The Identification of Piperine Alkaloid By Chromatography (TLC)

Practical pharmacognosy

lab.3

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- By the use of thin layer chromatography (T.L.C)
- The stationary phase = Silica gel G.
- The mobile phase =Toluene: Diethylether: Diaxon (62.5:21.5:16).
- The standard compound =
- The spray reagent =Dragendorff's reagent.
- Mechanism of separation = Adsorption.
- Developing = Ascending.
- Other mobile phases :
- Toluene: Ethyl acetate (70:30), Acetone Water: Ammonia (90:7:3).

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, 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.

Result:

- Orange spot will appear.

Tropane Alkaloids

Datura stramonium



Tropane Alkaloids

- Datura stramonium, known by the common names Jimson weed or datura, is a plant in the Solanaceae (nightshade) family. For centuries, datura has been used as a herbal medicine to relieve asthma symptoms and as an analgesic during surgery or bone setting.
- It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.

Constituents of datura are:

- Hyoscyamine and its isomer atropine, which is formed during extraction procedure. Also it contains **hyoscine** (**scopolamine**) alkaloid, which is found in trace amounts.
- The medicinal use is mostly due to the hyocsyamine (atropine), used as mydriatic, antispasmodic, antidote to the toxicity of cholinergic compound, decrease in the secretion (upper and lower respiratory tract) before surgery. While the use of scopolamine mostly in motion sickness.

Constituents of datura

- The tropane alkaloids (hyocsyamine and hyoscine) have the following structures:
- These alkaloids are also present in other plants as **Hyoscyamus niger** , **Atropa belladona** of the family Solanaceae, and others.
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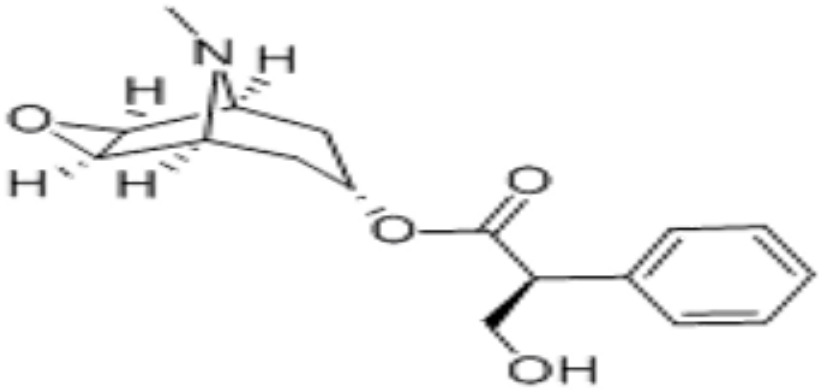
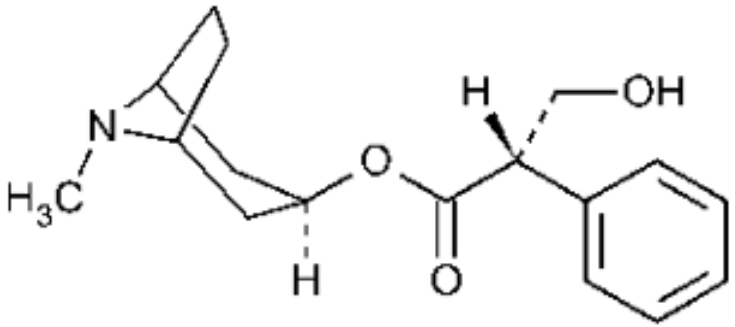


Hyoscine (scopolamine)



Hyoscyamine

Isolation and Identification of the Datura Alkaloids:

- **2.Extraction:**
- Aim: to isolate datura alkaloids.
- **Equipments:**

- Reflex apparatus. Conical flasks.
- Stirrer. Funnel.
- Separatory funnel. Water bath.
- Filter paper. Litmus paepr.

Reagents:

- 90% ethanol.
- 2% HCl.
- Ammonium hydroxide solution.
- Chloroform.

- **Procedure:**
- Method of extraction:.
- Plant used: Datura stramonium.
- Part used: fruits.

Procedure:

Extract ***50 gm*** of the datura fruits in ***150 ml*** of *90%* ***ethanol*** under Reflex condenser for **1 hrs.**

↓

Filtration

↓

Take ***20 ml*** of alc. Extract in conical flask and concentrate on the water bath to about ***2 ml*** to remove all of ethanol

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Pour the concentrated in to ***10 ml*** of ***2% HCl***

↓

Procedure:

Heat gently
(5 min,)

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Cool and filter the Acidic extract and place in a separatory funnel

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[Wash with *5 ml* of ***Chloroform***] two times

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Take supernatant (upper layer) and made alkaline by addition of

↓

Ammonium hydroxide solution (check by litmus paper)

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[Partition with *5 ml* of ***Chloroform***] two times

↓

Take the lower layer, dehydrate by adding ***anhydrous sod.***

Sulphate filter (or decant)., evaporate to dryness

The Identification of Datura Alkaloids:

Qualitative Analysis:

- A. The specific tests for tropane alkaloids:
- 1. Vitalli Marine Bu Test:
- Aim : to identify the tropane alkaloids from other alkaloids.

- Equipments and Reagents:
- Small beaker.
- Fuming nitric acid.
- Alcoholic KOH.

Procedure:

- Take few mls of the extract, add to it drops of fuming nitric acid and evaporate, then add 2 ml of alc. KOH.
- Result:
- A **violet** color will be developed.

2. Gerhard's Test:

- Aim: to identify the tropane alkaloids from other alkaloids.
- Equipment and Reagents:
- Small beaker.
- 2% HgCl2 in 50% aqueous ethanol.

- Procedure:
- Add 2% HgCl2 in 50% aqueous ethanol to 0.0006 g of atropine.
- Result : A deep red color will be developed.

Identification of Datura Alkaloids By Chromatography:

- By the use of thin layer chromatography (T.L.C)
- The stationary phase = Silica gel
- The mobile phase = Butanone: Methanol: Ammonia (60:70:10)
- Or Acetone: Water: Ammonia (90:7:3).
- The standard compound = atropine or hyoscine.
- The spray reagent = Dragendorff's reagent.
- Mechanism of separation = Adsorption.
- Developing = Ascending.

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 and the standard spots on the silica gel plates, on the base line by the use of capillary tube.
- 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 spray with the spraying reagent.

Study problems:

Give the reason of :

- 1. Using the reflux in extraction the tropane alkaloid from datura stramonium.
- 2. Making the evaporation step after filteration?
- 3.Addition of 2% HCl to the alcoholic extract?
- 4. Addition of ammonium hydroxide?
- 5. Using of chloroform in partitioning step?