Practice No. 2

ANALYSIS OF DRUGS CONTAINING POLYSACCHARIDES AND MUCILAGE

Polysaccharides consist of monosaccharides that are bound to each other by glycosidic bonds. They can be divided into homopolysaccharides and heteropolysaccharides depending on how many kinds of monosaccharides they consist of. Homopolysaccharides can be divided according to the type of the monomer units, for example glucans are polymers of glucose and galactans are polymers of galactose, etc. Polysaccharides unlike proteins and nucleic acids form both linear and branching polymers, because the glycosidic bond can happen at any hydroxyl group.

Oryzae amylum - Rice starch

Oryza sativa L., Poaceae, rice

Tritici amylum - Wheat starch

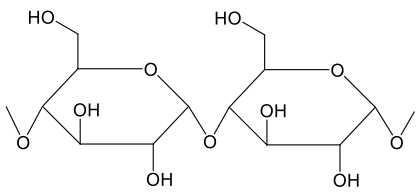
Triticum aestivum L., Poaceae, wheat

Solani amylum - Potatoes' starch

Solanum tuberosum L., Solanaceae, potatoes

Starch is a mixture of two D-glucans – linear glucan = *amylose* and branched glucan = *amylopectin* which are characteristic to starch grains in the plant cells. Amylose is mostly linear α-(1$\rightarrow$4)-D-glucan placed in a helix. The presence of amylose by iodine is based on the penetration of iodine into the helix. Starch shows strong absorbance – intensive blue color with iodine. Amylopectin is branched α-(1$\rightarrow$6)-D-glucan with a bush like structure with branching always about after 24-30 glucose units.

Starch doesn't dissolve in cold water or organic solvents. Starch grains swell in cold water, in warm water they dissolve and form the so-called starch wax which is exactly a colloidal solution of starch



Amylose

Identification

1- To 0.5 g of starch is added 25 ml of water and boiled 1 minute and cool down. The opalescent mucilage is formed (solution A).

2- 5 drops of iodine solution are added to 1 ml of the solution A; dark blue color is created which disappears after warming and appears again after cool down.

3- To 5 ml of the solution A is added 0.5 ml of 11% HCl and heated for 30 minutes in the water bath. Then it is alkalized to litmus using NaOH. 5mL of Fehling reagent is added and heated in the water bath. Reduction occurs and Cu_2O is formed.

Dextrinum - Dextrin

Dextrin is a mixture of variously branched D-glucans. It is formed by the partial hydrolysis of starch by mineral acids at 100-120°C or by enzymatic hydrolysis.

Identification

1- About 1 g of dextrin with 10 ml of water is heated in a water bath (with occasional shaking) long enough to form a muddy solution (solution A). This solution will be used for test 2 as well. Three drops of this solution are diluted with water to reach 5mL and 2 drops of iodine solution are added. The color will change to violet-red.

2- To the rest of solution A from the previous test 4 ml of Fehling's reagent are added and the mixture is boiled for a short while. It forms an orange-red powdered precipitate of Cu_2O.

Inulinum - Inulin

Inulin is a plant polysaccharide with β-glycosidic bonds between D-fructofuranose units. Glucose accounts for 5 % of the molecular weight of the complex. The composition of less than 100 units is a requirement for good solubility in water.

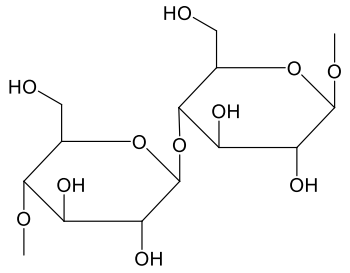
Identification

1- 1 g of inulin is dissolved by moderate heating in 20 ml of water (solution A). 0.05 g resorcinol is mixed with 1 ml of concentrated HCl, and added to 5 ml of the solution A, and boiled for 2 minutes. It forms a deep red color (because of the presence of fructose produced by the hydrolysis of inulin).

2- 0.5 ml phloroglucinol in HCl is added to 5 ml of the solution A from the previous test and boiled for 2 minutes. It forms yellow-brown to brown color (reaction of fructose).

Cellulosum ligni - Cellulose wadding

Bleached cellulose is made from coniferous and broad leaved trees. Cellulose is a linear polymer that consists of β-glycosidic bounded D-glucopyranose units.



Cellulose

Identification

1- To a piece of cellulose wadding is added zinc chloride and iodine solution. The color of the fiber will turn purple.

2- 0.02 M iodine solution is added to a piece of cellulose – after adding 80 % sulfuric acid, the color is changed into intensive green or blue-purple color.

3- To a piece of cellulose wadding 2 ml of diluted sulfuric acid are added and it is placed in a water bath for 15 minutes. After cooling down, it is neutralized by sodium hydroxide and added 1 ml of basic solution of picric acid. Then 1 ml of the mixture is heated in a water bath. It forms red color (picramic acid – a proof of the presence of glucose).

Tests of purity

1- A piece of wadding is moistened by the ethanolic solution of phoroglucinol in HCl (1.0 g/100 ml). The color of the sample doesn't change and it remains lightly pinkish (lignin).

2- Another piece is moistened by sodium hydroxide (20 g/100 ml). The color should either remain the same or can change into yellow (suberin).

Lana gossypii depurate - Purified cotton wool

Purified, free fat and bleached fibers from the seeds of *Gossypium, Bombacaceae*

Identification

1- Iodine solution is added to a few fibers of cotton – the color is changed into brown. If further sulfuric acid is added, the color of fibers will become blue-purple (cellulose).

2- 10 ml of zinc chloride in formic acid is added to 0.1 g of cotton wool, heated at 40 °C for 2.5 hours with occasional shaking. The sample shouldn't dissolve.

Drugs with content of mucilage and gum

Mucilage is a high molecular polysaccharide, which greatly swells up in water. It forms viscous colloidal hydrophilic solution that is compared to the solutions of gums, they aren't soluble in ethanol or organic solvents. They are hydrolyzed to hexoses and pentoses (most commonly galactose and arabinose) or to close derivates of sugars.

Gums are products of metabolism in the plant cell wall. They are amorphous, optically active compounds, which are soluble in water and form lightly acidic colloidal solution. If they aren't soluble directly, they swell up massively. They aren't soluble in ethanol or organic solvents.

They are composed of uronic acids, their salts and individual sugars. The main components are arabin, basorin and cerasin. These three yield sugars upon hydrolysis, especially galactose, arabinose, xylose and uronic acids. Arabin is soluble in water. By heating to 120°C it becomes cerasin, which is slightly soluble in water, but swells up. Basorin is almost insoluble in water, but swells up massively.

Proof of presence of mucilage in drugs

The presence of mucilage is possibly proven by color reactions in plant tissue, which are based on the change of color by some pigments, eg. ethanolic solution of methylene blue colors mucilage into blue, potassium hydroxide solution turns the color into yellow to yellow-green, alkaline solution of coralline changes the color into red, ethanolic solution of thionine changes the color into blue-purple, solution of ruthenium-red changes the color into purple.

Evaluation of mucilaginous drugs

Determination of the swelling capacity

Determination of the content of mucilage in drugs is based on the determination of the swelling capacity. It is the volume in mL, which 1 g of the drug occupies together with its mucilage after 6 hours of storing in water at room temperature.

The procedure of determination

1.0 g of the drug, whole or crushed according to the requirement of each experiment, is placed into a titration cylinder (25 ml) with ground-glass, height about 125 ± 5 mm and divided into 0.5 ml. If it's graduated in a different way, the drug is moistened by 1.0 ml of 96% ethanol, 25 ml of water is added and then it is closed. It is shaken during the first hour intensively every 10 minutes and then left for 3 hours to settle. After 90 minutes most of the liquid is bound to the drug. Floating parts of the drug on the surface are removed by mild vertical circular motion of the cylinder. It is the volume that the drug occupies with its mucilage. The measurement is performed three times and an average is taken.

Agar - Agar

Agar is dried mucilage from different species of seaweed especially from *Gelidium.*

Identification

1- A piece of the drug is boiled with potassium hydroxide. The color is changed into yellow-green.

2- 0.1 g of the drug is dissolved in 50 ml of water and heated in a water bath (if it isn't completely dissolved, it is possible to heat on a light flame in the end) (solution A). After cooling down, 3 ml of water is very carefully added to 1 ml of mucilage, so that they form two separated layers. Then 0.1 ml of 0.05 M iodine solution is added. The interface is changed into brown-purple, after shaking the liquid becomes light yellow.

3- 5 ml of the mucilage from previous test (solution A) are heated for 30 minutes in a water bath with 0.5 ml of 11% HCl. Then 1 ml of $BaCl_2$ is added. The white turbidity is formed within 30 minutes.

4- 5 drops of formaldehyde are added to 1 ml of agar solution – unlike gelatin, agar doesn't precipitate.

Determination of swelling capacity

It is determined according to the mentioned procedure for powdered drug. The content of mucilage should correspond minimally to the number 10.

Lini semen - Linseed

The dried seeds of *Linum usitatissimum L., Linaceae*.

Identification

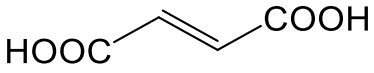
1- Put a drop of resorcinol solution on several seeds in a porcelain dish and leave it to evaporate until it is dry. Then add 2.0 mL of conc. HCl and heat the mixture in a water bath. It forms red to purple color (pentoses in mucilage).

Determination of swelling capacity

It is determined according to the previously mentioned procedure. The content of mucilage should correspond to the number 4.5 for the powdered drug and to the number 4 for the non-powdered drug.

Lichen islandicus - Islandic lichen

The whole or cut thallus of *Cetraria islandica L., Parmeliaceae*.



Fumaric acid

Identification

Microsublimation

The powdered drug gives microcrystal yellowish sublimate (fumaric acid) by microsublimation at 220-250^{O}C. That sublimate is dissolved in a drop of ammonium hydroxide and then the sample is dried. The needle-like crystals can be observed which are often connected together (ammonia fumarate).

Acaciae gummi - Acacia

Solidified gum from species *Acacia senegal L., Mimosaceae*

The gum is hydrolyzed to glucuronic acid, galactose, rhamnose and arabinose.

Identification

1- 0.5 g of drug is dissolve in 5 ml of water by intensive stirring. The solution is poured onto 10 ml of 95% ethanol acidified by concentrated acetic acid slowly. It forms a white precipitate (arabin). The precipitate is filtered out and ammonium oxalate is added to the filtered solution. It forms a white precipitate, easily dissolved in diluted HCl, insoluble in diluted ammonium hydroxide and concentrated acetic acid (Ca^{2+}).

2- A solution of the tested compound (1:10 000) is mixed with basic lead acetate. It forms a white precipitate after several minutes.

Althaeae radix - Marshmallow root

Dried, peeled or non-peeled roots of *Althaea officinalis L., Malvaceae*

Identification

1- A few drops of iodine solution are added to a fragment of the root. The color is changed to blue (starch).

2- A few drops of potassium hydroxide solution is added to a piece of the root. It becomes yellow (mucilage).

Determination of swelling capacity

It is determined according to the previously mentioned procedure. The content of mucilage should correspond minimally to the number 10.

Althaeae folium - Marshmallow leaf

Dried leaf of species *Althaea officinalis L., Malvaceae*

Determination of swelling capacity

Only 0.2 g of the powdered drug is used and to continue according previously described procedure. The content of mucilage should correspond to the number 12.

Farfarae folium – Coltsfoot leaf

Dried leaves of *Tussilago farfara L., Asteraceae*

Identification

2.0 g of the powdered drug are mixed with 20 ml of water and boiled for 2 minutes. Then it is filtered and 10 ml of the filtrate are mixed with 10 ml of 96% ethanol. After careful shaking, it is filtered again. The precipitate on a filtration paper is dissolved quantitatively in 10 ml of water, potential turbidity is removed by repeated filtration. 5.0 ml of the transparent filtrate are added to 5.0 ml of 96% ethanol. It forms an intense white opalescence (mucilage).

Determination of swelling capacity

It is determined according to the previously mentioned procedure. The content of mucilage should correspond minimally to the number 10.