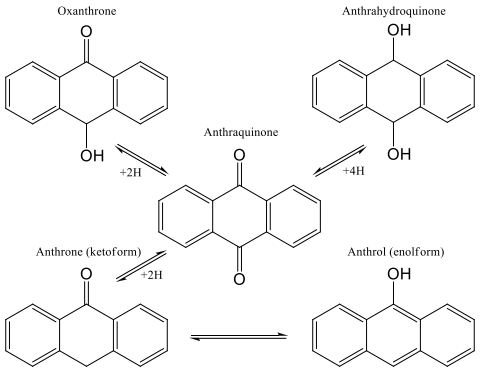
Practice No. 5

ANALYSIS OF DRUGS CONTAINING ANTHRAGLYCOSIDES

Anthraglycosides are glycosides with an aglycone derived from anthracene. The sugar component is mostly L-rhamnose and D-glucose. Extracts from plants containing Anthraquinone glycosides and derivatives have always been used to dye fabrics, in medicine they are used as laxatives.



Anthraquinone derivatives have two or more hydroxyl groups bound to the carbons of the aromatic ring. Hydroxyl hydrogens are partially dissociable and can be replaced with a metal. When reacting with alkaline hydroxides, these compounds create salts – phenols, which are soluble in water, forming intensely red solutions. The Bornträger-reaction, serving as evidence to the presence of anthraglycosides, is based on this principle. Only loose, oxidized aglycones can react.

The reaction is carried out so that the anthraglycosides present in the drug are hydrolyzed for a few minutes in a hot solution of mineral acids or alkali hydroxides. Anthracene derivatives, which are present in the the drug mostly in the form of glycosides are released and the derivatives of anthrone and anthranone are oxidized into anthraquinones. Aglycones are soluble in alkaline aqueous solutions (when they form phenol salts), from which they are displaced with sodium chloride and separated into organic solvents (liquid-liquid extraction). Acidification leads to the suppression of dissociation of the phenolic groups and the resulting lipophilic solutions easily pass into the organic solvents during shaking, which, due to the formation of phenolates, turn cherry red.

Only loose (oxidized) anthraquinones give positive Bornträger-reaction. Anthrones, anthranones, dianthrones and their glycosides don't react. To capture these derivatives with this reaction, compounds need to undergo pre-oxidation or hydrolysis.

The above described method of carrying out the Bornträger-reaction is not suitable for the *Folium sennae*. Slight coloring of the ammonia layer can be caused by the insufficient release and insufficient oxidation of the glycosidically bounded sennosides. Proof of the presence of these compounds, that are derived from dianthrones, is performed only after their preoxidation.

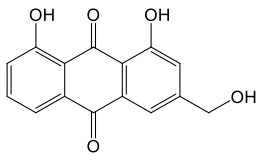
Bornträger-reaction can be used also for microchemical evidence of anthraglycosides in plant tissues. Slices are exposed to the ammonia vapor or they are inserted into a diluted lye solution. The resulting color is sometimes not pure red, but rather reddish, which is caused by the simultaneously present tannins.

Microsublimation can also be used as an evidence of the presence anthraglycosides. By microsublimating the powdered drug, crystals of anthraquinones derivatives are formed, which are dyed red by alkali hydroxide solution. Glycosides, present in the drugs, are partially hydrolyzed at higher temperatures, and at the same time, anthrones and anthranones are oxidized into athraquinone derivatives. These are identified in the sublimate with above mentioned Bornträger-reaction.

Aloe capensis, Aloe barbadensis

Concentrated juice from the leaves of *Aloe* species, especially *Aloe ferox, Liliaceae, Aloe barbadensis, Liliaceae*

Content compounds: anthraquinone derivatives: aloin, aloinoside A & B, aloe-emodin etc.



Aloe-emodin

<u>Identification</u>

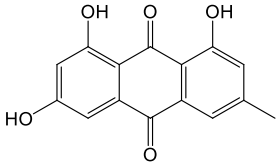
1- 0.1 g of the drug is boiled with 10 ml of water and then 0.1 g of talc is added to the solution and is filtered. 0.25 g of sodium tetraborate is added to 10 ml of the filtrate and is heated until dissolution. 2 ml of this solution are mixed with 20 ml of water. The solution fluoresceinates yellow-green, the fluorescence is particularly strong under UV light at 355 nm.

2- Add a few drops of nitric acid on a small fragment of the drug, within 3 minutes there will be only greenish, not reddish (other species of aloe) color around the fragment.

Frangulae cortex

Dried bark or debris from the trunks and branches of *Rhamnus frangula* L. (=*Frangula alnus* MILL.), *Rhamnaceae*

Content compounds: a mixture of anthraquinones (glucofrangulin A and B, frangulin, frangula-emodin, chrysophanol), tannins, bitter principles, saponins.



frangula-emodin

Identification

1- 1 drop of diluted sodium hydroxide solution is added to the inner part of the bark, which turns dark red. (ČL2002)

2- Approximately 0.5 g of the powdered drug is heated with 25 ml of diluted hydrochloric acid II and the mixture is heated for 15 minutes in a water bath. After cooling the mixture is filtered and the filtrate is shaken out with 20 ml of ether. The aqueous layer is removed. The ether layer is shaken out with 10 ml of diluted ammonia. The aqueous layer turns purple (anthraquinones). (ČL2002)

Diluted hydrochloric acid II: (0.37 g/l HCl) - 1 ml diluted hydrochloric acid I (according to Rhei radix procedure) is diluted with water to 200,0 ml.

Diluted ammonia: (100-104 g/l) - 41 g 26% NH_3 is diluted with water to 100 ml.

3- Powdered drug produces yellow sublimate by microsublimation at 150 – 160°C, which turn red after humidification with 1 drop of diluted potassium hydroxide solution.

4- The other part of microsublimate is moistened by the solution of ferric chloride in alcohol. A green tint is created.

5- Chromatographic analysis of anthraquinone derivatives

Test solution: A mixture of 0.500 g of the powdered drug and 5 ml of 70% alcohol is heated until boiling. After cooling, it's centrifuged and the clear top layer is separated and used for further testing within 30 minutes. (ethanolic extract of the drug)

Elution mixture: ethyl acelate : methanol : water (100 : 17 : 13)

Reference solution: aloin solution, frangula-emodin solution

Three solutions of substances are applied to a thin layer of silica gel in this order:

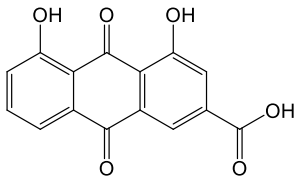
1. ethanolic extract of the drug,
2. aloin solution
3. frangula-emodin solution

Chromatogram is eluted for the distance of 10 cm. The layer is dried for 5 minutes at room temperature, then is sprayed with KOH solution in 50% alcohol (50 g/l) and is dried for 15 minutes at 100 – 105°C. It is observed under UV light at 365 nm. A brown-yellow spot corresponding to aloin is evident in the central part of the chromatogram. There is no stain fluorescing intensely yellow or orange to reddish which would correspond to aloin according to the RF on the chromatogram. There are still 2 or 3 red spots (frangulin A and B) visible between the spots of aloin and frangula-emodin and there are several orange-brown stains, of which the most significant are the 2 in the area ranging between 0.25-0.35.

Rhei radix

Whole or cut dried roots and rhizomes of species *Rheum palmatum* L.*, Rheum officinale, Polygonaceae,* or their hybrids or a mixture

Content compounds: Anthraquinone derivatives (chrysophanol, aloe-emodin, rhein, glucorhein, palmidin A and B and others), tannins, starch, calcium oxalate, flavonoids, organic acids, mineral salts, pectin and sterols



Rhein

Identification

1- Approximately 0.5 g of the powdered drug is heated with 25 ml of diluted hydrochloric acid II and the mixture is heated for 15 minutes in water bath. After cooling the mixture is filtered and the filtrate is shaken out with 20 ml of ether in separately funnel. The aqueous layer is removed. The ether layer is shaken out with 10 ml of diluted ammonia. The aqueous layer turns purple (anthraquinones).

Diluted hydrochloric acid II: (0.37 g/l HCl) - 1,0 ml diluted hydrochloric acid I (viz Rhei radix) is diluted with water to 200,0 ml.

Diluted ammonia: (100-104 g/l) - 41 g 26% NH_3 is diluted with water to 100 ml.

2- Yellow sublimate consisting of droplets or acicular crystals, which changes into a red color (anthraquinone derivatives) in a drop of diluted potassium hydroxide solution, appears when microsublimating at 140 – 160°C.

3- 10 ml of sodium hydroxide (0.1 mol/l) are added to approximately 0.1 g of the powdered drug and the mixture is boiled and then filtered after cooling. The filtrate is slightly acidified with diluted hydrochloric acid and shaken out with 10 ml of ether in separately funnel. Separated ether layer is shaken out with 5 ml of diluted ammonia solution, the aqueous layer stains in a wine-color (emodin), the ether layer remains yellow (chrysophanic acid).

4- Chromatographic analysis of anthraquinone derivatives

Test solution: 50 mg of powdered drug is mixed with diluted HCl (1 ml of 35% HCl and 30 ml of water) and heated for 15 minutes in a water bath. It is shaken out with 25 ml of ether in the separately funnel after cooling. The ether layer is dried with anhydrous sodium sulfate and then is filtered again. The filtrate is evaporated to dryness, the residue is dissolved in 0.5 ml of ether.

Reference solution*:* solution of emodin and 1,8-dihydroxyanthrachinon

Developing mixture: anhydrous formic acid : ethyl acetate : petroleum ether (1 : 25 : 75)

Detection reagent: KOH in methanol (100 g/l)

20 µl of solutions are applied on the plate and eluted for a distance of 10 cm. The layer is then dried at room temperature and observed under UV at 365 nm. An orange fluorescing stain (emodin) appears in the central part of the reference solution chromatogram. There is a spot corresponding to the spot of the reference solution chromatogram in the chromatogram of the tested solution, according to RF and color. There are two fluorescing orange spots (in ascending order of RF – physcion, chrysophanol) above the emodin stain and two more

orange fluorescing spots (in descending order of R_F – rhein, aloe-emodin) under the emodin stain. The layer is sprayed with KOH solution – all of the stains turn red to violet.

Chrysarobinum

purified extract from the *Andira araroba, Fabaceae* tree cavities

Content compounds: a mixture of anthracene derivatives, especially anthranones and anthrones, where the majority constitutes chrysophanol anthranone and chrysophanol anthrone

Identification

1- 0.01 g is boiled with 5 ml of water and 5 drops of diluted solution of potassium hydroxide. After cooling, the substance is filtered and the filtrate is then slightly acidified with concentrated HCl. Then the filtrate is shaken out with 10 ml of benzene in separately funnel. 5 ml of the separated benzene layer is shaken out with a mixture of 2 ml of water and 1 ml of the diluted solution of potassium hydroxide. The upper benzene layer turns yellow (chrysophanic acid), the bottom aqueous layer is stained red (anthracene derivatives)
2- 0.005 g is dissolved in 5 ml of concentrated sulfuric acid, the resulting crimson solution is poured into 50 ml of water, chrysarobin is precipitated as an orange-yellow precipitate.
3- About 2 mg are mixed with 2 drops of fuming nitric acid. The russet mixture turns purple-red by adding couple drops of diluted ammonia solution.

Sennae folium

dried leaves of *Cassia senna* L. *(=C. acutifolia), Cassia angustifolia* DELILE, *Caesalpiniaceae* species

Content compounds: dianthrone glycosides, especially sennosides A, B, C and D, small portion of anthraquinone glycosides, such as aloe-emodin-glucoside, mucilages and flavonoids.

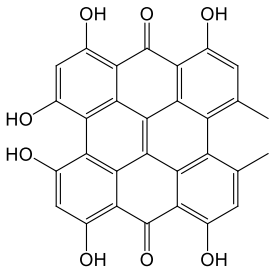
Identification

Microsublimation at 160 – 180 °C, the resulting sublimate turns red (anthraquinone derivatives)

Hyperici herba

Dried leaves of the *Hypericum perforatum* L*, Hypericaceae* species

Content compounds: dianthrone derivatives (hypericin, pseudohypericin), flavonoids, hyperforin



Hypericin

Determination of the content of hypericin in the drug:

Principle: Hypericin is extracted from the drug by polar solvents. The determination of the content of hypericin itself is performed spectrophotometrically.
Procedure: 1 g of the dried drug is extracted using Soxhlet apparatus by chloroform until colorless chloroform is trickling down. The fluid is removed and the drug is dried freely in the air stream. The same drug is extracted with acetone again, until colorless acetone is trickling down. The acetone is evaporated to dryness on a vacuum evaporator, the residue is dissolved in methanol, and transferred to a volumetric flask and diluted with methanol to 25 ml. The liquid is filtered and the first 2 ml of the filtrate are removed. 5 ml of filtrate is placed to volumetric flask and filled with methanol to 25 ml. The absorbance of the solution is measured at 590 nm using a spectrophotometer, methanol is used as the blank solution.
The content of hypericin is calculated in percentage as follows:

$$\frac{A \times 0.174}{m}$$

A – absorbance of the tested solution at 590 nm

m – weight of the drug in grams