Practice No. 10

ANALYSIS OF DRUGS CONTAINING ALKALOIDS PART I

Alkaloids are nitrogenous alkalies of plant origins. Nowadays, the group includes even other similar compounds that are similar to alkaloids, those obtained by the modification of natural products by semi-synthetic or synthetic means. It should be noted at the very beginning that alkaloids represent one of the most important groups of substances of plant origin, mainly because they show strong physiological effect.

Alkaloids are found mainly in dicotyledonous plants. The plant usually contains a number of alkaloids which are chemically related, often derivatives of one another. Exceptionally, there is only one alkaloid found in the plant. Alkaloids are seldom found free in the plant. Most are bound in the form of salts with organic acids, especially with malic acid, oxalic acid, citric acid and tartaric acid, and also with the acids specific to certain types of alkaloids such as the lysergic acid (ergot alkaloids), chelidonic acid (alkaloids from celadine) and meconic acid (opium alkaloids).

Chemically, alkaloids are organic nitrogenous bases. The vast majority of alkaloids contain a nitrogen included in a heterocycle. The group of phenylethylamines, which includes ephedrine, is the most important of those that make an exception to this rule. Alkaloids thus don't form a distinctive structurally related group as a whole, but they resemble amines of higher molecular weight with some of their properties. Alkaloid bases are mostly solid, colorless, crystalline substances. Rarely, they are liquids (eg oxygen-free nicotine or coniine – they also have characteristic smell) or colored (yellow berberine, red sanguinarine, purple cryptolepine). The free bases of alkaloids are either slightly water soluble or insoluble. However, they are usually well dissolved in organic solvents, especially in ether, chloroform, benzene, etc. With acids, the alkaloids form salts, which are usually water soluble. The most commonly used are chlorides, sulfates, nitrates, phosphates, bromides and tartrate. These salts are somehow hydrolyzed and react in a weakly acidic way in aqueous solutions. Alkaloid salts are insoluble in organic solvents. Many alkaloids are optically active, predominantly levorotatory. Almost all have bitter taste. (This is to be trusted, not TRIED!)

Determination of alkaloids in drugs

Quantitative determination of alkaloids in drugs is mostly limited to:

a) determining the total amount of alkaloids present in the drug
b) determination of the main alkaloid

Individual methods are elaborated based on the overall chemical nature of the determined substances. Considerations are taken regarding solubility, the ability to form salts with different degrees of hydrolysability, dissociation constant of the base, the ability to form crystals, whose weights could be used for quantitative determination. The ability of some alkaloids to provide permanent coloring with reagents, suitable for the colometric determination is also used. The results of the determination may only be compared if it is being done under the same conditions. The procedure of quantitative determination of alkaloids in drugs can be divided into three parts:

1. Preparation of the drug and extraction of alkaloids
2. Purification of the extract
3. The quantitative determination of the alkaloids

Preparation of the drug and extraction of alkaloids

The alkaloids are found virtually in all parts of the alkaloid containing plants. The individual plant organs used for extraction are especially rich. It cannot be generalized which organ is the richest in alkaloids. Sometimes it is the root (Radix veratri, scopoliae, ipecacuanhae, hydrastidis), sometimes the leaf (Folium belladonnaee, hyoscyami, stramonii, theae), bark (Cortext Chinae, granati), fruit (Fructus capsici, Conii), herb (Herba lobeliae) or seed (Semen colae strychni).

According to the localization of the maximum amount of alkaloids in the specific organ it is apparent that, it will be easier to extract the alkaloids for example, from the leaves, which are made of an easily crushable, soft mesh, than from the hard tissue of the bark. Therefore, the powdered drug is always used for the extraction of alkaloids to be as completele as possible. The powdering is usually carried out just before the determination. The drug has to be sieved through a required sifter (usually V or VI).

Some alkaloid drugs - mostly drugs made up of seeds (Semen strychni, colchica) must be defatted using petroleum ether prior to the determination. There is a large amount of oils and fats in the egg-white an the cotyledons, which could make the proposed determination of alkoloids considerably more difficult, if not impossible. Weighing of the drug for quantitative determination is carried out at least with the precision of one hundredth of a gram, unless it is stated otherwise in the instructions.

The extraction of the drug is done by maceration, percolation or continuous extraction, frequently in the Soxhlet apparatus. Total exclusion of alkaloids is tested with Mayer's reagent, which is applied to the couple last drops of the filtered extract or the percolate. (The colchicine and purine bases, which react with Mayer's reagent only in significant concetration, are the exception).

The test is usually done by letting the drug residue dissolve in very small amount of 0,5 M hydrochloric acid solution and a drop of Mayer's reagent. When determining colchicine or purine bases a drop of Lugol's reagent is added.

The extraction of alkaloids is carried out mostly using organic solvents (ether, chloroform, petroleum ether, benzene, pentane, dichloroethane, etc.) while alkalizing the mixture, which releases the bases of alkaloids from the bonds with the organic acids. Alkalization is mostly carried out with ammonia solution, which has the advantage of not soaping the fats present in the drug against alkali hydroxides. Some methods use sodium carbonate for alkalizing, which supposedly penetrates the hard tissue of the endosperm better (eg. Semen strychni). The exception to the general procedure (alkalization – release of the base of the alkaloids – shaking out into the organic solvent) is Cortex Chinae. Here the alkaloids are bound to tannins in the form called alkaloid ethanate. This bond can be broken using hydrochloric acid. First, the hydrochloric acid solution is applied to Cortex Chinae, the alkaloids are set free from the bond with tannin and they form hydrochlorides + tannic acid. Adding sodium hydroxide to the extract partly recreates the alkaloid salts with tannins, however, those are not stable and tend to decompose due to the excess of alkalies.

Apart from the above-mentioned organic acids, diluted ethanol can also be used as an infusion agent of alkaloids, (alkaloids present in the plants of Solanaceae family) and so can be the calcium hydroxide (extraction of opium). Distillation by water steam is used to get the volatile alkaloids present in the drugs (Fructus conii, Folium nicotianae).

Purification of the infusion

The extract from the drug has to be purified, because it contains a significant amount of impurities, which are formed by the side content substances. Especially saccharides, polysaccharides, lipids, wax, resins, saponins, colors, and with the above-the-ground parts of plants even chlorophylls are present there. The purification has to be carried out especially punctually, if the content of alkaloids is to be determined quantitatively.

Schematically, purification of the residue can be described like this:

The solution of alkaloids in the organic solvent is shaken out several times with acidified water, which makes the alkaloids turn into salts in the aqueous extracts and most of the lipophilic substances stay in the organic solvent. The acidic aqueous extract is alkalized again and shaken out with approximately half the volume of appropriate organic solvent, which makes the alkaloids move to the organic acids again as alkalies. The alkaloids are purified by repeated acidification of the solution, its alkalization and shaking out into the organic solvent. This way of purification is appropriate because it is not time consuming. Better results could be achieved through column chromatography, but this procedure takes longer. If the extract contains soft suspended substances or substances, which lower the superficial tension (saponins), bad separation of organic solvent from the aqueous layer occurs, because emulsion is formed during the shaking. That's why it's recommended to add some powdered tragacanth into the extract, which attracts all the impurities after the shaking, resulting into separation of two unmixable solvents. The starch present in the drug also functions as a purifying and swelling agent (Radix belladonnae, ipecacuanhae).

The extract used for the determination has to always be colorless, otherwise the proper result of determination is not guaranteed. The chlorophyll present in the drugs from above-the-ground parts of the plants causes harmful interference during the titration and colorimetric determinations. Its elimination is done by precipitating 10% citric acid solution, which adjusts the pH of the infusion to the value of 3. Citric acid is added to the concentrated alcohol extract of the drug. Precipitated chlorophyll is filtered. The filtrate is alkalized with sodium carbonate and sodium hydroxide to pH 9 and shaken out into chloroform, where the alkaloids migrate.

The quantitative determination of alkaloids itself

Alkaloids are usually determined by titration. Acidimetric determination is used for Cortex Chinae, Cortex granati, plant alkaloids from the Solanaceae family, also Radix ipecacuanhae, Semen strychni, Tuber aconiti and others. Acidimetric determination of alkaloids consists of titration of a weak base by a strong acid, the equivalent point is usually reached between the pH values of 3 and 6. For this reason, the suitable indicators for the titration of alkaloids are methyl red, methyl orange, and depending on the type of basis even other acidic indicators, such as erythrosine.

Most cases even multihydric alkaloids can be titrated as the monohydric alkalies, because their second dissociation constant is usually very small (eg. quinine, brucine, strychnine). All of this applies to aqueous solutions. Most of the alkaloid bases are insoluble in water, but dissolves well in 50% ethanol. However, ethanol at this concentration changes their dissociation constants in an apparent way. This sometimes causes the color change of the methyl red at the end of the titration not clear enough. In such cases the Bromothymol blue can be used.

Common analytical practice is usually about the determination of alkaloids and similar bases in purified extracts obtained from drugs or from various galenic preparations by shaking them from the alkaline environment out with ether, chloroform etc. Organic bases isolated in such way are then either dissolved in alcohol and titrated directly using methyl red or Bromothymol blue as an indicator, or we proceed so that we dissolve the obtained base in an excess of

hydrochloric acid solution and titrate it back through the unused acid with sodium hydroxide solution to methyl red.

In the gravimetric determination, the extract containing alkaloids needs to be thoroughly purified. The alkaloids are then determined directly by drying the residue to a constant weight, or the weight of the precipitate formed by adding a suitable reagent (eg. phosphowolframic, picric, phosphomolybdic acid) to the purified fusion is determined.

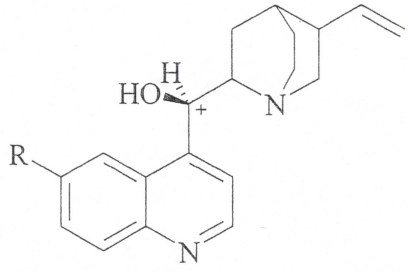
The physico-chemical methods most frequently used for the determination of alkaloids in drugs are colorimetry and nephelometry. Colorimetry can be especially used for determination of such alkaloids, that provide specific color compound with suitable reagents (eg. ergotamine with p-dimethylaminobenzaldehyde in sulfuric acid environment gives blue color). The color intensity is then compared with the intensity of the solution color and known concentration based on different light absorption. In the nephelometric determination of alkaloids in drugs we measure the turbidity, which is caused by the insoluble particles in the fluid (eg. turbidity formed in very diluted solutions of alkaloids after adding a suitable coagulating reagents to alkaloids) and compare it to a fluid with a known amount of the suspended particles. Some reagents used for the evidence of alkaloids, such as Mayer's reagent, phosphowolframic acid, picric acid, etc., are also used for precipitating alkaloids containing extracts. During the last 10-15 years the chromatography, coupled with some physico-chemical method, mostly with colorimetry, has significantly affected even the quantitative determination of alkaloids. This method is particularly suitable because it allows the determination of one alkaloid (separated by chromatorgraphy).

QUINOLINE ALKALOIDS

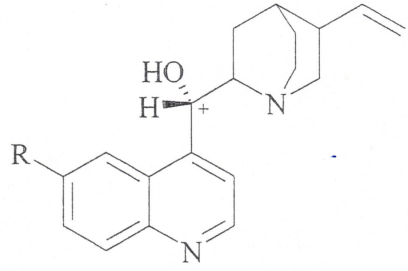
Cinchonae cortex

Cinchona sp., Rubiaceae

Content substances: quinoline alkaloids (quinine, quinidine + their 6'-demethoxyderivatives, cinchonodine, cinchonine, chinicine, cinchonicine), quinic acid, cinchotannin, glycoside quinovin, small amount of anthraquinones



Quinine R= OCH3
Cinchonidine R=H



Quinidine R=OCH3
Cinchonine R=H

Identification:

1- 0.2 g of drug is carefully heated over a direct flame in a dry horizontally positioned test tube, violet vapors develop, which condensate to blood-red tar in the colder parts of the tube. After cooling, the tar is dissolved in 5 ml of 70% ethanol. The solution fluoresces intensely blue under UV at 365 nm. By adding a few drops of concentrated hydrochloric acid the fluorescence disappears immediately.

2- 0.1 g of powdered drug is shaken out with 5 ml of 15% sulfuric acid for 1 minute and is filtered. Few drops of Mayer's reagent are added to ml of the filtrate, yellowish precipitate is formed immediately (alkaloids).

3- Thin layer chromatography

Tested solution: 0,1 g of the powdered drug is mixed with 0.1 ml of 26% ammonia and 5 ml of $CHCl_3$. The solution is left to stand with ocassional shaking for 30 minutes, then it is filtered and evaporated. The residue is dissolved in 1 ml of 96% alcohol.

Reference solution: 17.5 mg of quinine is dissolved in 5 ml of 96% alcohol.

Developing mixture: diethylamine: chloroform (10:90)

Detection reagent I: anhydrous formic acid

The layer is sprayed by detection reagent and observed under UV at 254 nm after evolving

4- Talleioquine reaction:

1.250 g of powdered drug (V) is mixed with 2.0 ml of concentrated formic acid and 15 ml of water and heated in a water bath for 30 minutes. After cooling, 15 ml of chloroform are added together with 60 ml of ether and after strong shaking, also 3,5 ml of concentrated sodium hydroxide solution. The mixture is strongly shaked and then left to stand for 30 more minutes with frequent shaking. After this time, 2 g of powdered tragacanth are added and everything is shaked until clarification. The almost clear solution is filtered with a dry filter. Bromine water is added dropwise to 5 ml of the solution, if the resulting precipitate is dissolved by shaking, and immediately the excess of diluted ammonia is added, blue-green precipitate is excreted (quinine, quinidine). Other oxidizing agents (hydrogen peroxide, hypochlorite solutions) can be used instead of bromine water. Excretion of methanol and oxidation at both the core and the vinyl group probably occur during the reaction. The resulting green pigment is called talleioquine. The color of the content turns red if diluted sulfuric acid is added to the green solution. The talleioquine passes into chlorofom in a blue-violet color. The precondition to the formation of talleioquine is the presence of quinoline and quinuclidine nuclei, and also the methoxyl at position 6. Cinchonine and cinchonidine, which don't contain methoxyl, don't provide talleioquine reaction. Recently it was found, that besides talleioquine, another substance, showing the same color properties as talleioquine, is formed by the conjugation of two quinoline nuclei during the reaction.

TROPANE ALKALOIDS

Belladonnae folium

Atropa bella-donna, Solanaceae

Content compounds : 0.3 – 0.60% alkaloids (mainly hyoscyamine, which changes into propylene atropine by inconsiderate handling, further scopolamine, apoatropine, belladonine), free volatile nitrogenous bases (pyridine, N-methylpyrrolidine), traces of nicotine, coumarin derivatives (scopolin, scopoletin), flavonoid glycosides, tannins.

Hyoscyami folium

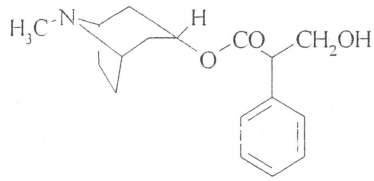
Hyoscyamus niger, Solanaceae

Content compounds : 0.04 – 0.17 % tropane alkaloids (mainly hyoscyamine, or atropine, scopolamine, further cuscohygrine), flavonoids, coumarins, tannins

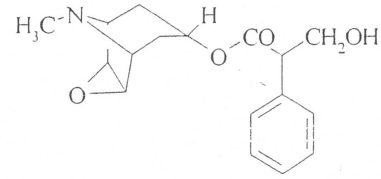
Stramonii folium

Datura stramonium, Solanaceae

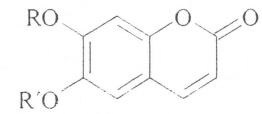
Content compounds : 0.2 – 0.6 % tropane alkaloids (mainly hyoscyamine, hyoscine, or propylene atropine, further scopolamine)



Hyoscyamine



Scopolamine



Scopoletin R=H, R'=CH3
Scopolin R=Glu, R'=CH3

Identification:

1- 1.0 g of powdered drug (IV) is shaked with 10 ml of diluted sulfuric acid (0.05 mol/l) for 3 minutes and is then filtered. The filtrate is also used for the test 2. Add 5 drops of Mayer's reagent to 1 ml of the filtrate, the precipitation occurs (alkaloids).

2- Add 1 ml of 26% ammonia and 5 ml of water to the rest of the filtrate from the test 1. Gently shake 15 ml of ether to avoid formation of emulsions. The ether layer is dried by anhydrous sodium sulfate, then filtered and the ether evaporates inn the porcelain dish. Add 0.5 ml of fuming nitric acid (or some different nitrating agent) to the residue and let it evaporate to dryness on a water bath. Add 10 ml of acetone to the residue and also potassium hydroxide (30 g/l) in 96% alcohol dropwise; dark purple color (hyoscymine) occurs.

This reaction is referred to as Vitali reaction. It is provided by acid esters, whose structure is similar to tropic acid. Applying nitric acid causes nitration of the phenyl residue. Alcoholic infusion is a condition for the formation of quinoid structure of the corresponding nitro-derivative. The presence of tropine core is not necessary for the ocurrance of the reaction, but it is beneficial to the stability of the color. Vitali reaction can be used for distinguishing the tropane alkaloids of atropine type from the cocaine alkaloid types. The same Vitali reaction course as atropine can be observed with scopolamine, whose molecule contains the corresponding structural grouping. In contrast, homatropine, does not meet the above-mentioned structural condition and yields orange-yellow color during the Vitali reaction.

3- Thin layer chromatography

Tested solution: 0.6 g of powdered drug is shaken with 20 ml H_2SO_4 (0,05 mol/l) for 15 minutes and is filtered. The filtrate is washed with 0.05 mol/l sulfuric acid to a final volume of 25 ml. Add 1 ml of 26% ammonia to the filtrate and shake it with 2x 10 ml of peroxide free ether. If necessary, the layers are separated by centrifugation. United ether layers are dried with anhydrous sodium sulfate, filtered and evaporated to dryness on a water bath. The residue is dissolved in 0.5 ml of methanol.

Reference solution: hyoscyaminesulfate, scopolamine bromide in MeOH

Developing mixture: ammonia : water : acetone (3: 7: 90)

Detection reagent I: potassium bismuth iodide (Dragendorff reagent)

Detection reagent II: sodium nitrite

20 µl of both solutions are applied separately on the thin layer and developed over a distance of 10 cm. The layer is dried at 100 – 105°C for 15 minutes. After cooling, it is sprayed with detection reagent I until orange or brown spots appear on a yellow background. The layer is then sprayed with detection reagent II so that it becomes transparent, and it is observed after 15 minutes. The spots corresponding to hyoscyamine turn reddish-brown, but not gray-blue (atropine).

4- Content determination

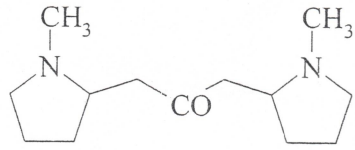
2.00 g of the powdered drug (VI) are mixed with 50 ml of ether in a 250 ml flask. After 5 minutes, 4 ml of 11% ammonia solution and 16 ml of water are added and the mixture is shaken strongly for 30 minutes. After 5 minutes of standing still the ether layer is rapidly filtered through cotton wool and 30.0 g of the filtrate (equivalent to 1.20 g of the drug) is evaporated in the flask. We add 1.0 ml of 95% ethanol to the residue and dry for 2 hours at

105°C. The residue is then dissolved in 1.0 ml of chloroform, we add 5.00 ml of 0.01 M sulfuric acid solution and the mixture is heated in a water bath until the chlorofom evaporates. We add 5 ml of water, 3 drops of the mixture indicator and titrate with 0.02 M sodium hydroxide solution from the microburette. The consumption is substracted from the blank test consumption.
Blank test: 5 ml of 0.01 M sulfuric acid solution, 5 ml of water, 3 drops of the mixture indicator
1 ml of 0.01 M sulfuric acid solution corresponds to 0.005788 g of hyoscyamine.

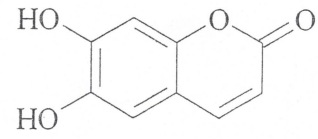
Belladonnae radix

Atropa bella-donna, Solanaceae

Content compounds : 0.4 – 0.8 % of alkaloids (mainly hyoscyamine, which changes into propylene atropine by inconsiderate handling, further scopolamine, hygrine, hygroline, cuscohygrine, tropinone, tropine, pseudotropine), calcium oxalate, starch, coumarin derivatives (scopoletin, esculetin)



Cuscohygrine



Esculetin

Identification:

1- Approximately 1.0 g of the powdered drug (IV) is shaken with 3 ml of diluted hydrochloric acid and 5 ml of water for 3 minutes and then filtered. The filtrate is used for the test 2 as well. 5 drops of Mayer's reagent are added to 1 ml of the filtrate, precipitation occurs (alkaloids).

2- The rest of the filtrate from test 1 is evaporated in a porcelain dish to dryness, we add 10 drops of concentrated nitric acid and again, the mixture is evaporated until dryness. The residue is moistened with an alcohol solution of potassium hydroxide, it turns red-violet (hyoscyamine).

3- The powdered drug is extracted with ammoniacal chloroform in a porcelain dish. The solvent is evaporated on a water bath and the drug is removed. A mixture of perhydrol and concentrated sulfuric acid (1: 10) is allowed to run through the residue. Olive green to dirty green zones appear (hyoscyamine).