

Cardio-active glycosides

Lab II Chemical tests for identification of cardio-active glycosides

Color reactions of cardio-active glycosides for cardinolides

1. Reaction due to (--CH₂--)group of the lactone ring

➤ Baljet's Test

Equipment & 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.

➤ Raymond 's Reaction:

Equipment and 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.

➤ Kedde's Reaction :

Equipment 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.

Result: **Pink** color.

➤ **Legal's Reaction:**

Few mls 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 a test for the unsaturated lactone ring of the genin.

2. Reaction due to the steroidal nucleus:

➤ **Lieberman's Sterol Reaction:**

Equipment 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, 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).

3. Reaction due to presence of 2-deoxy sugar

➤ **Keller- Killian's Test**

Equipment & 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. **H₂SO₄** 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 (H₂SO₄ layer). The junction appears as a **reddish – brown** ring.

Identification of cardioactive 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).**

Or **Ethyl acetate: Methanol: Water (81:11:8)**

The standard compound = **Oleandrin**.

The spray reagent = Lieberman's reagent **or** sulphuric acid reagent (5% ethanolic H₂SO₄)

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 °C** for **5-10 min_s** in the oven.
6. Note the spots, and calculate the R_f value for each spot.

Study problems:

Q1. What is the meaning of CATSC, explain with structure?

Q2. Give the reasons for:

- a) The addition of lead sub acetate, sodium phosphate and anhydrous sodium sulphate to the extract?
- b) The use of chloroform: ethanol in partitioning of fraction A?
- c) The use of HCl in the extraction procedure of cardio-active glycosides (Fraction B)?
- d) The use of chloroform alone in partitioning of fraction B?
- e) The use of picric acid in the performance of Baljet's test?
- f) The use of glacial acetic acid in Keller Killian test procedure?
- g) The addition of conc.H₂SO₄ in Keller-Kellian test& Lieberman's sterol reactions?

Q3. How can you identify an extract containing cardio-active glycosides?