MACROMORPHOLOGICAL INVESTIGATIONS

Frangulae cortex–Frangula bark Ph. Eur.

Frangula alnus Mill. (Rhamnaceae)

The bark occurs in curved, almost flat or rolled fragments; the outer surface is greyish-brown or dark brown and covered with numerous greyish, transversely elongated lenticels. The orange-brown or reddish-brown inner surface becomes red when treated with alkali. The fracture is short, fibrous in the inner part.

Rhei radix–Rhubarb Ph. Eur.

Rheum palmatum L. *var. tanguticum*; *Rheum officinale* Baill. (Polygonaceae)

The crude drug consists of the peeled rhizome. Its color is brownish-red, the surface is covered with a layerof brownish-yellow powder. It shows a reticulum of darker lines. The fractureis granular.

Sennae folium – Senna leaf Ph. Eur.

Cassia angustifolia Vahl.; *C. senna* L. (Leguminosae)

Greyish-green or brownish-green, thin, fragile leaflets, lanceolate, mucronate, asymmetrical at the base and fine at the peak. Pinnate venation is visible mainly on the lower surface, with lateral veins.

Sennae fructus angustifoliae – Senna pods, Tinnevelly Ph. Eur.

Cassia angustifolia Vahl.; *C. senna* L. (Leguminosae)

Flattened, slightly reniform pods, brown with dark brown patches at the positions corresponding to the seeds. At one end is a stylar point and at the other a short stalk. The pods contain 5-8 flattened and obovate seeds, with incomplete, wavy, transverse ridges on the testa.

Aloe capensis – Aloe,cape Ph. Eur.

Aloe ferox Mill. (Xanthorrhoeaceae)

Dark brown masses tinged with green and having a shiny conchoidal fracture, or greenish-brown powder.

Aloe barbadensis–Aloe, barbados Ph. Eur.

Aloe barbadensis Mill. (Xanthorrhoeaceae)

Dark brown masses, slightly shiny or opaque with a conchoidal fracture, or brown powder.

Hyperici herba –St. John's wort Ph. Eur.

Hypericum perforatum L. (Hypericaceae)

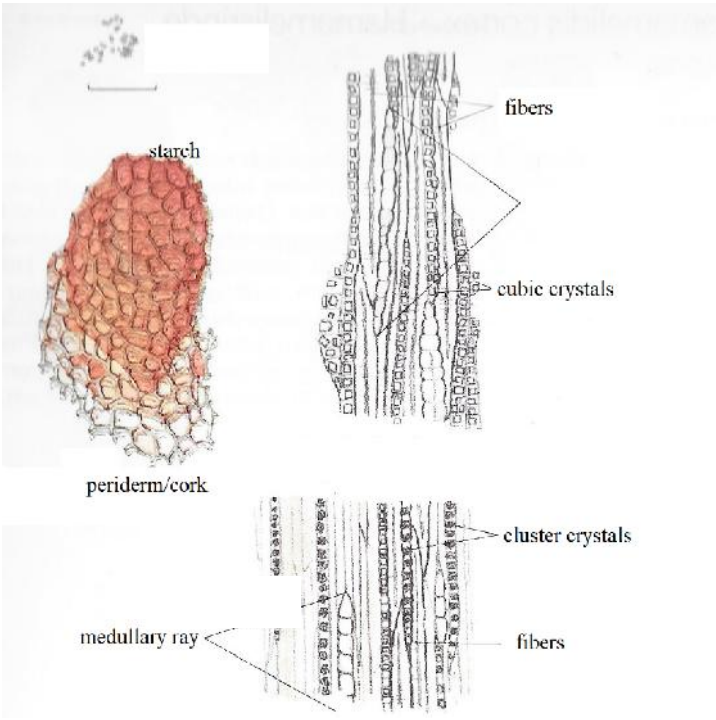
Leaves are opposite, ovate-elliptic with clearly visible translucent oil-dots on the surface. Flowers are bright yellow, with 5 yellow petals and numerous stamens. Yellowish green pieces of stem are hollow and often have two longitudinal ridges opposite each other.

Rhamni purshianae cortex – Cascara bark Ph. Eur.

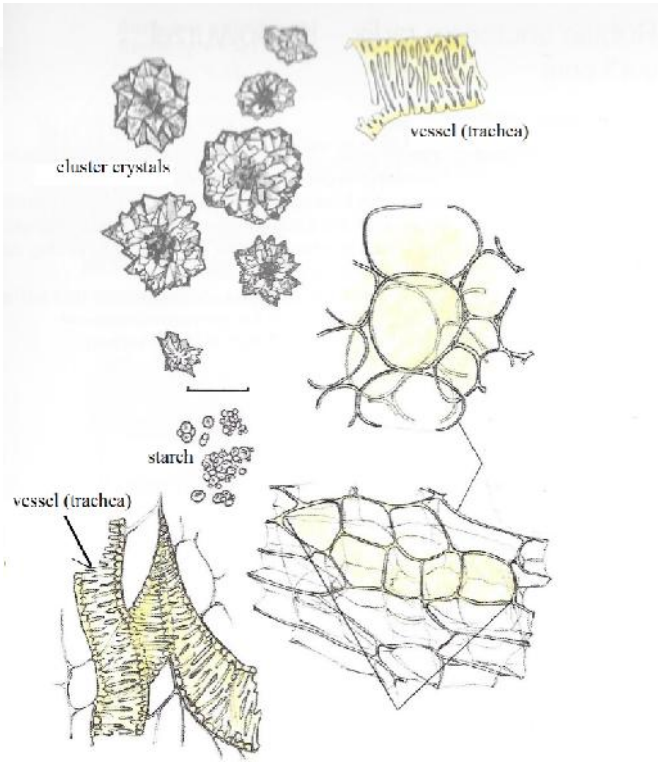
Rhamnus purshiana D.C. (Rhamnaceae)

The bark occurs in slightly channelled or nearly flat pieces. The outer surface is grey or dark greyish-brown and shows occasional lenticels. It is usually more or less completely covered by a whitish coat of lichens, epiphytic moss and foliaceous liverwort. The inner surface is yellow or reddish-brown or almost black with fine longitudinal striations; it turns red when treated with alkali. The yellow fracture is short and granular in the outer part and somewhat fibrous in the inner part.

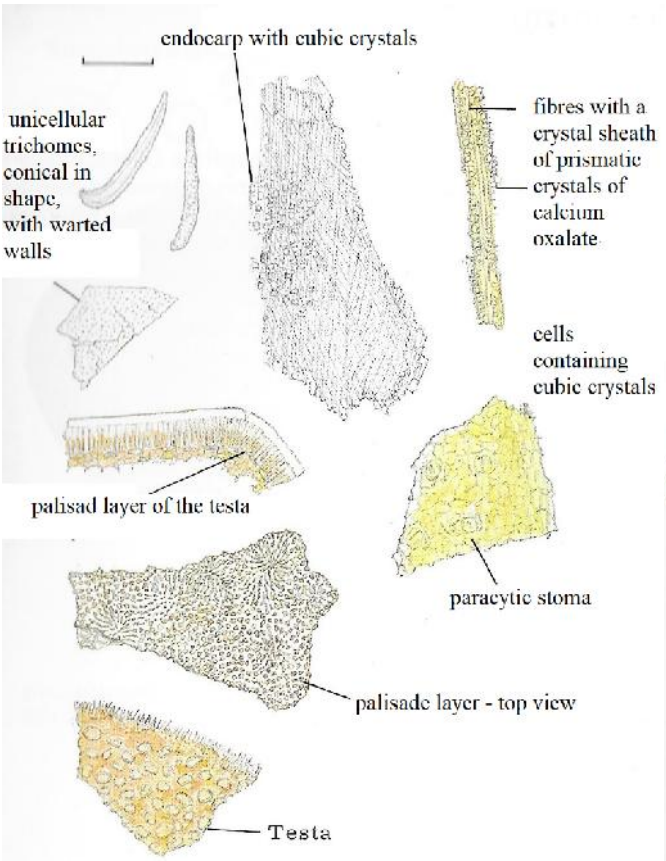
MICROSCOPICAL INVESTIGATIONS



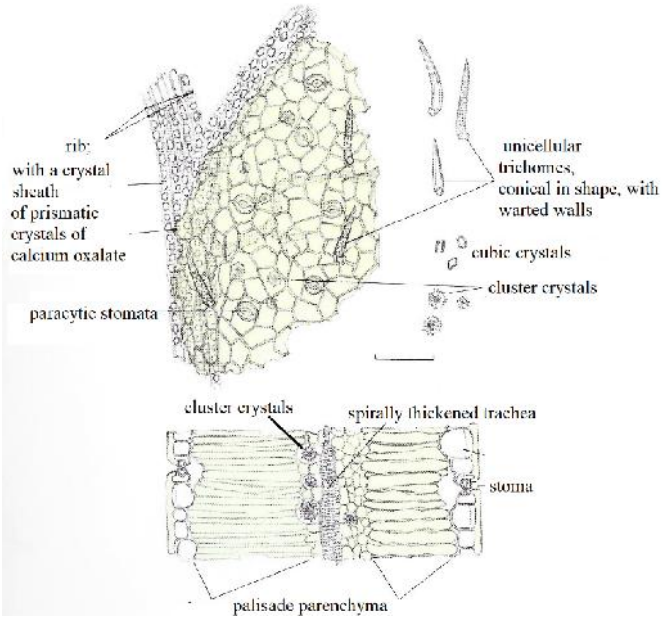
Frangulae cortex



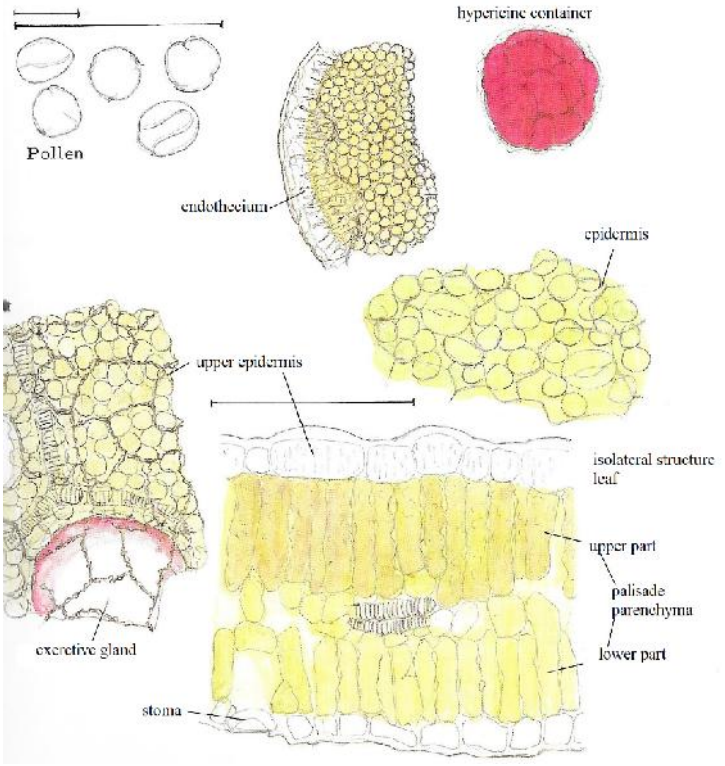
Rhei radix



Sennae folium



Sennae fructus



Hypericiherba

CHEMICALSTUDIES

1. Identification of anthraquinone derivatives by simplereactions

Frangulae cortex, Rhamni purshianae cortex

With a drop of R-NaOH, the inner surface takes on a red colour (Bornträger reaction).

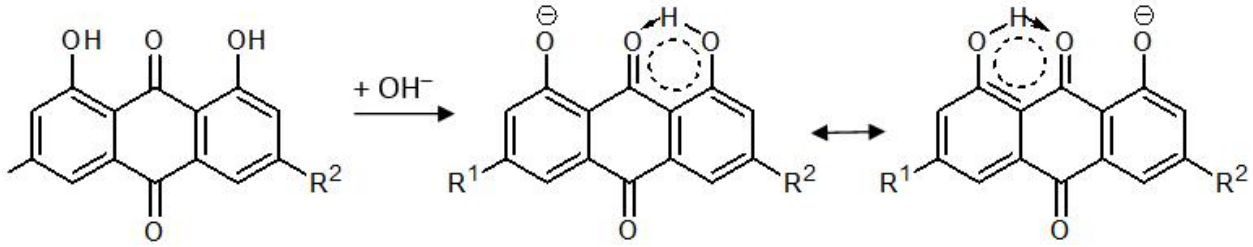
2. Identification of emodines

2.1. Bornträger-reaction

Frangulae cortex, Rhei radix, Sennae folium, Sennae fructus

Extract 0.5 g of powdered drug with 5 ml $CHCl_3$ for 5 min by shaking.After filtration add 5 ml of R-ammonia solution and mix it. The water- phase (upper phase) will turn bright red.

The free anthraquinone derivatives (aglycones = emodins = 1,8-dihydroxy-anthraquinone derivates) are soluble in $CHCl_3$. After re-extraction with alkali solution they turn to red due to the mesomeric structures of phenolates.



2.2. Detection of free anthraqionones, anthraquinone-*O*-glycosides and *C*-glycosides with Bornträger-reaction

Rhamni purshianae cortex, Frangulae cortex, Sennae folium

1 g crude drug powder is extracted with 10 ml chloroform, shaking a few times. Filter the extract, put the drug into a porcelaine dish and evaporate the solvent on water-bath, under the hood.Detect the anthraqinone content of the chloroformic extract adding 1 ml R ammoniac solution, after shaking (1).

Add 10 ml R HCl solution to the drug powder and heat for 10 min. on water-bath.After cooling, filter into separatory funnel and shake with 2×5 ml chloroform. Pay attention to the emulsification during the first shaking! Detect the anthraquinone content of the chloroformic layer, adding 1 ml R NH_3 solution and shaking (2).

Add 5 ml Fe(III)-chloride solution to the wateric phase and heat for 5 min. on water-bath (100°C).Filter cool down, put into a separatory funnel, and shake with 2×5 ml chloroform. Pay

attention to the emulsification during the first shaking! Detect the anthraquinone content of the chloroformic layer, adding 1 ml R NH_3 solution and shaking (3).

Explanation: 1) First, we detect the free aglycones..

2) Heating in acidic condition, O-glycosides are hydrolised and we get aglycones

3) C-glycosides also hydrolyse adding oxidiser (FeIII).

2.3. Preparative layerchromatography: Isolation of frangula-emodin, purification, UV-VIS and MS spectra.

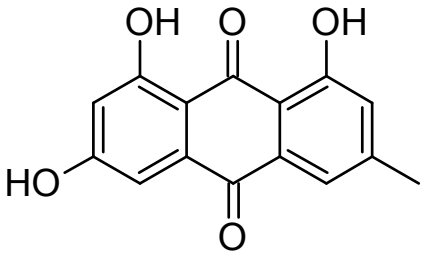
l.0 g powder of air-dry drug is heated in a 100 ml spherical flask equipped with a reflux condenser with 10 ml of R-hydrochloric acid for 20 min. on a hot water bath. Add through the condenser 50 ml of ethyl-acetateand boil for 30 min. Filter the mixture through a cotton plug into a 250 ml separatory funnel, wash the flask and the cotton with 2 x 10 ml of ethyl-acetate. Separate the ethyl-acetate layer, dry over Na_2SO_4 sicc. and evaporate the solvent. The residue is dissolved in methanol (2 x 1.0 ml) and put into a test tube (little one).

150 µl of this solution is used for layer chromatography. (12 cm bands on silica gel G). Solvent system: chloroform-ethylacetate (93:7) *Reference solution*: frangulaemodin.

After evaporation of the solvent the band (orange yellow) corresponding to the reference frangula emodin (≈ R_f = 0.4) is scraped and eluted with chloroform- methanol (2:1) solvent (20 ml) by simple shaking. After filtration the solvent is evaporated under reduced pressure and the residue dissolved in 2×1 ml methanol. Dilute (~ 1:100) with methanol. Identity is proved by spectroscopy (200-600 mm). 1 ml is used for the mass spectrometric study.

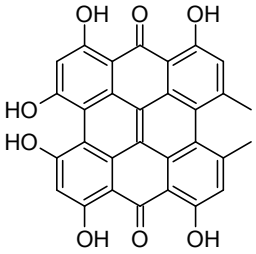
Spectral characteristics: λ_{max}: 224, 253, 268, 290, 442 nm

λ_{min}: 350



frangula emodin

3. Identification of hypericin (dianthrone) in *Hyperici herba*.



hipericin

0.2 g powered drug gives a green colour with 1 ml of 2 % potassium hydroxide.

QUANTITATIVE DETERMINATIONS

4. Determination of anthraglycosides (Ph.Eur.)

Sennae folium, Sennae fructus

Carry out the assay protected from bright light.

Place 0.300 g of the powdered drug in a 100 ml Erlenmeyer flask. Add 30.0 ml of water (1) and place in the water-bath. Heat under a reflux condenser for 15 min. Allow to cool, put in a 50 ml cylinder and adjust to the original volume if needed, with water. Transfer 20.0 ml of the supernatant liquid to a 50 ml separating funnel.

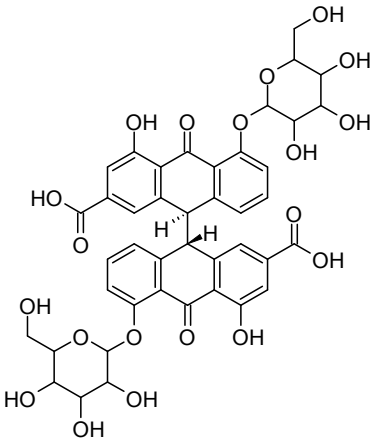
Add 0.10 ml of *2M hydrochloric acid* and shake with three quantities of *chloroform*, each of 15 ml. Allow to separate and discard the chloroform layer (2). Add 0.10 g of *sodium hydrogen carbonate* (pH 7-8) and shake for 3 min. Let it sediment and transfer 10.0 ml of the supernatant liquid to a 100 ml round- bottomed flask with a ground-glass neck. Add 20 ml of 0.4 M *ferric chloride solution* and mix. Heat for 20 min. under a reflux condenser in water-bath; add 1 ml of *hydrochloric acid* and heat for a further 20 min., with frequent shaking, to dissolve the precipitate (3).

Cool, transfer the mixture to a separating funnel and shake with three quantitiesof 25 ml*ethyl-acetate*previously used to rinse the flask (4). Combine the *ethyl-acetate*layers and wash with two quantities, each of 15 ml, of *water*. Transfer the *ethyl-acetate*layers to a volumetric flask and dilute to 100.0 ml. Evaporate 10.0 ml carefully to dryness and dissolve the residue in 10.0 ml of a 5 g/l solution of *magnesium acetate/methanol.* Measure the absorbance at 515 nm using *methanol* as the compensation liquid (5).

Calculate the percentage content of anthraglycosides expressed in sennoside B.

Note: Ph.Eur. use this method for the quantitative determination of Frangulae cortex and Rhei radix.

specific absorbance: $A^{1\%}_{1cm,\ sennoside\ B} = 240$.



sennoside B

1. Sennosides (dianthrone-glycosides) are water soluble glycosides due to -COOHgroup+ sugar moieties

2. To remove the free aglycones and the semipolarsubstances (the aim is to determine glycosides)

3. Due to the –C-C- bond of dianthrones, oxidative hydrolyses isneeded.

4. The free aglycones are solulbe in apolar solvents, e.g. ethyl acetate, chloroform. Note: in Ph.Eur. ether is used for the extraction.

5. Mg-complex isformed. Color is proportional with the concentration

3.1 Determination of anthraglycosides and anthraquinonederivatives

Frangulae cortex, Rhei radix

Heat 0.05 g powder of air-dry drug accurately weighed, in a 100 ml spherical flask equipped with a reflux condenser with 6 ml of R-concentrated acetic acidfor15minutes on a hot water bath (1). Add through the condenser 30 ml of ethyl-acetateto the cooled liquid and boil for 15 minutes (2). Filter the mixture through a cotton plug into a 250 ml separatory funnel, wash the flask and the cotton with 2 x 5 ml of ethyl-acetate. Return the cotton into the flask and boil again with a mixture of 2 ml of concentrated R-acetic acid and 30 ml of ethyl-acetatefor further 10 minutes. Filter the ethyl-acetate extract through a new cotton plug in the separatory funnel used before. Wash the flask and the cotton again with 2 x 5 ml of ethyl-acetate. Cool it,add 15 ml of 30 per cent sodium hydroxide solution and 25 ml of ammoniacal sodium hydroxide solution (82 ml of sodium hydroxide solution + 18 cc. NH_3 solution of 25 per cent ammonia) to the combined chloroform extract (3). Shake the separatory funnel while cooling under the water tap. Transfer the red-coloured alkaline layer after 5 minutes of standstill into a 100 ml volumetric flask. Extract the ethyl-acetate fraction with 2 x 20 ml of ammoniac sodium hydroxide solution, allow to stand for 5 minutes and transfer the alkaline layer into the volumetric flask. Heat the volumetric flask for 20 minutes on hot water bath, and make up to 100.0 ml with cc. ammonia aftercooling.

Measure the extinction of the solution at 530 mm in a 1 cm cell, against water as the blank. Calculate the total contents of anthraquinone and anthranol derivatives expressed in 1,8-dihydroxy-anthraquinone:

$m/m\ \%_{total\ anthraquinone\ and\ anthranol\ derivatives} = A / (4{,}72 \times m)$

m = g of drug (≈ 0.05)

A = absorbance

Explanation: 1. We determine both glycosides and aglycones in aglycone form (emodin), glycosides are hydrolysed by acidic treatment.

2.Emodins are soluble in apolar solvents, like ethyl-acette.

3. Bornträger-reaction.

Note: Anthraglycosides and anthraquinone derivatives are soluble in organic solvents and in basic conditions in water due to the phenolate structure. They can be extracted from organic solvents into alkalic water; from alcalic water – after making the conditions acidic - , with organic solvent. Anthraglycosides are soluble in polar organic solvents; their aglycons (emodins) in semipolar organic solvent. Sennosides are soluble in water due to theircarboxyl group.

4.3. Calculation of anthraglycosid-doses based on the results ofassay (anthraglycoside + anthraquinone determination 4.2.)

Anthraglycoside-containing drugs are stimulant **laxatives**; as they act directly on the intestinal mucosa by influencing several pharmacological targets. The long-term use or extreme doses of anthranoids may result in a (reversible) blackening of the colon (*Pseudomelanosis coli*), which is due to the incorporation of metabolites of the anthranoids. It is thought to be associated with an increased risk of colon carcinoma.

The recommended doses of hydroxyanthracene derivatives is **10 mg/day**, for **maximum of 2 weeks**. After 2-3 months the cure can be repeated.

What are the recommended **onefold** and **daily**doses of *Frangulae cortex* or *Rhei radix*(4.2. assay), if consumed 3 times a day?

Results in report:

1. Evaluation of test tube reactions
2. Anthraglycoside content
3. Anthraglycoside and anthraquinone derivative content
4. UV-VIS and MS data of isolated frangula-emodin
5. Calculation of doses (maximal dose/one time, and maximal dose/day) based ont he results of assay.