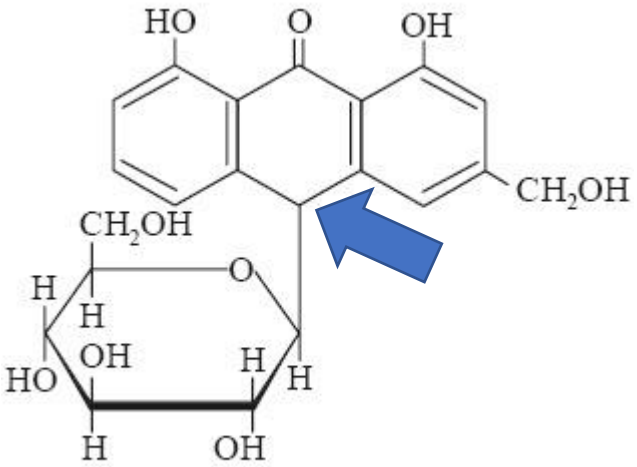
classification according to type of linkage

C- glycosides : where the sugar part is linked to the C atom of the aglycon by a C-C bond

O-glycosides: where the sugar part is linked to the O atom of the aglycon by a O-C bond

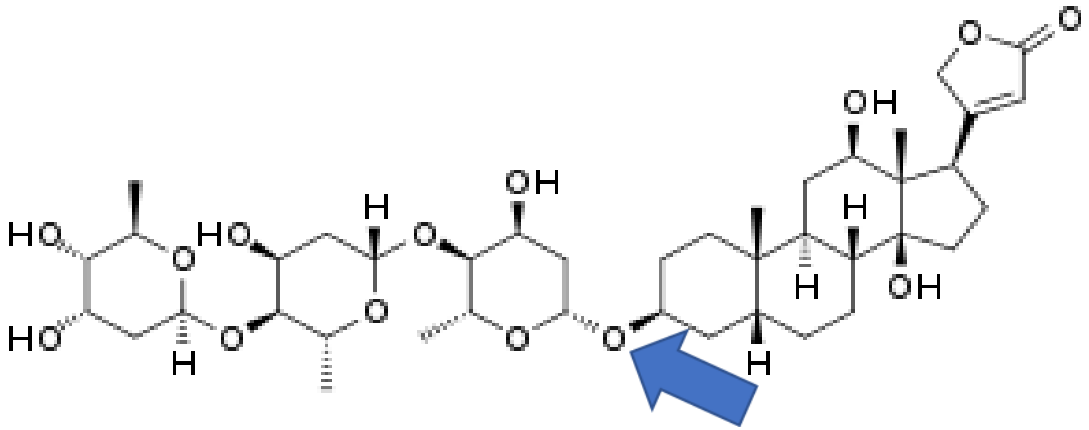
N-glycosides: where the sugar part is linked to the N atom of the aglycon by a N-C bond

S-glycosides: where the sugar part is linked to the S atom of the aglycon by a S-C bond



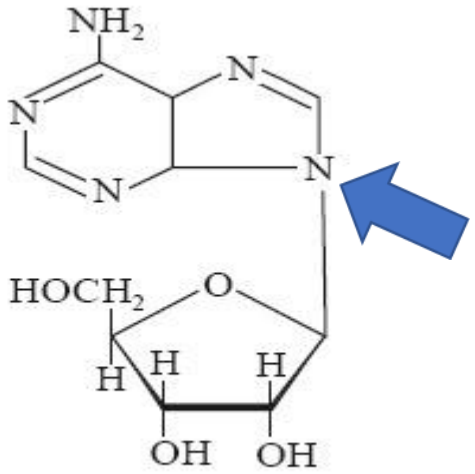
Aloin (or Barbaloin)

C-glycoside



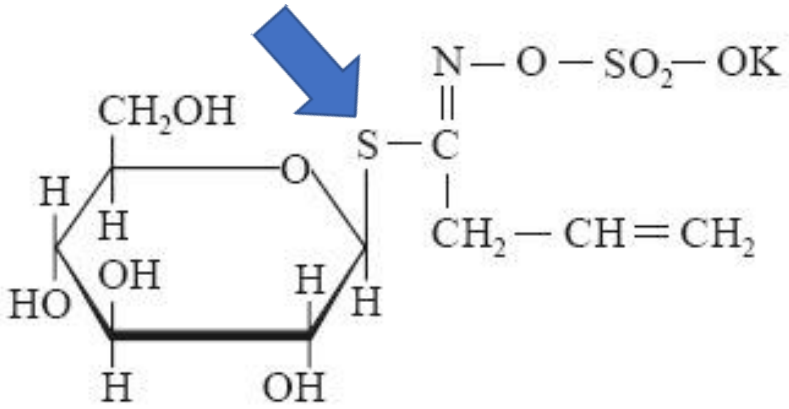
Digoxin

O-glycoside



Adenosine

N-glycoside (nucleosides in RNA)



Sinigrin

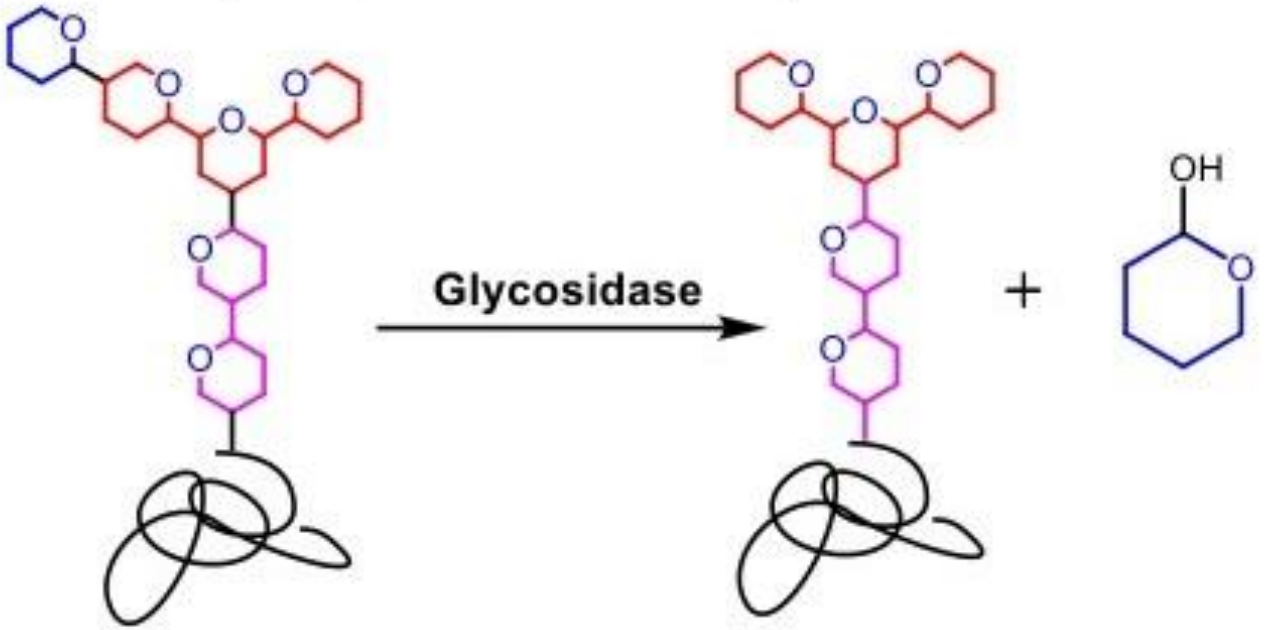
S-glycoside

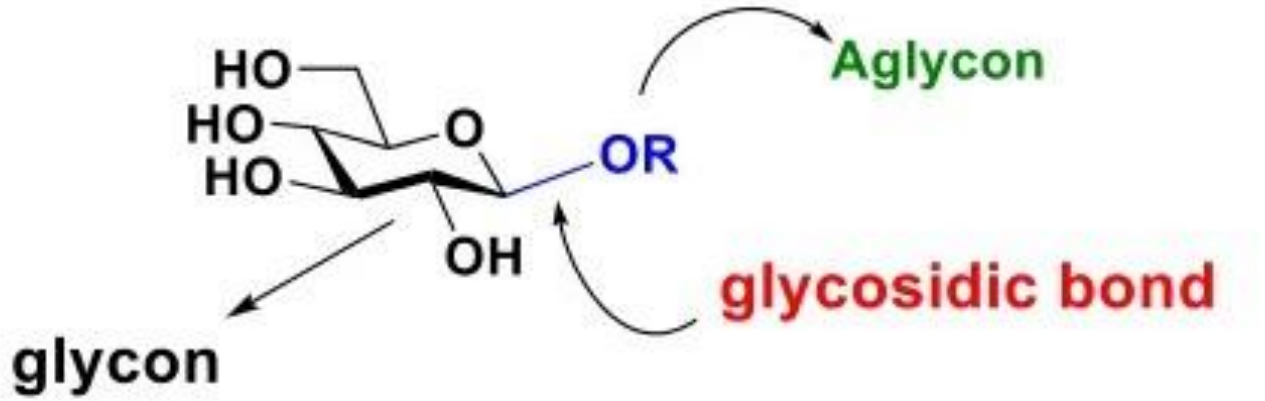
glycosidases

- Generally all glycosides are hydrolyzed by boiling with mineral acids.
- Others are hydrolyzed by the presence of specific enzymes found in the plant tissue (glycosidases that cleave the glycosidic linkage between sugar and aglycon)
- such as: emulsin enzyme present in almond kernel and myrosin enzyme found in black mustard seeds

Pharmacist Ayah AlQrimli

Glycosidases





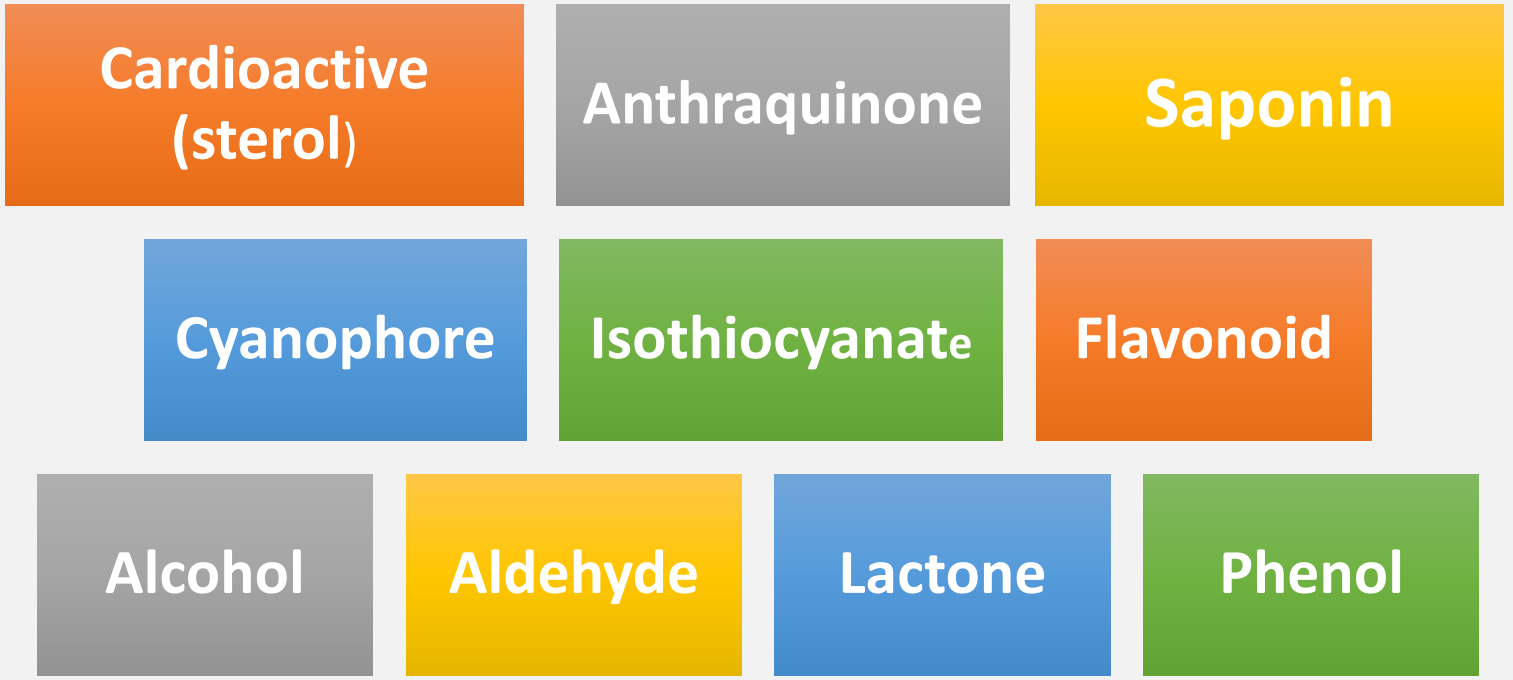
Considerations when extraction of glycosides

A **polar solvent** , mainly **alcohol** but not water , since water may cause fermentation and requires high temperature due to its high boiling point

Using a **base** to neutralize the extract , as acids lead to glycoside hydrolysis

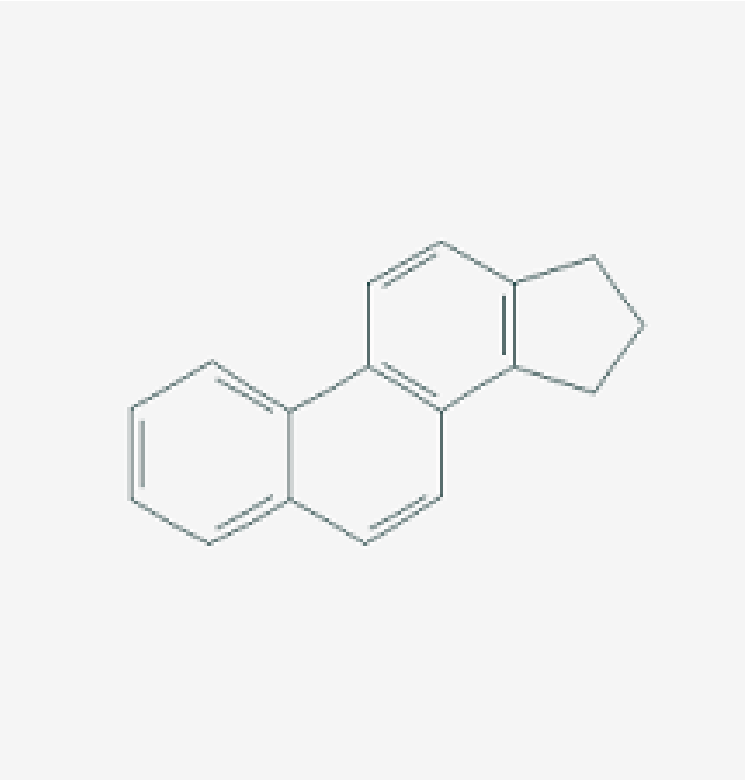
Heating the extract to inhibit the activity of hydrolytic enzymes present in plant cells

Classification according to chemical structure of the aglycon



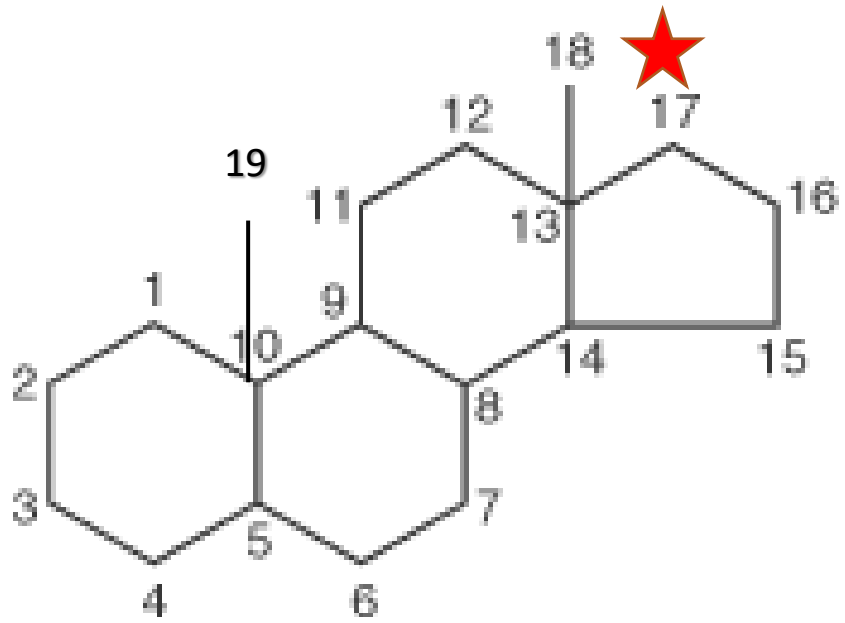
Cardioactive glycosides

Sterol aglycon
cyclo-pentaphenathrene



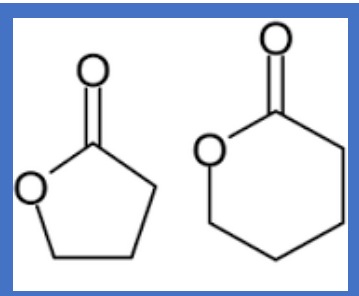
- They are called cardioactive due to their action on the heart muscle as they increase the output force of the heart and decrease its rate of contractions by acting on the cellular **sodium-potassium ATPase pump.**
- The aglycon here is a steroidal aglycon having the chemical structure **cyclo-penta-phenathrene** which is sterol basic nuclei for most steroids

Cyclopentaphenanthrene nucleus numbering system



Cyclopentanophenanthrene nucleus

Steroidal aglycons are of two types : according to C17 substitution



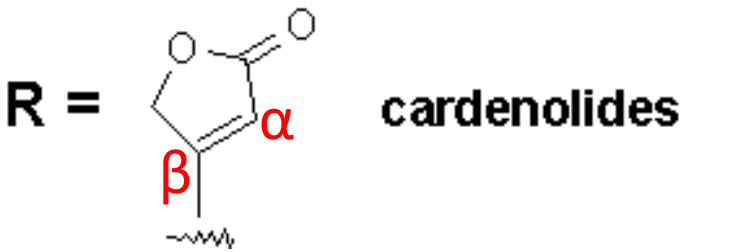
- **Cardenolides**

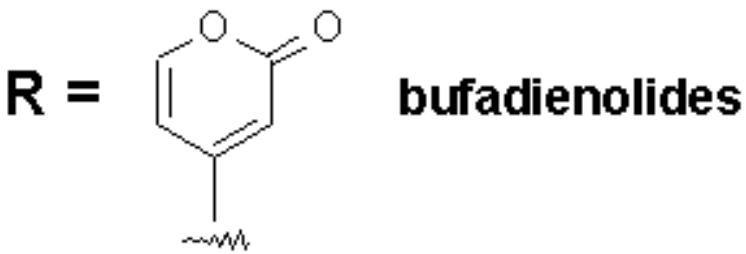
(α-β unsaturated 5-membered lactone ring)

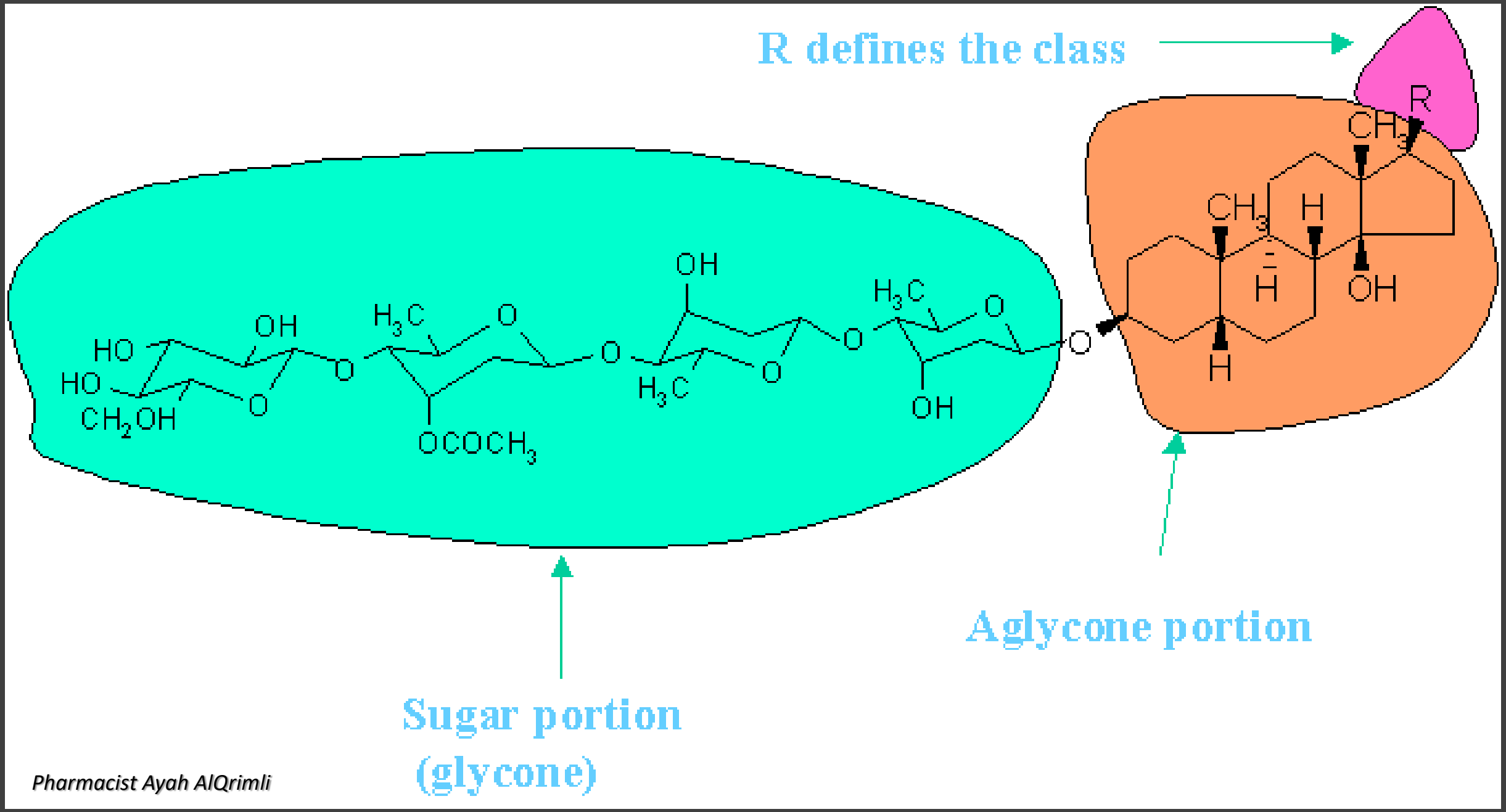
- **Bufadienolides**

(doubly unsaturated 6-membered lactone ring)

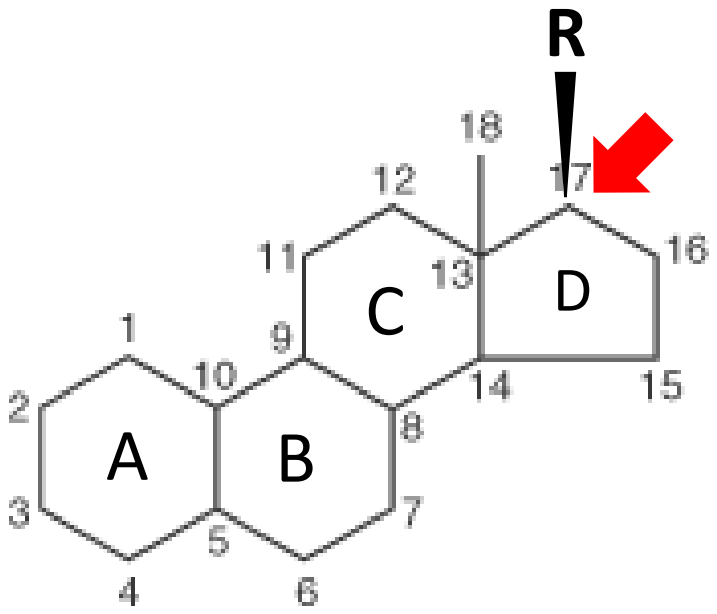
- The more prevalent in nature is cardenolide type



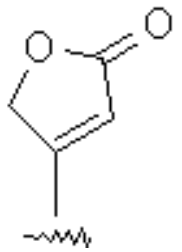



R defines the class
Sugar portion
(glycone)
Aglycone portion

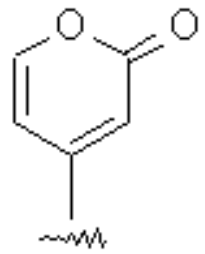
Numbering system of cardioactive glycosides



Cyclopentanophenanthrene nucleus

R =  **cardenolides**

(α-β unsaturated 5-membered lactone ring)

R =  **bufadienolides**

(doubly unsaturated 6-membered lactone ring)

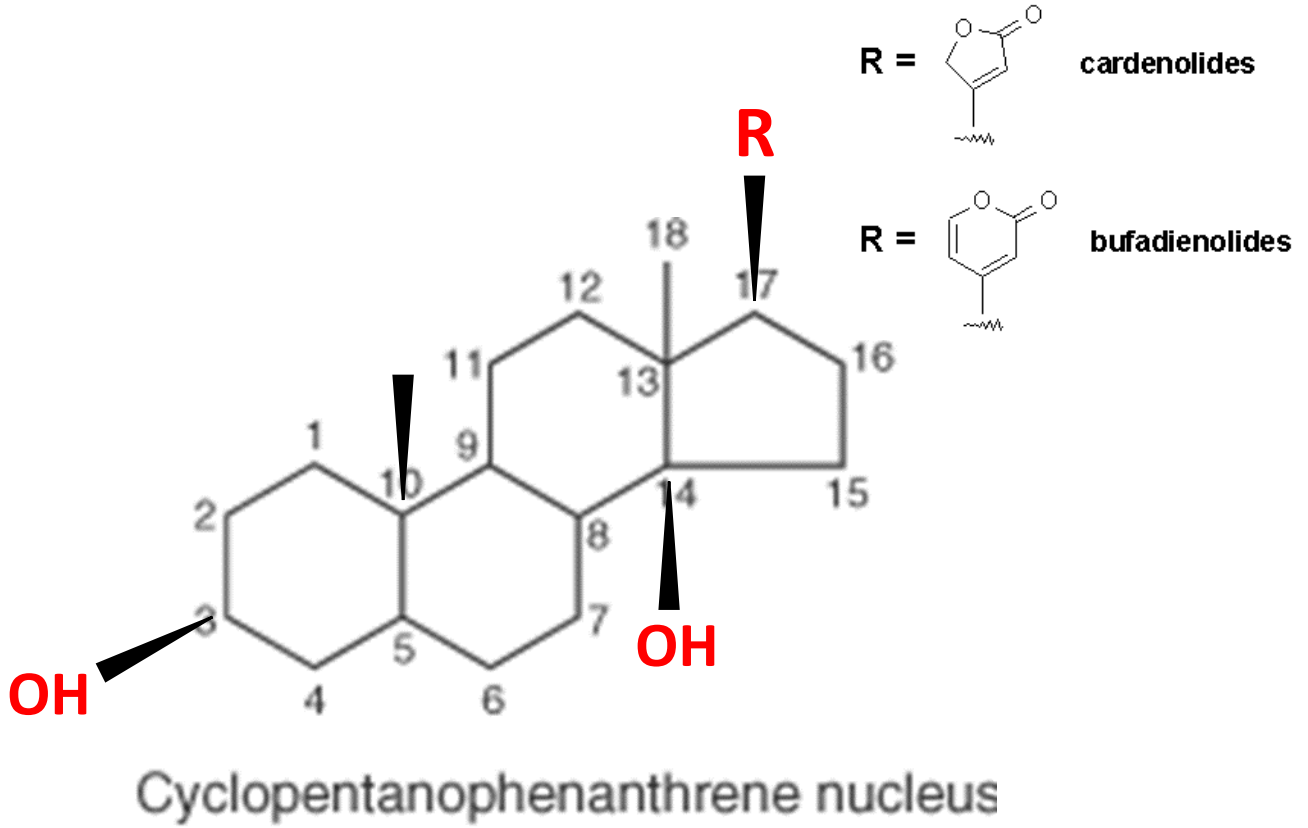


stereochemistry

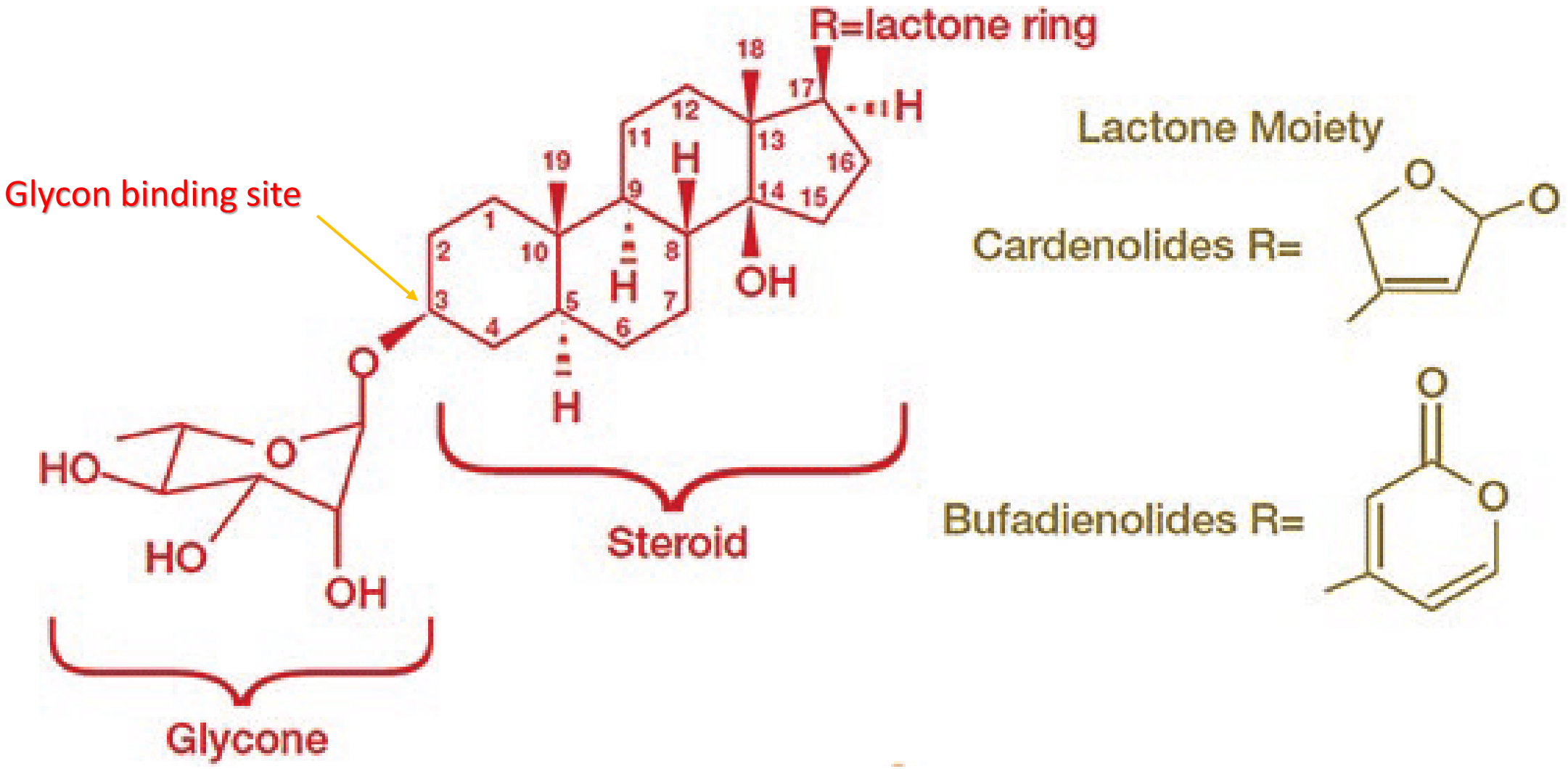
- Alpha α : below the plane
- Beta β : above the plane

Structure requirements for maximum activity of cardioactive glycosides:

- Cyclopentaphenanthrene ring
- 17-β-lactone ring (cardenolide or bufadinolide)
- 3-β-OH (glycon binding site)
- 14-β-OH



Cyclopentanophenanthrene nucleus



Pharmacist Ayah AlQrimli

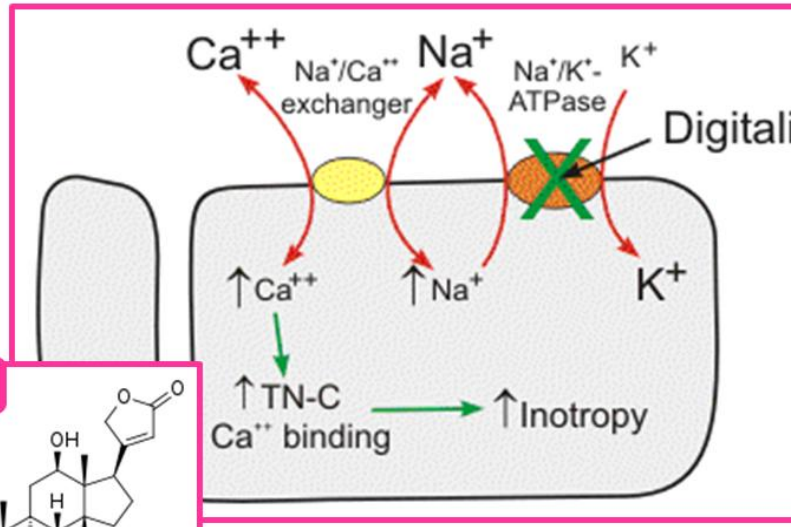
Plants containing cardioactive glycosides

Pharmacist Ayah AlQrimli

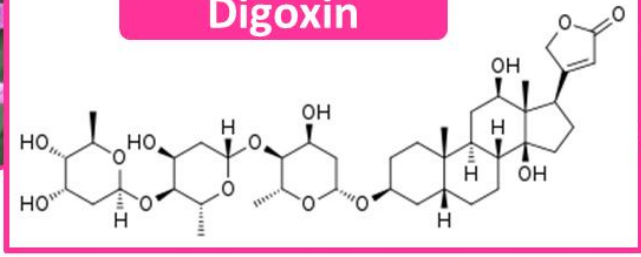
Digitalis purpurea







Digoxin



November 16

In November 16th 1954 the FDA approved as a new molecular entity the active pharmaceutical ingredient **Digoxin** under the trade name **Lanoxin**

Pharmacist Ayah AlQrimli

Digitalis lanata

(white)

twice to four times as potent cardioactive glycoside lannatoside A/B



Pharmacist Ayah AlQrimli

Nerium oleander

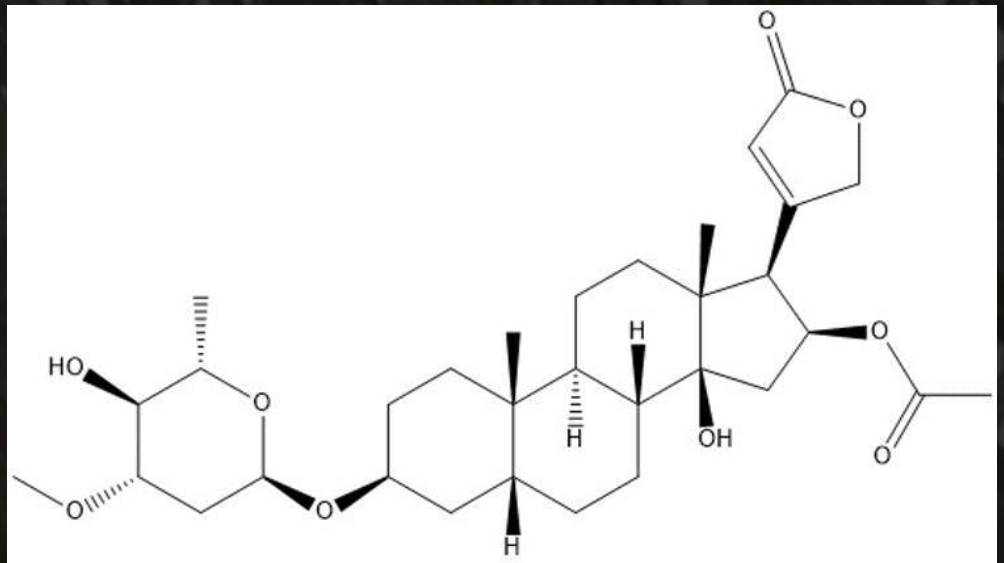
oleandrin





Pharmacist Ayah AlQrimli

Nerium oleander F:apocyanaceae



oleandrin

- Toxic in all its parts
- Oleander is one of the most poisonous commonly grown garden plants.
- Ingestion of this plant can affect the gastrointestinal system, the heart, and the central nervous system.

Pharmacist Ayah AlQrimli



EXPERIMENTAL WORK

Pharmacist Ayah AlQrimli

Isolation and identification of cardioactive glycosides

1 – Extraction :

- Aim: isolation of the cardioactive glycosides
- Procedure :
- Method of extraction: maceration (soaking)
- Plant used :**Nerium oleander**
- Part used: **dry leaves**





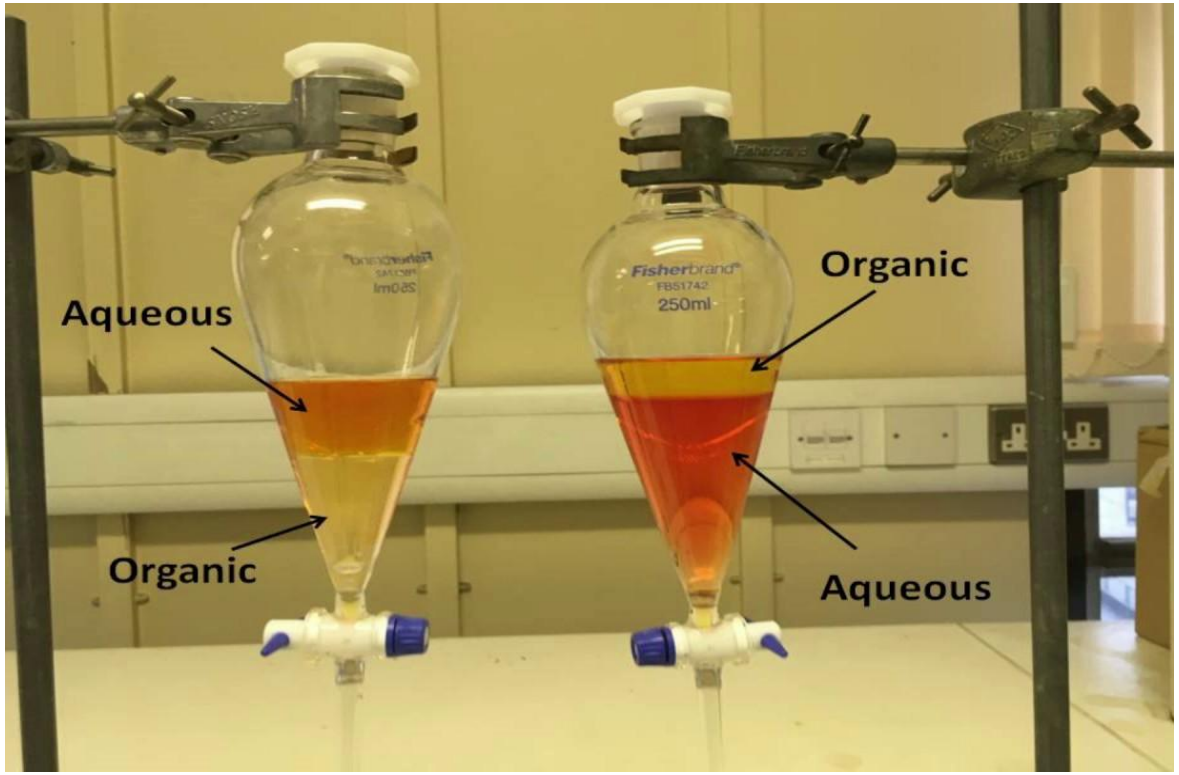


Maceration

Procedure

- Maceration 10gm of the powdered leaf in 100ml of 70% ethanol for 24hrs (prepared previously)
- Take 20ml of the alcoholic extract in a conical flask
- Add 10ml lead sub acetate solution (mix and stand for 5 min)
- Centrifuge (5 min)
- Add 10 ml of sodium phosphate solution
- Decant and take supernatant (upper layer)
- Divide supernatant into two divisions

Fraction A

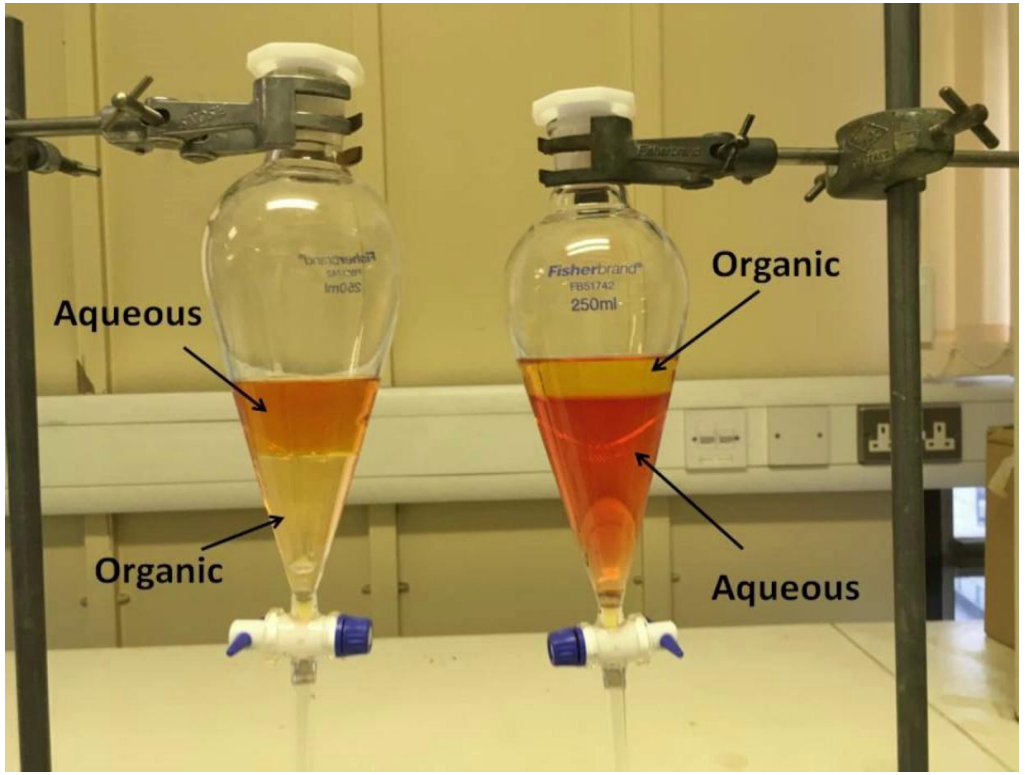


- Take one division and put in the separatory funnel
- Add 10ml of

chloroform : ethanol (3:1 v/v) twice

- Shake and stand
- Take the organic lower layer and put it in the conical flask (lower layer)
- Add anhydrous sodium sulphate and stand for few minutes until clear then decant >>> Fraction A

Fraction B



- Take the other division of the extract in the conical flask
- Add 3ml of **4N HCL**
- Boiling in water bath for 15 min
- Cool and transfer to a separatory funnel
- Add 10 ml of **chloroform** twice
- Take lower layer (chloroform layer)
- Add anhydrous sodium sulphate and stand for few minutes until clear then decant >>> Fraction B

Results:

Fraction A: contain the whole glycosides

Fraction B: contain the aglycon (genin) part only

DISCUSSION

- Lead sub acetate is added to precipitate tannins and other un wanted material.
- Sodium phosphate is added to take the excess of lead sub acetate.
- Use of chloroform –ethanol in partition is due to the fact that the chloroform will take the genin part while the ethanol will take the glycoside there will be no loss in the glycoside.
- Anhydrous sodium sulphate is added in during mixture since the anhydrous form will act as an adsorbent.
- 4N HCL is used to hydrolyse the glycoside to glycone and aglycone parts.
- Boiling is to accelerate the process of hydrolysis.
- Use of chloroform alone is to extract the genin part .