Practice No.12

ANALYSIS OF DRUGS CONTAINING BITTER COMPOUNDS AND BITTER GLYCOSIDES

Bitter compounds or amara form a biogenetic and structurally diverse group of natural substances whose common property is the bitter taste. Names are often derived from the Greek πικροσ = bitter. In the past, bitter mixtures were widely used to increase appetite. Bitter substances act on taste receptors in the mouth and so they psychologically stimulate the secretion of gastric juices.

We can divide them structurally into several groups – iridoids and secoiridoids bitter compounds (aucubin, loganin, gencianin, genciopicrin, amarogenin, harpagoside...), sesquiterpens (absithin, cynarin...), diterpens (picrosalvin...), triterpens (quassiin, cucurbitacin...), steroids (condurangins...), non-terpenoid bitter compounds (neohesperidin, naringin, humulon, lupulon...).

Content of bitter compounds in the drugs or in mixtures is established by the taste test "determination of bitterness".

Determination of bitterness:

Degree of bitterness is defined as a reciprocal value of the diluted water extract of the drug or mixture which still tastes bitter. For comparison, we use chininium chloride solution, its number is 200 000.

Taste test is provided so that the person, who makes the test, washes his mouth by water heated to 20 °C, and then tastes 10 ml of the investigated solution for 10 seconds until the solution comes in contact with the surface of the tongue. Then the mouth is washed by water again to eliminate the bitter taste. Next solution is possible to be tested after 15 minutes.

Procedure

a) Determination of the sensitivity to bitterness

Because the taste is strongly influenced by the subjective perception of people, it is necessary to provide a personal orientational self-testing to determine the sensitivity before testing the bitterness.

Solution A: 0.0100 g of chininium chloride is dissolved in a 500 ml volumetric flask and it is completed by water to the mark.

Solution B: 100.0 ml of solution A are put in a 200 ml volumetric flask and it is completed by water.

It starts with solution B and if the investigator doesn't mark that solution as clearly bitter, it is continued with solution A. If the investigator doesn't mark solution A as bitter, they are excluded because they're not able to determine bitterness because of low sensitivity.

b) Self-determination of bitterness

Investigated solution: 1 g of the powdered drug is poured into 1000 ml of boiling water and is heated with continuous stirring in a water bath for 30 minutes. After cooling down, it is

completed to 1000 ml, mixed thoroughly and then filtered. The first 20 ml of filtrate are excluded.

Reference solution: 0.100 g chininium chloride is dissolved in water and diluted to 100 ml by water. 1 ml of this solution is diluted with water to 100 ml.

Series of the reference solutions are prepared so that in the first flask are 4.2 ml of basic chininium chloride solution and in each subsequent tube, the volume of the solution increases by 0.2 ml up to 5.8 ml. The volume of each tube is completed to 10 ml by water. The lowest concentration is determined so that the solution still tastes bitter. 10 ml of the least concentrated solution are rolled in the mouth for 30 seconds to that solution comes into contact with the tongue. If the solution does not taste bitter, it is spit out and the mouth is washed with water after 1 min. After 10 minutes, the next solution with higher concentration is tested in the same way

Correction factor is to be calculated as follows:

$$\frac{5{,}00}{n}$$

where:

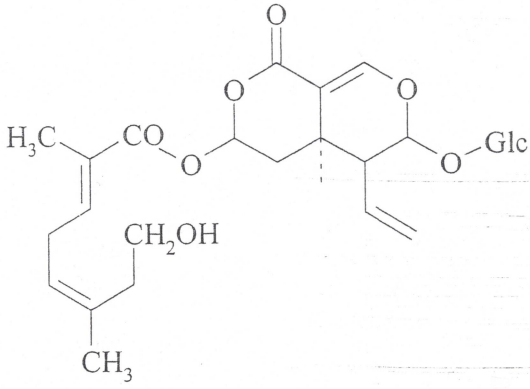
n – volume in ml of the basic solution of chininium chloride, which still tastes bitter.

$10/k$ ml of tested solution is diluted to 100 ml by water (individual to each drug). 10,0 ml of that solution tastes bitter.

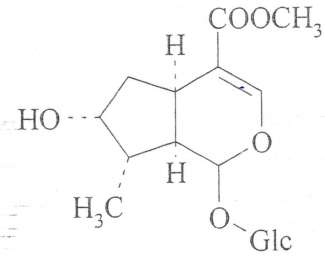
Trifolii fibrini folium

Menyanthes trifoliata, Menyathaceae

Content compounds: bitter compounds - secoiridoids glycosids (foliamenthin, menthiafolin, sweroside), iridoids glycoside loganin, tannins, flavonoids (rutin)



foliamenthin



loganin

Identification

1- Thin-layer chromatography

Tested solution: 1 g of the powdered drug is mixed with 10 ml of methanol and it is heated at 60 °C in a water bath under reflux condenser for 5 minutes and filtered after cooling down.

Elution mixture: water : methanol : ethylacetate (8 : 15 : 77)

Detection: To 100 ml of the vanillin solution in ethanol 96% (10 g/l), 2 ml of concentrated sulfuric acid are added dropwise carefully. It can be used fort 48 hours.

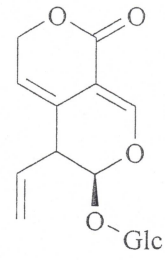
Apply 20 µl of tested solution on the TLC layer and it is eluted for a distance of 12 cm. The layer is dried at room temperature and it is sprayed by detection reagent. It is dried at 100-105 °C for 5-10 minutes. On the chromatogram of the tested solution, there are visible intense purple to dark blue spots (foliamenthin, menthiafolin, dehydrofoliamenthin). There can be other visible spots.

2- Determination of bitterness

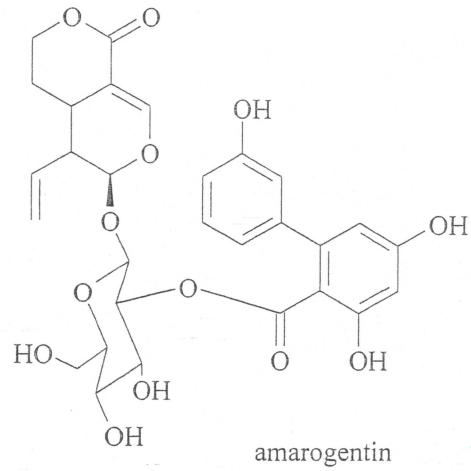
Gentinae radix

Genus *Gentiana* especially *Gentiana lutea, Gentianaceae*

Content compounds: bitter glycosides (especially secoiridoids gentiopicroside, amarogentin) alkaloids (gentianine, gentialutine), yellow pigments (xanthones gentisin, isogentisin, gentioside...), sugars (trisaccharides gentianose, disaccharides gentiobiose and sucrose), pectin, fats



gentiopicroside



amarogentin

Identification

1- By microsublimation of the powdered drug, light yellow needles and crystals are formed, which are dissolved in a drop of 6.5% KOH solution to form a yellow, not red, solution (opposite to roots of *Rumex*).

2- Thin-layer chromatography

Tested solution: 2 g of the powdered drug is shaken with 50 ml of methanol for 20 minutes and then filtered quickly to eliminate the solvent loss. 25 ml of the filtrate is dried using vacuum evaporator at a temperature lower than 50 °C. The residue is dissolved in a small volume of methanol to get 5 ml of the solution. Some precipitation can be found in the solution.

Reference solution: 0.05% rutin solution in methanol

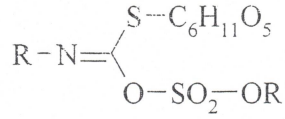
Elution mixture: water : methanol : ethylacetate (8 : 15 : 77)

Detection: To 100 ml of the vanillin solution in ethanol 96% (10 g/l), 2 ml of concentrated sulfuric acid are added dropwise carefully. It can be used fort 48 hours.

ANALYSIS OF DRUGS CONTAINING THIOGLYCOSIDES

Thioglycosides or glucosinolates form a group of glycosides, where the molecule of sugar is bound through a sulfur atom to the aglycone. They are found in plants from the families *Brassicaceae*, *Resedaceae*, *Capparidaceae*, and *Tropeolaceae*. Their hydrolytic products – isothiocyanates – have characteristic pungent odor and adverse effects on the digestive tract; also can cause symptoms of goiter.

The basic structure of these compounds



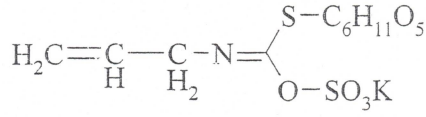
where R is an aliphatic aromatic substituent and R´ is an alkali metal or ester-bound phenolic derivate. Biosynthesis of the thioglycosides is from aminoacids.

Sinapis semen

Brassica nigra, Brassicaceae

Content compounds: oils, glucosinolate sinigrin, mustard essential oils, proteins, mucilage, step of hydrosulfate sinapine

Only artificial mustard essential oil is used according to ČL97 (Sinapis etheroleum artificale)



sinigrin

Identification

1- 0.5 g of the powdered seeds are crushed with 1 ml of water in a bowl, after a minute, mustard essential oil smells

2- 1 g of the powdered seeds is slightly heated with 1.5 ml of water. After cooling down, the extract is shaken with 10 ml of ether. The ether layer is filtered and the filtrate is dried on a water bath. Ethanolic ammoniacal $AgNO_3$ solution is added to the residue. The solution darkens after a while by the excluded silver sulphide.

Test of purity

1- Water macerate of the drug (1 : 10) with 15 drops of Millon reagent cannot change the color to red (sinalbin).

2- Decoction of the drug cannot be colored to blue by the addition of a few drops of iodine solution (starch).

Identification

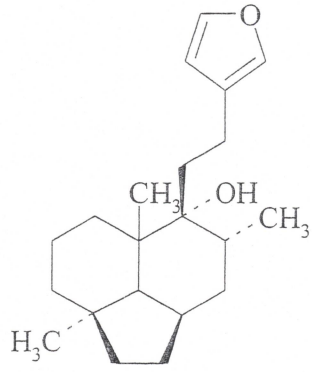
1- Cca 0.3 g of the powdered drug (IV) are shaken with 15 ml of chloroform in a ground-glass flask for 5 minutes and the extract is filtered over cotton wool. The filtrate is dried in a water bath to 1 ml and 5 ml of dimethylaminobenzaldehyde solution in acetic and phosphoric acid are added and the mixture is heated for 5 minutes in a water bath. The color of the solution is changed into blue-green to dark green. (artabsin). (ČsL 4)

2- Determination of bitterness

Marrubii herba

Marrubium vulgare, Lamiaceae

Content compounds: diterpenic bitter substances (marrubiin, marrubiol, marrubenol), tannins, essential oils, saponins, mucilage



marrubiin

Identification

1- Thin-layer chromatography

Tested solution: 1 g of the powdered drug is mixed with 10 ml of methanol and it is heated at 60 °C in a water bath under reflux condenser for 10 minutes and it is filtered after cooling down.

Elution mixture: methanol : ~~chloroform~~ TOLUEN (5 : 95)

Detection: vanillin reagent

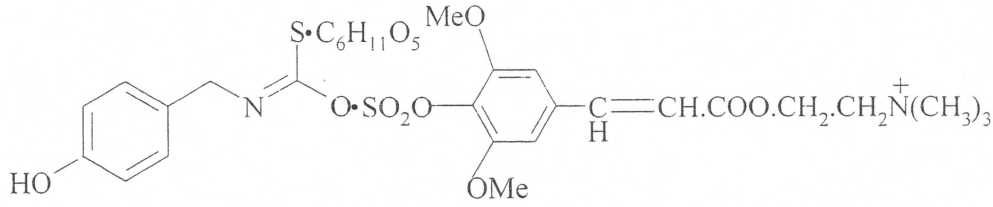
Apply 20 µl of tested solution on the TLC layer and it is eluted for a distance of 12 cm. The layer is dried at room temperature and it is sprayed by detection reagent. It is dried at 100-105 °C for 5-10 minutes. On the middle part of the chromatogram, there is a visible intense purple spot (premarrubiin). There can be other less visible spots.

2- Determination of bitterness

Sinapis albae semen

Sinapis alba, Brassicaceae

Content compounds: glucosinolate sinalbin, oil, proteins



sinalbin

Identification

1- Water macerate of the drug (1 : 10) is filtered and liquid-liquid extraction with petrolether is performed. Petroleum ether is evaporated at room temperature and the residue gives a red color with Millon reagent upon slight heating (sinalbin).

2- Section of the drug or powder is colored by conc. nitric acid into yellow-red (sinalbin).

3- Section of the drug or powder is colored by 13% KOH solution into red-orange, after slight heating to red-brown (sinalbin).

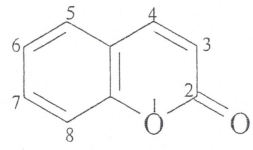
ANALYSIS OF DRUGS CONTAINING COUMARINES

Coumarines are lactones of o-hydroxycinnamic acid. o-Hydroxycinnamic acid is present in plants as a glycosidic precursor and is able to form lactone cycles after hydrolysis. Coumarines can be either free or in the form of glycosides.

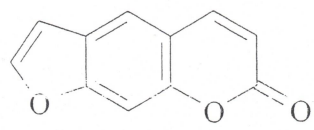
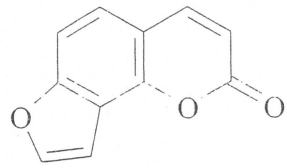
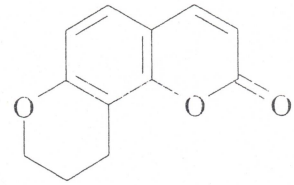
Coumarins are quite soluble in hot water, in ethanol. They don't precipitate with neutral lead acetate, when they don't have phenolic hydroxyl groups in their molecule. But they precipitate with basic lead acetate. Their chemical structure shows that they absorb UV light, they fluorescent and their fluorescence is pH-dependent.

Classification of the coumarines according to the chemical structure:

a) simple



b) condensed coumarines, which are divided to furan-coumarines and pyran-coumarines

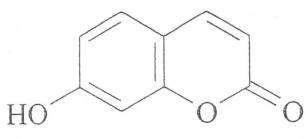
Coumarines cause depression of the CNS and they have a fever-lowering effect. They have spasmolytic, sedative, diuretic, antiseptic effects. Few of them can cause photo-sensitivity of skin and others are used as a protection against intense sunshine (e.g. esculin, esculetin).

Dicumarol is formed by bacteria in the freshly collected drug, which has a strong anticoagulant and hemorrhagic effects and it is used in pharmacotherapy and prophylaxis of thrombosis.

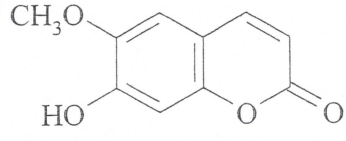
Asperulae herba

Galium odoratum (syn. *Asperula odorata*), *Rubiaceae*

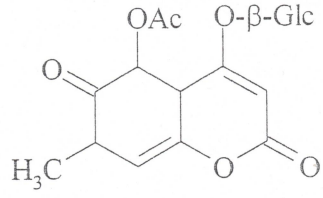
Content compounds: non-substituted coumarine, other coumarines umbelliferone and scopoletin, glycoside asperuloside, bitter compounds, tannins



umbelliferon



scopoletin



asperulosid

Identification

Thin-layer chromatography

Tested solution: 1 g of the powdered drug is extracted with 10 ml of methanol in a water bath with continual stirring for 30 minutes. The extract is filtered and dried to 1 ml.

Reference solution: 1% methanolic bergapten solution

Elution mixture: toluene : ether (1 : 1), saturated by 10% acetic acid

Detection: 10% KOH solution in ethanol

Apply 10 µl of both solutions on the TLC layer and it is eluted for a distance of 12 cm. After drying, it is sprayed by the detection reagent and investigated under UV light at 365 nm. On the chromatogram of the tested solution, there is a visible yellow-green spot of coumarin closely above spot of the bergapten in the reference chromatogram. There can be other slightly visible spots of umbelliferone (R_f = 0.4) and scopoletin (R_f = 0.25).

Herniariae herba

Herniaria glabra, Herniaria hirsuta, Caryophylacea

Content compounds: coumarines (herniarin = umbelliferone and methylumbelliferone), neutral saponins (mixture of derivates of medicagenic, gypsogenic acid etc.), acidic saponines, flavonoids, tannins

Identification

1- By microsublimation at 100 °C, sublimate with melting temperature 116-117 °C is formed. The sublimate is dissolved in water and 1 drop of diluted ammonia solution is added. The solution fluorescents by blue color (methylumbelliferone).

2- Thin-layer chromatography

Tested solution: 1 g of the powdered drug is extracted with 10 ml of methanol in a water bath with continual stirring for 30 minutes. The extract is filtered and dried to 1 ml.

Reference solution: 1% methanolic bergapten solution

Elution mixture: toluene : ether (1 : 1), saturated by 10% acetic acid

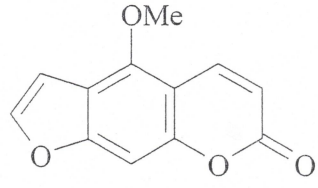
Detection: 10% KOH solution in ethanol

Apply 10 µl of both solutions on the TLC layer and it is eluted for a distance of 12 cm. After drying, it is sprayed by the detection reagent and investigated under UV light at 365 nm. On the chromatogram of the tested solution, there is a visible light blue spot of herniarin at a similar level as bergapten in the reference solution and umbelliferone (R_f = 0.4).

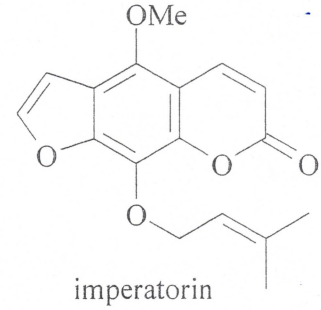
Levistici radix (ČL 2002)

Levisticum officinalle, Apiaceae

Content compounds: furan-coumarines (bergapten, isoimperatorin), essential oils (phenylpropanoids - apiol, myristicin), flavonoids (apiin), mucilage, saccharides



bergapten



imperatorin

Identification

Thin-layer chromatography

Tested solution: 1 g of the powdered drug is extracted with 10 ml of methanol in a water bath with continual stirring for 30 minutes. The extract is filtered and dried to 1 ml.

Reference solution: 1% methanolic bergapten solution

Elution mixture: toluene : ether (1 : 1), saturated by 10% acetic acid

Detection: 10% KOH solution in ethanol

Apply 10 µl of both solutions on the TLC layer and it is eluted for a distance of 12 cm. After drying, it is sprayed by the detection reagent and investigated under UV light at 365 nm. On the chromatogram of the tested solution, there is a visible blue spot of bergapten, sometimes a green spot of imperatorin and umbelliferone (R_f = 0.4).

Distinct blue spot with R_f = 0.9 is 3- butylidenephthalide.

Bergamottae etheroleum

Essential oils obtained by the pressing of fresh pericarp of *Citrus aurantium* L. subsp. *bergamia*

Content compounds: essential oils (linalylacetate, geraniol, linalool), coumarines (bergapten, citroptene)

Identification

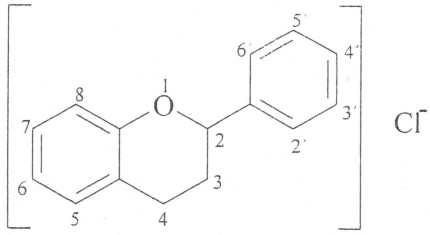
Thin-layer chromatography – as other drugs

Tested solution: essential oils

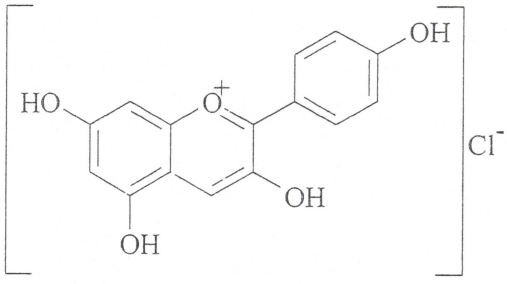
Apply 10 µl of both solutions on the TLC layer and it is eluted for a distance of 12 cm. After drying, it is sprayed by the detection reagent and investigated under UV light at 365 nm. On the chromatogram of the tested solution, there is a visible blue spot of bergapten, and then a visible spot of citroptene and sometimes other spots.

ANALYSIS OF DRUGS CONTAINING ANTHOCYANINS

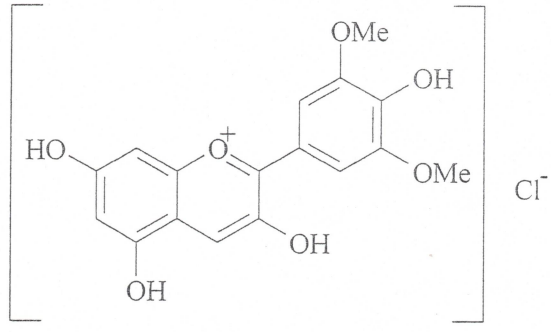
Anthocyanins are red, purple or blue pigments of flowers, leaves and fruits of many plants (from the Greek: ανθοσ = flower κιανεοσ = blue). In plants there are in the form of glycosides whose aglycones are called anthocyanidins. Structurally are derived from flavylium-chloride.



All these aglycones have hydroxyl groups at positions 4', 5, 7, and nearly all at position 3. More hydroxyl groups may be in positions 3 'and 5' etherified by methyl group.



pelargonidin



malvidin

Changing the pH of the solutions of these compounds, changes their structure and their altered chromophore system manifests itself by changing color. For example, the aglycone cyanidin is a pigment in red roses and blue cornflowers.

Isolation of anthocyanins is carried out by the extraction with ethanol or methanol containing 1-5% hydrogen chloride. The chloride is precipitated from crude extract by adding ether etc. Adsorption chromatography, especially on Al_2O_3, is a good technique for the isolation of anthocyanins

Qualitative determination:

Color reactions

Adding sodium carbonate or sodium acetate to the aqueous acidic solution changes the color from red or purple into blue or variously stable blue-purple.

The pigment is shaken out from the aqueous extract into amyl-alcohol and a little sodium acetate, a drop of diluted solution ferric chloride is added to the alcoholic extract. Intense blue color is characteristic for those anthocyanins, which have two adjacent free hydroxyl groups (delphinidin, cyanidin, petunidin).

N-hydroxylation. The most common glycosides are amygdalin, prunasin and sambunigrin which are present in the family *Rosaceae, Fabaceae, Poaceae, Araceae, Euphorbiaceae* and Passionflower.

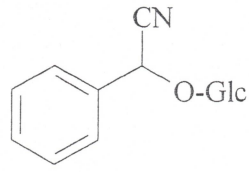
Identification of cyanogenic glycosides is performed by using the filter paper strip impregnated with the reagent, which provides the color reaction with hydrogen cyanide.

Quantitative determination of cyanogenic glycosides is carried out by titration. The drug is suspended in acidified water and water steam distilation is performed. The distillate that contains hydrogen cyanide is titrated with silver nitrate. Gas chromatography is used very often for the determination.

Laurocerasi folium

Prunus laurocerasus, Rosaceae

Content compounds: 1.2 – 1.8% cyanogenic glycosides (prunasin = (-)-(R)-mandelonitril-β-D- glucoside)



prunasin

Identification

The crushed drug is placed into a tube, moistened by water and the filter paper impregnated with sodium picrate is placed onto the mouth of the tube. We observe the transition of the yellow pigmentation into dark red (sodium isopurpurate).