General information about alkaloids

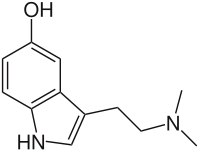
The plant kingdom is a rich source of biologically active compounds. Alkaloids are a class of biologically active substances. Alkaloids are basic nitrogenous compounds of plant origin, that are physiologically active and produce appropriate salts with acids. The name "alkaloid" is derived from two words: Arabic «al-gali» for alkali and Greek «eidos» - meaning -like.

Simple nitrogenous compounds (methylamin and other amins), and also aminoacids are not attributed to alkaloids. However, tyramine and betains (stachydrine, trigonelline and oth.) are considered as transitional compounds.

In addition to carbon, hydrogen and nitrogen, alkaloids may also contain sulfur, more rarely other elements such as chlorine, bromine or phosphorus.

The basicity of alkaloids is derived from the presence of a nitrogen atom. Nitrogen is mostly present as heteroatom, sometimes – in the form of amino nitrogen. All properties of organic bases are characteristic for alkaloids. So, the free alkaloids (bases) are easily soluble in organic solvents, and alkaloidal salts are soluble in water.

Alkaloids are produced by a large variety of living organisms. They are widely distributed in higher plants. About 10 to 25% of higher plants contain alkaloids. Alkaloids are found in some animal species. Bufotenine in the skin of some species of toads can be presented as an example. An alkaloid psilocybin is also isolated from Psilocybe mushrooms. Some biogenic amines such as adrenaline and serotonin which play an important role in human and animal organism are similar to alkaloids in their structure and biosynthesis.



Bufotenine

Most alkaloids are present in plants in the form of salts of organic acids, rarely – in the form of inorganic acids. Alkaloids are very often present in the form of salts of oxalic, malic, succinic, citric acids. In some plants alkaloids are associated with special acids, for example meconic acid in opium, quinic acid in cinchona.

Biological activities of alklaoids are closely correlated with their structures. The study of the structure -activity relationship of alkaloids with certain pharmacological action is a reason of production of most synthetic preparations.

Alkaloids – containing plants have been used by humans since ancient times.  A Chinese book on houseplants written in 1st–3rd centuries BC mentioned a medical use of [Ephedra](https://en.wikipedia.org/wiki/Ephedra) and [opium poppies](https://en.wikipedia.org/wiki/Opium_poppy). Also, [coca](https://en.wikipedia.org/wiki/Coca) leaves have been used by [South American](https://en.wikipedia.org/wiki/South_America) Indians since ancient times. [Extracts](https://en.wikipedia.org/wiki/Extracts) from plants containing toxic alkaloids, such as [tubocurarine](https://en.wikipedia.org/wiki/Tubocurarine) and oth., were used since antiquity for poisoning arrows.

Studies of alkaloids began in the 19th century. At the end of the 18th century some plant material (opium, bark of cinchona tree and oth.) was studied chemically and some purified substances were obtained. Morphine was first isolated from opium poppy plant in 1804 by Segen, then in 1806 Serturner isolated morhpine crystalls and described it as alkali. He studied isolated substance comprehensively and in 1817 he described this substance as a plant alkali. Before this discovery it was considered that the plant organism secretes only acidic and neutral substances. The discovery of alkaloid morphine led to extensive research on base substances. The term "morphine", used at present, was given by the French physicist [Louis Gay-Lussac](https://en.wikipedia.org/wiki/Joseph_Louis_Gay-Lussac).

A significant contribution to the chemistry of alkaloids was made by the French researchers [Pierre Pelletier](https://en.wikipedia.org/wiki/Pierre_Joseph_Pelletier) and [Joseph Cavento](https://en.wikipedia.org/wiki/Joseph_Bienaim%C3%A9_Caventou). They discovered strychnine (1818) and quinine (1820).[Xanthine](https://en.wikipedia.org/wiki/Xanthine)(1817), [atropine](https://en.wikipedia.org/wiki/Atropine) (1819), [caffeine](https://en.wikipedia.org/wiki/Caffeine) (1820), [coniine](https://en.wikipedia.org/wiki/Coniine) (1827), [nicotine](https://en.wikipedia.org/wiki/Nicotine) (1828), [colchicine](https://en.wikipedia.org/wiki/Colchicine) (1833), [sparteine](https://en.wikipedia.org/wiki/Sparteine) (1851), and [cocaine](https://en.wikipedia.org/wiki/Cocaine) (1860) and other alkaloids are isolated around that time.

Production of alkaloids including opium alkaloids is associated with the activities of Chichibabin and Rodionov. The isolation and study of alkaloids from plant material is associated with A.P. Orekhov.

It must be pointed out that at present the number of alkaloids discovered exceeded 6000, only 50 alkaloids are found in materials of animal origin.

**Classification of alkaloids**

There are different type of alkaloids:

1. Botanical or phylogenetic classification. This classification is based on the systematic affiliation of plants from which alkaloids are isolated to certain genus or family (Solanaceae, Apocynaceae, Papaveraceae and oth.).

2. Pharmacological classification. This classification is based on the mechanism of action of alkaloids on organism, for example narcotic analgesics, M-cholinomimetics, stimulators of CNS and oth.

3. Chemical classification. This classification is based on the structure of heterocycle and biosynthesis of alkaloids. According to this rule alkaloids are divided into 3 groups:

1). True alkaloids which contain nitrogen in a heterocycle. True alkaloids originate from amino acids, sometimes – from nicotinic or anthranilic acids.

2). Proto-alkaloids which contain nitrogen in the side chain of heterocycle. They originate from amino acids.

3). Pseudo- alkaloids (isoprenoid alkaloids). They originate from mevalonic acid according to isoprenoid synthesis. That’s why they are grouped irrespective on the basis of the heterocycle presence.

True alkaloids are classified according to the structure of heterocycle and biogenetic precursor. They are formed by the decarboxylation of the amino acids.

Biogenetic precursors of most alkaloids are amino acids: ornithine is involved in the biosynthesis of pyrrolidine, pyrrolizidine, tropane and some pyridine alkaloids; lysine – chinolysidine ( family Fabaceae, lupin -type) and some piperidine alkaloids; tyrosine – most isoquinoline alkaloids; tryptamine – indole, from isoquinolone alkaloids – cynchonos, some pyridine and piperidine alkaloids; histidine – imidazole alkaloids of pilocarpine; glycine and asparaginic acid – in the biosynthesis of purine alkaloid.

Nicotinic acid is involved in the synthesis of some alkaloids (table 4). Proto-alkaloids include aliphatic, phenolic, cyclic, polycyclic carboline compounds containing nitrogen in the side chain. (isomers of ephedrine, alkaloids norpseudoepehdrine, capsanoides, colchicine solated from the colchicum, ephedra and pepper genus).

Pseudoalkaloids (isoprenoids) are classified in the following way:

Monoterpenes (Actinidia);

Sesquiterpenes (Nuphar, Dendrobium);

Diterpenes (Aconitum, Delphinum);

Triterpenes (Taxus).

Steroidal or glycoalkaloids (Solanum, Veratrum, Holarrhena).

In addition to the monomeric alkaloids, there are also dimeric, trimeric and even tetrameric alkaloids. They are formed upon condensation of two (three and four) monomeric alkaloids. Dimeric alkaloids are usually formed from monomers of the same type. The main mechanisms for dimerisation of alkaloids:

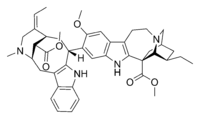
Mannich reaction. The example is bisindole alkaloid – voacamine.

Michael reaction. Alkaloid villalstonine.

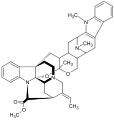
Condensation of aldehydes with amines. Alkaloid toxiferine.

Oxidative addition of phenols. Alkaloids dauricine and tubocurarine.

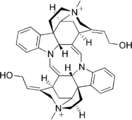
Lactonization. Alkaloid carpaine.



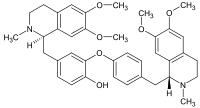
Voacamine



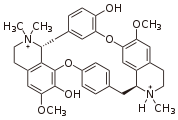
Villalstonine



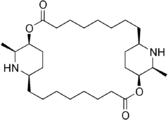
Toxiferine



Dauricine



Tubocurarine



Carpaine

There are alkaloids containing isoprenoid carbon skeleton– steroidal alkaloids. In this case pathway of this group of alkaloids begins with carbohydrates through mevalonic acid and geranylpyrophosphate.

The formation of any heterocycle depends on the precursors – amino acids. Strong development of the alkaloid biosynthesis was possible in the 50s of the twentieth century after the isotopic method was applied.

Most alkaloids contain oxygen and they are colorless, crystalline or amorphous substances, odorless, bitter taste. They are optically active (especially L-isomers). Some alkaloids are colored (like alkaloid berberine from barberry leaves is coloured yellow).

Oxygen-free alkaloids are volatile liquids with specific unpleasant odor. This group of alkaloids includes tobacco (nicotiana iavasit) and nicotine in the leaves of wild tobacco (Nicotiana rustica), coniine isolated from hemlock (Conium maculatum), pelletierine from the bark of pomegranate (Punica qranatim), pilocarpine from leaves of pilocarpus (Pilocarpus), anabasine from Anabasis aphylla. The free alkaloids (bases) are insoluble in water, readily soluble in organic solvents, produce salts with acids. Alkaloid salts are readily soluble in water, insoluble in organic solvents. Alkaloid salts are used in medicine, because they are soluble in water. For example, atropine-sulphate, strychnine-nitrate and oth.

Pyridine and piperidine alkaloids.

This group includes pyridine derivatives. This group also includes alkaloids of toxic plant – posion hemlock - Conium maculatum: coniine, N-methylconiine and oth.; alkaloids of lobelia – Lobelia inflata: lobeline and lobelanine. Lobeline and lobelanine exert stimulating effect on the respiratory centre and is involved in the regulation of respiration in medicine. Alkaloids of this group are present in Solanaceae, Equisetaceae and Lycopodiaceae.

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pyridine piperidine quinolysine quinolizidine

Examples of pyridine alkaloids include nicotine and anabasine. Nicotine is extracted form tobacco leaves (Nicotiana tabacum L.), and anabasine – from echinochloa (Anabasis aphylla L.). Both alkaloids can be used for the synthesis of nicotinic acid. In addition they are used in the form of sulfates in pest control.

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**DEMO**

nicotine anabasine

Tablets of Anabasine Hydrochloride are used in medicine (Tabulettae Anabasini hydrochloridi 0,003) internally or under the tongue as an agent for smoking cessation. Recently the application of some nicotine salts and its dosage forms for the treatment of psychiatric disorders is offered.

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**DEMO**

Cytisine

Cytisine contains 1,2,3,4- tetrahydroquinolizine cycle condensed with piperidine. It can be considered as 1,2,3,4-tetrahydroquinolizone-6 derivative because it contains ketogroup in position 6. Cytisine is present in most plants of the family Fabaceae. For example, in the seeds of Cytisus (Cytius laburnum), thermopsis (Thermopsis lanceolata R.Br). White or slightly yellowish crystalline powder, it is easily soluble in water, alcohol and chloroform.

Tablets of «Tabex» contains cytisine  (0,0015 g)  and used for smoking cessation.

Cytiton is used intravenously for stimulation of respiration and blood circulation. Maximal single dose is 1 ml, daily dose – 3 ml.

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Pachicarpine hydroiodide

Pachicarpine was first isolated from the aerial parts of Sophora japonica, and then from thermopsis. 3% solution of preparation in freshly boiled and cooled water should be colorless, transparent and has a neutral reaction. This solution should remain colorless in the presence of phenolphthalein, but in the presence of bromthymol blue it turns green or blue.

Pachicarpine hydroiodide is ganglion-blocking substance, eliminates the peripheral blood vessels spasm, enhances uterine muscle contraction. Tablets (0,1 g) and 3% of injection (subcutaneous or intramuscular) are used. It is used for the treatment of hypertensive emergencies, myopathy, and also during the labor to enhance of uterine muscle tone. Preparation (0,1 g) is produced in the form of rectal suppositories for the application in obstetrics.

Preparation and its dosage forms (list B) are stored in a place protected from light. Maximal single dose is 0,2 g internally, subcutaneous – 0,15 g, maximal daily does –0,6 g internally, subcutaneous – 0,45 g.

The tropane alkaloids used in medicine are divided into two classes:

1. Alcohol tropine deirvatives (hyoscyamine, atropine, scopolamine);

2. Ecgonine or tropine-2-carboxylic acid derivatives (cocaine).

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**DEMO**

tropane tropine ecgonine

Tropane alkaloids such as hyoscyamine and atropine are esters of alcohol tropine with tropic acid. Atropine is a racemic mixture of d,l-tropic acid, but hyoscyamine is formed from optically active l-tropic acid and alcohol tropine. Scopolamine is ester of scopine alcohol with l-tropine acid. Scopine differs from tropine by the oxygen bridge between C-6 and C-7.

Atropine sulfate is a monoacid tertiary base and forms salts highly soluble in acids. The rhizomes with roots of Scopolia carniolica are used in the industrial manufacture of atropine. Plant material contains about 0,9% alkaloids, mainly l-hyoscyamine ansd scopolamine, but atropine in small quantities. Alkaloids are extracted from plant material in the base form of, i.e. the material is first treated with ammonium solution, then it is isolated by organic solvent. When the isolated hyoscyamine is treated with water-alcohol solution of sodium hydroxide, it is converted into the racemate – atropine. Atropine-base obtained thus is purified, dissolved in anhydrous alcohol and converted into atropine sulfate by pure sulfuric acid.

Atropine is a cholinolytic substance, it includes in antispasmodic and mydriatic drugs. In contrast to atropine scopolamine has a sedative effect on the central nervous system. The mixture of camphorate scopolamine and camphorate hyoscyamine is included into “Aeron” tablets which are used as sedative and antiemetic agent.

The main ecgonine alkaloid is cocaine. It is present in the leaves of wild coca shrub - - Erythroxylon coca Lam., growing in America (about 1,5%).

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**DEMO**

cocaine

The content of cocaine in total alkaloids isolated from coca plant is only 40-50%. It is profitable to prepare the cocaine in industry by semisynthesis from ecgonine. Cocaine hydrochloride is used in medical practice. One of the negative effect of cocaine is addiction cocainism. At present synthetic analgesic analogues of cocaine is more widely used in medical practice (procaine, dicaine, soucaine, trimecaine and oth.).

Pyrrolizidine is resulted from the condensation of two pyrrolidine rings. This group includes about 40 alkaloids isolated form different species of groundsel (Senecio) and other plants. 1-methylpyrrolizidine or heliotridane form the basis for these alkaloids.

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pyrrolizidine 1-methylpyrrolizidine

These alkaloids yiled aliphatic mono- or dicarboxylic acids (necin acids) and aminoalcohols (necines) on hydrolysis. At the present time there are about 10 various necines that are srtucturally or stereochemically different from each other.

Platyphyllin and sarracine – are the derivatives of heliotridane that are important in medicine. Seneciphylline is used for the synthesis of curarelike preparation “Diplacin”.

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**DEMO**

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Platyphylline hydrotartrate

White odorless crystalline powder with weak or specific smell and bitter taste. Easily soluble in water, very littler soluble in alcohol; practically insoluble in chloroform and ether.

Platyphylline is a cholinolytic, spasmolytic and mydriatic agent. It is similar to atropine in its influence on the peripheral cholinoreactive system, but it is less active than atropine. It exerts sedative effect on central nervous system.

## The structure of quinoline alkaloids is based on quinoline and quinuclidine nucleus.

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**DEMO**

or

*quinoline* quinuclidine

Alkaloids isolated from the cinchona tree bark (quinine and quinidine) and equinopsine obtained from globe thistle are used in medicine.

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**DEMO**

Equinopsine nitrate

Equinopsine was first isolated from the fruits of globe thistle (Echinops ritro L.; Echinops sphaerocephalus L.) by Gresgof in 1900. The content of alkaloids reaches 1%. It is similar to strychnine in pharmacological properties, it stimulates CNS, exerts a tonic action.

Cinchona tree bark contains about 30 alkaloids. The major Cinchona alkaloids are quinine, quinidine, cinchonine and cinchonidine. The alkaloid content varies within wide limits depending on the species of cinchona tree- 6,5-20%, for example Cinchona succirubra Rav. - 6,5 %, in Cinchona ledgeriana Moens. -20 %.

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**DEMO**

Quinic acid quinine

Compared to quinine cinchonine doesn’t have a methoxy-group. So, quinine may be regarded a methoxy cinchonidine and quinidine as methoxy cinchonine. Methoxy-groups of quinine and quinidine are converted into phenolic hydroxyls by alkaline hydrolysis, these compounds produce azo dye with diazonium salts. Quinine is a diacidic base and forms both acid and neutral salts. The following salts are used in medical practice: quinine sulfate, quinine hydrochloride, quinine dihydrochloride..

Most alkaloids include isoquinoline and its derivatives. The following preparations of this group alkaloids are used in medical practice: isoquinoline, 1,2,3,4-tetrahydroisoquinoline, 1-benzyltetrahydroisoquinoline, 1-benzylisoquinoline, the derivatives of morphinan (phenantrenisoquinoline) and aporphine. , isoquinoline 1,2,3,4-tetrahydroisoquinoline 1-benzylisoquinoline

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**DEMO**

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**DEMO**

aporphine morphinan

papaverine R = H morphine thebaine

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R = CH3 codeine

The derivatives of tetrahydroisoquinoline are alkaloids salsonine and salsolidine, the derivatives of 1-benzyl-tetrahydroisoquinoline – narcotine, hydrastine, cotarnine-chloride, the derivatives of 1-benzylisoquinoline – papaverine and its preparations, the derivatives of morphinan – morphine, codeine and thebaine, the deirvatives of aporhine – apomorphine and morphothebaine.

There are many important indole alkaloids in medicine, for example physostigmine, strychnine, reserpine, aymalin, vincamine, brevicoiline and oth.

Most indole-alkaloids are toxic compounds, have a high biological activity and are present in many plant families (Fabaceae, Loganiaceae, Apocynaceae və s.).

The indole nucleus can be regarded as the condensation of the pyrrole and benzene rings.:

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**DEMO**

indole indolizidine (piperolidine) β-carboline

The pilocarpine derived form imidazole is used in medicine. Pilocarpine is first isolated from the leaves of Pilocarpus Jaborandi Holm., growing in Africa.

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R1

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**DEMO**

imidazole pilocarpine – R=CH3; R1=C2H5

pilocarpidine – R=H; R1=C2H5

pilozin – R=CH3; R1=C6H5 – CHOH

Pilocarpine is oxidized with the formation of pilopic and homopilopic acids, imidazole is cleaved with the formation of methylurea, ammonium, methylamin and carbon dioxide. Pilocarpine hydrochloride (Pilocarpini hydrochloridum) is used in medical practice. Pilocarpine is cholinolytic substance. It is used as eye drops or ointments (1-2%). Rarely it is injected subcutaneously. It is widely used as anti-glaucomatous agent (reduce intraocular pressure). The main purine alkaloids are caffein, theobromine and theophylline. Purine ring underlies the structure of both these essential alkaloids and complex proteins which play important role in the vital acitivity of human and animal organism. The end-product of methabolism is uric acid – the origin of which is purine. These alkaloids are the derivatives of xanthine – the product of oxidation of purine ring. Purine nucleus is made of imidazole and pyridine rings.

A particular characteristic of these 3 alkaloids is stimulating action on the nervous system and heart acitivities. It is more expressed in caffeine, but theobromine has a diuretic and vasodilator aciton (blood vesse, widener). All three alkalids are present in the leaves of tea, cofe and kola nut, and also in cocoa beans. The leaves of tea contains 1-3% of caffeine, cocoa beans beans contains about 3% theobromine.

Nitrogen is present in acyclic alkaloids in the side chain, not as a heteroatom, i.e. exocyclic amine nitrogen. The alkaloids used in medicine are spherosine, ephedrine and colchamine. Alkaloids colchamine is isolated from colchicum bulbs (Colchicum) of liliaceae family; 0,5% colchamine ointment (or omain) (Unguentum colchamini 0,5 %) is used for the treatment of skin cancer.

**Alkaloid biosynthesis**

The first complete synthesis of an alkaloid was achieved in 1886 by the German chemist Albert Ladenburg.

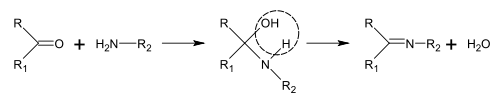
Biological precursors of most alkaloids are amino acids, such as ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, aspartic acid and anthranilic acid. All these amino acids except anthranilic acid are proteinogenic. Nicotinic acid can be synthesized from tryptophan or aspartic acid.

Ways of alkaloid biosynthesis are too numerous. That’s why it can not be easily classified. However there are a few typical reactions involved in the biosynthesis of various classes of alkaloids:

- synthesis of Schiff bases

- [Mannich](https://ru.wikipedia.org/wiki/%D0%A0%D0%B5%D0%B0%D0%BA%D1%86%D0%B8%D1%8F_%D0%9C%D0%B0%D0%BD%D0%BD%D0%B8%D1%85%D0%B0) reaction

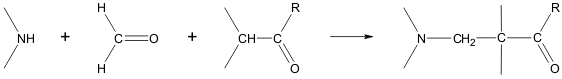
Schiff bases can be obtained by reacting amines with ketones or aldehydes. These reactions are a common method of producing C=N bonds.



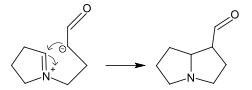
In the biosynthesis of alkaloids, such reactiond may take place within a molecule, such as in the synthesis of piperidine.



An integral component of the Mannich reaction, in addition to an amine and a carbonyl compound, is a carbanion, which plays the role of the nucleophile in the nucleophilic addition to the ion fomred by the reaction of the amine and the carbonyl..



The Mannich reaction can proceed both intermolecularly and intramolecularly. The example of intramolecular Mannich reaction is the synthesis of pirrolizidine nucleus.



The example of intramolecular Mannich reaction is reaction Piktet-Spengler – cyclisation of Schiff bases, derived from phenylethylamines followed by formation of the tetrahydroisoquinoline system. The biosynthesis of alkaloids in plants always involves enzymes. However, non-enzymatic synthesis of isoquinoline alkaloids is also found. Two consistent steps – synthesis of Schiff bases from catecholamines and aldehyde; the Pictet-Spengler reaction proceed. Both reactions can proceed under physiological conditions and in the absence of enzymes. Non-enzymatic synthesis usually occurs during metabolic disorder or intoxications, when the excess of amines or aldehydes are present in the body.

In contrast to polyphenols and terpenoides alkaloids are secondary metabolites grouped on the basis of chemical structure – they include various natural compounds, containing nitrogen and therefore possess basic properties.

Some universal principles are characteristic for the formation of alkaloids. This is due to the fact that the common to most alkaloids is the presence of simple five- or sixmembered nitrogen-containing heterocycles– for example pyrrolidine, piperidine or pyridine type or simple N-heterocycles condensed with other carbo- and heterocycles with the formation more complex polycyclic structures. Thus the alkaloid structure is based on a relatively small number of standard structural elements. Their formation is not associated in which compounds they are included in the further biosynthesis steps, because this process is carried out by the same precursors through intermediate steps.

Decarboxylation, oxidative-deamination or aminoacid transamination are primary reactions of biosynthesis. The the direct transmethylation of the obtained intermediate compounds comes and precursors aliphatic chains cycle into various hetero and carbocyclic structures takes place.

The complication of the structure by introduction of additional methyl groups can occur at any stage of alkaloid biosynthesis. This reaction often occurs precisely at the level of aliphatic precursors. The main point of alkaloid biosynthesis is methylation. Thus methylation doesn’t only precede cyclisation and condensation, but also directs it. The method of ring closure into carbocyclic or heterocyclic fragments of alkaloid depends on the position of CH3-groups of precursor.

The reactions that lead to the formation of N-heterocycle structures with aliphatic joined nitrogen of amino acid have general significance.  They are connected with the formation of C-N-bind as a result of different reactions. Different intermolecular and intramolecular reactions can lead to these bonds, but the reactions of azomethines formation (Schiff’s bases) and the reaction of Mannich condensation type are the most significant.

Azomethanes can be formed spontaneously or by fermentation from amino- and carbon groups. (scheme 1).  Amines that form Shiff’s bases (A) are synthesised in aminoacids decarboxylation, but carbonic compounds are synthesized due to transamination and oxidation-deamination.. In Mannih condensation C-N- bind formation of similar functional groups occurs in interstitial creation of N-hydroxymethyl derivative or acid amine depending on the application of carbonil compound: aldehyde or acetyl -KoA .

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**4**

**5**

**4**

**5**

Scheme I.The formation of C-N bond, an important reaction in alkaloid biosynthesis

 The processes of aliphatic chains cycle in heterocycles are supplemented by condensation processes at the next stages: separate rings are combined and more complex structures are formed, often polycyclic.  The formation of alkaloids are combined with splitting of previously formed cyclic structures due to C-C, C-N or C-O binds breaking. Skeletal complication is achieved by internal molecular regrouping with old binds breaking and formation of new C-C; C-N binds.

Limited cyclic variants and regrouping in biosynthesis of alkaloids are combined mainly with including of functional groups and substitutes during different stages of metabolism, it leads to the development of different structural types of alkaloids in nature.

The most important step of any alkaloid biosynthesis is undoubtedly the primary cyclization of their aliphatic precursors, leading to the formation of those protozoan nitrogen-containing heterocycles. The main cyclic nucleus is built from them in various combinations. The classificaiton of alkalodis is based on the presence of certain heterocyclic structures.

A precursor for the pyrrolidine ring is amino acid ornithine. The symmetric diamine putrescine is formed directly from the decarboxylation of ornithine in the first step. (scheme 2). One of the aminogroup of putrescine is then methylated, after – it is oxidatively deaminated to N-methylaminobutanal which generates the N-methyl-pyrrolinium cation, a central precursor of numerous alkaloids containing five-membered nitrogen heterocycle.

In addition of ornithine the precursor of pyrrolidine ring can be some other compounds which are associated with metabolic transformations. It can also be derived from the amino acid arginine.

Pyrrolizidine ring of alkaloids is a cyclic structure, composed of two pyrrolidine rings which share a common nitrogen. Pyrrolizidine ring is also derived from ornithine via the intermediate product – putrescine. In this case diamine is firstly oxidatively deaminated or interaminated to 4-aminobutanol. Then 2 molecules of 4-aminobutanol link together forming Schiff’s bases (scheme 2). Then it is cyclized followed by the cleavage of aminogroups, complete reduction of cyclic ring and mostly hydroxylation. Specific bicyclic derivatives of pyrrolizidine alcohols or necine bases, which are main structural elements of all pyrrolizidine alkaloids. Individual alkaloids of this group are esters consisting of a necine base esterified with one or two specific organic acids which occur only in plants and have ability to synthesize this group of alkaloids. They are called necic acids – mono- or di-carboxylic acids with branched carbon chains and they are derived from branched amino acids (valine, isoleucine).

Biosynthesis of bicyclic ring of tropane alkaloids can be considered as a continuation of the biosynthesis of pyrrolidine ring (scheme 2).

Scheme II. Biosynthesis of pyrrolidine, pyrrolizidine and tropane alkaloids

The N-methyl-pyrrolinium cation is thought to condense with acetoacetic to yield hygriene-a-carboxylic acid. After decarboxylation of this acid hygriene is produced, which in one-two intermediate steps it is converted into tropine. Tropine is a characterized by a byciclic structure, this compound is a condesate of pyrrolidine and piperidine rings which share a common nitrogen. Tropine is included into a series of tropane alkaloids, thus it is a direct precursor of this group. Most tropane alkaloids are characterized by the presence of ester bond with acid via OH-group of tropine. Tropic acid often serves as a acid component (especially in solanaceae). Tropic acid a aromatic amino acid – phenylalanine derivative and it is formed from phenylalanine by an intramolecular rearrangement of the side chain.

Pyperidine ring (almost half of all known alkaloids include it) is widely distributed among the alkaloids and synthesized by 2 different ways: from the amino acid lysine, its metabolites or its chemical equivalent –cadaverine, or from acetate. “Lysine” and “acetate” ways are not isolated and can function in parallel in biosynthesis of some alkaloids. “Lysine” way is dominated and thus piperidine ring of most alkaloids is derived amino acid.

Lysine can be converted into piperidine by three ways (scheme 3). According to the first way a-amino group is cleaved from lysine by oxidative deamination. a-amino-α-ketokapronic acid (2) is formed, which cyclizes to form piperidine-2-carboxylic acid (3). Piperidine is produced by decarboxylation from piperidine-2-carboxylic acid (4). Piperidine is an immediate precursor of piperidine ring of alkaloids. According to other way the formation of the same precursor is begun with the cleavage of terminal amino-group. In this case semi aldehyde of a-aminoadipic (5) and piperidine-6-carboxylic acids are the intermediate compounds. Finally, the decarboxylation of lysine into the symmetric amine –cadaverine (7) is also possible. Then this mechanism followed by deamination of cadaverine leads to 5-aminopentanal (8) with further closure of aliphatic chain of aminoaldehyde and piperideine formation (4).

İn the further biosynthesis steps of piperidine alkaloids piperidine can react with other metabolites, then the additional condensation, cyclization, oxidation and oth. reactions are followed. A wide range of piperidine alkaloids are formed, most of them has a complex di-, tri- or tetracyclic structure. Alkaloids with major structural elements- single- or double quinolizidine ring – cyclic structure composed of 2 condensed rings of piperidine with a common nitrogen (comparing with pirrolizidine) are the most characteristic.

Scheme III. The biosynthesis of piperidine ring from lysine

However, the formation of piperidine ring of alkaloids can be derived from lysine. Bicyclic nucleus of simple lupin-type quinolizidine alkaloids can be synthesized through the intermediate stage of 5-aminopentanal by reactions (similar with reactions which are observed during the biosynthesis of pyrrolizidine alkaloids) (scheme 2). The formation of the piperidine ring from “acetate” is specific for the biosynthesis of coniine-type alkaloids. Polyketide chain is synthesized from 4 acetates during this process (scheme 4). Then it is converted into octanoic acid. Octanoic acid is reduced to an appropriate aldehyde – oktanal. Then the oxidation of aldehyde into 5-ketoderivative and conversion into an amine are followed. The cyclization with the coniine formation takes place. Due to this specificity of biosynthesis the presence of tricarbon side chain attached to the carbon of heterocyclic nucleus of piperidin adjacent to nitrogen is characteristic for conniine-type alkaloids.

Scheme IV. The biosynthesis of piperidine ring (coniine-type alkaloids) from acetate Only a few alkaloids contain a piperidine ring (nicotine, anabasine). However, in addition it is included into the structure of some essential and universal for all organisms - pyridine nucleotides (NAD, NADP and oth.). The immediate precursor of this ring is always nicotinic acid. In plants nicotinic acid is formed from more simple aliphatic compounds, however in human, animal and most microorganisms it is derived from amino acid tryptophan.

Scheme V. Biosynthesis of pyridine ring

In plants it is aspartic acid and glycerol or its phosphorylated  derivative -  phosphoglyceraldehyde (scheme 5). Quinolinic acid is formed from these compounds by condensation and some intermediate reactions. Then quinolinic acid is involved into the reactions of pyridine nucleotide cycle, as a result carbon dioxide is split off and it is converted into nicotinic acid. The latter is an intermediate precursor of pyridine alkaloids. In nicotine biosynthesis this acid is condensed with N-methyl-pirrolinium cation (with loss of COOH-group as a result of carbon dioxide splitting off). In anabasine synthesis nicotinic acid is condensed with piperidine.

In biosynthesis of isoquinoline nucleus that is a major structural element of isoquinoline alkaloids with various and complex chemical structures, the precursor is a aromatic amino acid tyrosine. This group also includes important alkaloids containing in opium poppy (papaver somniferum). During this process tyrosine is firstly oxidized into 3,4-dihydroxyphenylalanine (DOPA) followed by decarboxylation to dopamine. Dopamine thus formed reacts with carbonyl compound that leads to the heterocyclic ring closure and formation of isoquinoline nucleus. Pyruvic acid can be as a carbonyl component. Condensation of pyruvic acid with dopamine produces simple tetrahydroisoquinoline type isoquinoline alkaloids (for example salsolin) (scheme 6). However dopamine mostly reacts with carbonyl derivative of tyrosine – 3,4-dihydroxyphenylpyruvic acid (formed by oxidative deamination and inclusion of additional hydroxyl group into aromatic ring). As a result three-membered ring benzylisoquinoline type isoquinoline alkaloids are formed followed by the formation of the rest complex isoquinoline alkaloids by different modification of the structure.

Further complication of the structure of benzylisoquinolines is mainly condensation of the existing cyclic elements and intramolecular rearrangement, as a result of which new circular structures with different configurations are formed. In particular, when aromatic rings of benzylisoquinoline are condensed, a third six-membered carbon ring is produced with the formation of four-membered ring aporphine type isoquinoline alkaloids. When another cyclization is taken place via nitrogen, protoberberine type alkaloids are derived from benzylisoquinolines. In addition to isoquinoline nucleus the quinolizidine nucleus is included into four-membered ring structure. After additional regrouping and modifications the protopine, benzophenanthridine, roedina and papaverubin type isoquinoline alkaloids are formed from protoberberines.

Scheme VI. Biosynthesis of isoquinoline alkaloids

The most important isoquinoline alkaloids - Morphinanes are also derived from benzylisoquinoline precursor. The oxidative cyclisation of terminal carbon skeleton takes place which is accompanied by formation of new C-C –bond and certain reorganizaiton of heterocycle.  
  
Biosynthesis of the quinoline nucleus of alkaloids has not yet been deciphered. However it is identified that the amino acid tryptophan (alkaloids of cinchona tree) or anthranilic acid – one of the intermediate compound of biosynthesis (alkaloids of globe thistle – Rutaceae family) is an initial precursor of this process.   
  
Indole nucleus of widely distributed indole alkaloids arise from tryptophan. Tryptophan is decarboxylated and converted into triptamine in the first step of biosynthesis (scheme 7). Then different type of condensations of tryptamine (or its N-methyl derivative) with various metabolites can be followed. This process is accompanied by the formation of six-membered or five-membered N-heterocycle. Some other cyclic structures also can be formed. Thus, the condensation of tryptamine and activated acetate forms garman-type indole alkaloids. The condensation between tryptamine and a monoterpene secologanin leads to the formation of numerous different structures of indole alkaloids.

Scheme VII. Biosynthesis of garman (a) and ergoalkaloids (b) type indole alkaloids

However indole alkaloids can originate from tryptophan without its preliminary decarboxylation into tryptamine. In particular biosynthesis of ergoalkaloids (alkaloids of claviceps purpurea) begins by the condensation of tryptophan with the “activated isoprene” – dimethylallyl form of isopentenyldiphosphate. Then polycyclic compounds with 2 heterocycles – lysergic and isolysergic acids (stereoisomers), which are the main structural element of all ergoalkaloids, are form from these 2 components after some complex reactions. An important group of purine alkaloids differ form other alkaloids in the precursors which are the intermediate products of nucleic acid biosynthesis instead of amino acids.   
Xanthosine is converted into caffeine by intermediate steps N-methylxanthosine, N-methylxanthine and teobromine (scheme 8). Teophyllin is formed from caffeine by dymethylation of five-membered of latter.

Scheme VIII. Biosynthesis of purine alkaloids

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**Isolation and detection of alkaloids**

In plant cell alkaloids are found dissolved salts in the cell sap. Alkaloids can be isolated from the plant material in their base and salt form.

*Isolation from plant material.* Their content in medicinal plant material ranges from thousands particles of per cents to 10-15 per cents*.* They are grouped in 20 and more alkaloids, most of them are similar in chemical structure. Alkaloids can be extracted from the medicinal plant material as a free base or a salt form.

Plant mateiral is treated with water or alcohol with the addition of 1-2% acid (hydrochloric, sulfuric, tartaric, acetic and ith.) for the isolation of alkaloids in a salt form. The extract is alkalized and free alkaloids (bases) thus obtained are extracted with a water-immiscible organic solvent (chloroform, dichlorethan, benzene and oth.) for purification from ballast hydrophilic substances. Purification process is repeated several times. Then the organic solvent is removed, the residue containing total alkaloids is broken into individual compounds by chromatography if it is necessary.

Plant material is treated with ammonium solution or hydrocarbonate sodium that liberates the free bases. The free alkaloids (bases) thus obtained are extracted with organic solvent. Some lipophilic impurities pass into extract. Then the purification is carried out by conversion of alkaloids into salts, then again into bases.

Alkaloids can be isolated by chromatographic adsorption. Ion-exchange resin, coal, natural clay and oth. can be used as sorbents. Molecular and ion-exchange adsorption are used. In first case transition of molecules of dissolved substance from mobile into stationary phase (solid) occurs. Adsorption occurs on the surface of solid sorbent without chemical reaction. Desorption (elution) is carried out with the appropriate solvent. In the second case the ion-exchange of dissolved substance with ions of sorbents occurs. Chromatographic adsorption is widely used in industry.

In most cases isolation process of alkaloids from plant material is divided into 3 main stages: 1) isolation of alkaloids from plant material; 2) purification of extractions; 3) separation of total alkaloids and purification of alkaloids.

Extraction of alkaloids in a base form

Alkaloids are usually present in the salt form in plant material, that’s why before extraction it is necessary to convert alkaloid salts into free bases. The material is treated with different alkalines (NH4OH, NaOH, Са(ОН)2,, Ва(ОН)2, and oth.).  for this purpose. The properties of alkaloids are taken into account in the choice of the alkaline. Strong alkalis for example NaOH are used for the isolation of strong free alkaloids (bases) and alkaloids which are firmly connecting with tannins (the bark of the cinchona tree, pomegranate bark and oth.). However it is not used for the isolation of alkaloids containing phenolic hydroxyls. Morphine, salsolin, some alkaloids of claviceps are not extracted due to the formation of phenolates by organic solvent, becuase phenolattes are readily soluble in water and insoluble in organic solvents. Ammonium is used for transformation them into bases. Ammonium and other weak alkalis are used for isolation of ester alkaloids (atropine, hyosciamine, scopolamine and oth.), because strong alkalis can cause the decomposition of the alkaloids. Sodium hydroxide is not recommended for the isolation of alkaloids from seeds containing fixed oils, because caustic alkali cause the saponification of oils. Soaps promote the formation of an emulsion.

When sodium carbonate is used, it is necessary to remove carbon dioxide completely (by shaking Because it can react with alkaloids and produce salts that creates the danger of incomplete extraction of alkaloids.

The isolation of free alkaloids (bases) from plant sources is carried out with various solvents. The solvent with a better dissolving capacity should be chosen for their complete isolation. It is recommended to choose the solvent with the best dissolving capability for more complete extraction. Dichlorethane, chloroform, ethyl ether, benzene and oth. are often used. Along with alkaloids, the accompanying substances are transferred into the extraction: resins, fatty oils, chlorophyll and other pigments, from which it is necessary to separate the alkaloids.

Extraction of alkaloids in the salt form

Alkaloid salts are readily soluble in water and alcohols (ethanol, methanol). Thus one of the solvent containing 1-2% any acid is used for the extraction of alkaloids from plant material. Sulfuric, hydrochloric, tartaric, acetic or other acid that makes water- or alcohol- soluble salts are used for acidification. The extraction occurs quickly and adequately, but a large amount of accompanying substances are extracted with alkaloids (tanninhs, mucilages, saponins, proteins and oth.).

Purification of extracts

Purification of extracts based on the various solubility of free alkaloids (bases) and their salts.

1. Extract of alkaloids from plant material obtained by alkaline (after alkalization) extraction with organic solvent (water-immiscible) is treated with 1-5% acid. The free alkaloids (bases) form appropriate salts with acids, that dissolve in water and pass into water layer, but the major part of accompanying substances remain in organic solvent. Alkaline is added to the aqueous solution of alkaloid salts to convert alkaloid salts into base form. If the content of alkaloids is high then the free alkaloids precipitate that can be collected on filter. But water extractions are often sprayed with organic solvent immiscible with water after alkalization.

Free alkaloids (bases) pass into organic solvent. If it is necessary this process is repeated 2 or more times to separate alkaloids from accompanying substances.

Organic solvent is removed. The residue left after removal of the solvent is total alkaloids.

2.  The extract of alkaloids from plant material obtained by isolation with 1-2% acidic solution is alkalized and then the free alkaloids are extracted with organic solvent. If the alkaloids were extracted with alcohol (ethanol, methanol), alcohol is removed, and the residue is dissolved in water. The alkaloid salts are dissolved in water, and water-insoluble part of accompanying substances is separated by filtration. Aqueous solution of alkaloid salts is subjected to further purification, as already mentioned. .

Purification of extracitons by column chromatographic technique.

Adsorption chromatography is based on the ability of solid substances – adsorbents to selectively adsorb one or several substances from solutions or vapor-gas mixture. Chromatographic technique of purification and separation of alkaloids is applied both to aqueous solutions of alkaloid salts and free alkaloids (bases) in organic solvents. Adsorption processes used in chemical-pharmaceutical industry are divided into 2 groups: 1) purification processes, in which impurities (accompanying substances) are adsorbed, but the alkaloids remain in solution; 2) purification processes, in which alkaloids are absorbed, but the accompanying substances remain in solution. There are 2 types of adsorption: molecular and ion-exchanging.

In the first case transition of molecules of dissolved substances from mobile into stationary phase – solid occurs. Adsorption process is carried out on the surface of solid sorbent without chemical reaction.

In the second case the ion-exchange of dissolved substance with ions of sorbents occurs. Thus, ion-exchange chromatography is a method in which the process of ion-exchange between dissolved substance and ion-exchange sorbents is used. Depending on the nature ion-exchange sorbents are divided into inorganic and organic, depending upon the character of the exchangeable ions – anionites and kationites.

Ion-exchnage high molecular compounds – acidic or base ion-exchange resins insoluble in water and organic solvents are usually used as ionites. Extractions is passed through column filled with sorbent. Sorbent and conditions of adsorption process should be chosen for selective and maximal adsorption of the extraction. Desorption (elution) of alkaloids is carried out by appropriate solvent that provides maximum elution.

*The separation of total alkaloids*

Plant material usually contains several alkaloids, all or most alkaloids (sum) pass into extract by the treating plant material. To separate the alkaloid from a mixture and break down total alkaloids into individual compounds is very difficult. Most alkaloids have various physical and chemical properties, it is difficult to offer the single separation scheme. A large number of separation of alkaloids are described. We will mention only the main principles of separation of total alkaloids.

According to the solubility difference of alkaloids to separate

1. In some cases, partial separation occurs even when the organic solvent is treated with an initial water-acidic extraction after alkalization. During its treatment for example with ethyl ether, only part of alkaloids can pass into organic solvent. The remaining alkaloids in primary solutin can be extracted by other organic solvents (chloroform, dichlorethan anf oth.). Sometimes good results can be achieved by this method. However, it should be taken into account that the solubility difference of alkaloids of one plant is not often expressed sharply and that’s why only partial separation is achieved.

2. The separation of the total alkaloids can be sometimes achieved by the consistent treatment of residue (total alkaloids) obtained after the removal of solvent with different organic solvents (petroleum ether, benzene, chloroform and oth.).

Volatile alkaloids such as nicotine, coniine can be obtained by steam distillation. Alkaloid distillate is treated with organic solvents, then the organic solvent is removed.

General precipitate (group) and specific reaction are used for detection of alkaloids in plant material.

General reactions are based on the ability of alkaloids as bases to give simple or complex salts with different acids, salts of heavy metals and oth. These products are insoluble in water, so called precipitate.

General reactions are:

Vagner, Bushard and Lyugol reagents (solution of iodine in aqueous potassium iodide). ). These reagents produce a brown precipitate with most alkaloids in weak-acidic solutions.

Dragendorff’s reagent (solution of bismuth sub-nitrate, potassium iodide and acetic acid) Most alkaloids produce an orange-red or brick-red precipitate in acidic solutions.

Mayer’s reagent (a mixture of mercuric chloride and potassium chloride in water). This reagent produces a white or yellowish precipitate with most alkaloids in weak-acidic and neutral solutions. The sensitivity of alkaloids to this reagent is very different: strychnine and brucine at a dilution of 1:150 000, morphine – 1:25 000, and caffeine, colchicine don’t show a precipitation with Mayer’s reagent.

Marme reagent (solution of cadmium iodide in potassium iodide solution). Marme reagent with alkaloids produces a white or yellowish precipitate, often soluble in the excess of the reagent. Atropine, colchicine, veratrine and some other alkaloids are precipitated from relatively concentrated solutions, but caffeine doesn’t show a precipitation with this reagent.

Sonnenschein’s reagent (phospho-molybdic acid)H3PO4 x MoO3 x 2H2O)) is one of the most sensitive reagents. It produces amorphous yellowish precipitate, which in time become blue or green colour (reducing of molybdic acid).

Sheibler’s reagent is a solution of phosrphornotungustic acid (H3PO4 x WoO3 x 2H2O). It produces a whitish precipitate with most alkaloids.

Berthran reagent is a solution of silico-tungustic acid (SiO2 x 12WoO3 x 4H2O) Most alkaloids are very sensitive to this reagent and produce whitish precipitates in weak-acidic solutions.

Hager’s reagent (saturated solution of picric acid). Picric acid produce yellow precipitates (picrates) with some alkaloids. Some alkaloids are not precipitated by picrric acid (caffeine, morphine, aconitine, theobromin), others are only precipitated from concentrated solutions (for example, atropine).

Solution of picrolonic acid. Picrolonic acid produces a yellow precipitate with most alkaloids (picrolonates).

A freshly prepared tannic acid solution (5%) gives a coloured precipitate with alkaloids. Alkaloids give whitish or yellowish precipitates with tannins in acidified solutions.

Other organic compounds containing nitrogen can react with precipitation reagents, but caffeine and some purine alkaloids do not react.

Colour reactions: alkaloids with inorganic acids (nitric, sulfuric) or its mixture produce characteristic colored solutions. These reacitons are based on the chemical structures of alkaloids which are specific for group of alkaloids.

They include the following reagents:

Erdmann’s reagent (a mixture of concentrated sulfuric and nitric acid)

Frede reagent (solution of molybdate ammonium in the concentrated sulfuric acid)

Marki reagent (solution of formaldehyde in the concentrated acid)

Wasicky’s reganet (solution of p-dimethylaminobenzaldehyde in the concentrated sulfuric acid)

Caffeine and other purine derivatives are detected by the specific Murexide test. When colchicine is treated with mineral acids it gives yellow colour. Indole alkaloids (alkaloids of claviceps) reacts with 60% sulfuric acid and p-dimethylaminobenzaldehyde and produce blue-violet or red colour.

Vitali-Moren reaction is used to identify the tropane alkaloids. Modification of this reaction enables to determine the alkaloid cocaine.

Alkaloids containing a phenol group (morphine) produce with iron chloride blue colour. Vanillin is a reagent for the detection of indole cycle.

Sodium nitroprusside gives a characteristic colour reaction with pilocarpine, theophylline, pachicarpine and spherophysine.

Various types of chromatography are used for detection and qualitative determination of alkaloids in plant material. Alkaloids have blue (dark blue) and yellow fluorescence under UV-light. When the chromatograms are treated with chromogenic reagents the fluorescence of spots are changed and mostly the colour visible in non-electric light is formed.

Chromatographic analysis

Paper and thin layer chromatography play a leading role among the analytical methods in phytochemical analysis. These methods are especially used for detection, identification and control of degree of the purity and separation into individual substances for analysis of alkaloid-containing plant material.

Extract preparation from plant material

1 g of crushed plant material (leaves of belladonna, datua, thermopsis) is placed in a 100 ml flask, 25 ml of 1% hydrochloric acid is poured and periodically mix for one hour or heat in boiling water bath for 5 min. After cooling down the extract is filtered through cotton into a 100 ml separatory funnel.  Filtrate is alkalized with concentrated solution of ammonia till alkaline reaction to phenolphthalein and alkaloids are extracted with 5 ml of chlorophorm (extract B).

1. Thin layer chromatography

Thin- layer chromatography can be used for identification and quantitative determination of alkaloids in plant material. Chromatography is carried out on plates with fixed or unfixed layer of sorbent. Aluminium oxide for thin-layer chromatography, silica gel KSK and oth. are used as sorbent.

CaSO,·НаО and glass plates with size 12—20X8—15 sm are used as retainers for preparation of plates with fixed layer of sorbent.

Extract and "witness" solution are applied with a capillary or a special pipette to the starting line, which is 1.5-2 cm from the bottom edge of the plate. Liquid layer should be about 5 mm. Ascending chromatography is usually used for separation. The lower part of plate is placed in liquid, which is poured into chromatographic column. Plate with fixed layer is placed into chromatographic column, saturated with solvent vapours, vertically, with unfixed layer – at an angle 15-20. An exposition time is from 30 min to 1,5 hour.

The following systems of solvents are often used: 1) chloroform – aceton-dyethylamin (5:4:1); 2) chloroform-diethylamine (9:1); 3) n-butanol-methanol-diethylamin (17:1:2); 4) chlrooform-methanol-acetic acid <18 : 1 : 1); 5) benzene-methanol (19 ι 1); 8) chloroform –ethanol (8:2); chloroform-aceton-diethylamine (5:4:1); acetone-diethylamine (5:4:1); acetone-ammonia solution (95:5).

After drying thin-layer chromatograms are treated with the reagents of paper chromatography.

1. Paper chromatography.

There are a lot of techniques of paper chromatography. Ascending, descending and radial chromatography are the simplest and most widely used methods. In ascending and descending chromatography, the extraction and the "witness" solution are applied to the starting line of the strip of chromatographic paper with a capillary or a special pipette.

The method of fixing of the prepared chromatogram in a chromatographic chamber depends on the chromatographic method. Solvent system should provide maximum separation of alkaloids containing in extract. When the chromatogram contacts the liquid, the solvent begins to propagate slowly along the paper. When the solvent passes through the area where the total alkaloids are applied, the substances are dissolved and they move together with the liquid. In each section of the chromatogram, a repeated redistribution of substance takes place between mobile phase and stationary phase. That’s why the rate of movement of substances along the paper is different and depends on the distribution coefficient. The distance between starting line and front can be different (20-40 sm). It depends on the difference between Rf of substances containing in the extract. If the difference between Rf is smaller, then the distance from the starting line to the front is greater. Exposition time is from 3 to 20 hours, that is determined by the brand of chromatographic paper, solvent system and oth. The following system solvents are used: 1) n-butanol-acetic acid-water (5:1:4); 2) n-butanol-acetic acid-water (10:2:5); 3) n-butanol- hydrochloric acid –water (100:4 water until saturation); 4) ethylacetate –acetic acid- water (11 ι 21 : 85); 5) n-butanol-pyridine-water (10:2:5) and oth.

For deteciton of alkaloids dried chromatogram is treated with any reagent which gives coloured compounds with them. Dragendorff’s reagent is often used. When the chromatogram is treated with this reagent orange or orange-red spots (alkaloids) are formed on yellow background. Iodine vapours can be used for the detection of alkaloids (brown spots are produced). Saturated chloroform solution of antimony trichloride with the further heating at 105 C can be used for detection of steroidal alkaloids and brick-red colour is produced..

*Quantitative determination.* The individual method for the determination of alkaloid content is established for each material. This method includes isolation, purification and quantitative determination.

The methods of quantitative determination are:

* Acid-base titration in anhydrous medium for all forms of alkaloids (salts and bases of pachicarpin, tropane alkaloids, cocaine, platyphylline, salsoline, morphine, spherosine, ephedrine and oth.)
* Neutralization: а) direct titration of free alkaloids (bases) by acidic solution; б) back titration of acid excess by alkaline solution; в) direct titration of alkaloids by iodine solution or other complxing reagent, with whom alkaloids form insoluble compounds. The quantitative determination of caffeine, theobromine and theophyllin can be identified by the formation of insoluble salts, for example polyiodides or nitrates
* gravimetry
* methods based on individual chemical properties of alkaloids;;
* physico-chemical (photometry, polarography, polarimetry, spectrophotometry and oth.)

**Physical and chemical properties of alkaloids**

Alkaloids have the properties of ammonium compounds. Thus they are present in a salt a base form. Primary amines (mescalin), secondary amines (ephedrine), tertiary amines (atropine) and the derivatives of quaternary ammonium compounds are present. Tertiary amines are the most numerous.

Most alkaloids contain carbon, hydrogen, nitrogen and oxygen. In addition some alkaloids contain sulfur (alkaloids of nuphar lutea), rarely- chlorine or bromine.

Oxygen-containing alkaloids are usually crystalline or amorphous substances, odorless, colorless, with bitter taste. Optically active (especially L-isomers).

Coloured alkaloids can be found rarely (for example, berberine has a yellow colour in the leaves of barberry).

Oxygen-free alkaloids are volatile oily liquids with unpleasant smell. Oksigensiz alkaloidlərin duzları kristal şəklində olur. This group of alkaloids includes tobacco (nicotiana iavasit) and nicotine in the leaves of wild tobacco (Nicotiana rustica), coniine isolated from hemlock (Conium maculatum), pelletierine from the bark of pomegranate (Punica qranatim), pilocarpine from leaves of pilocarpus (Pilocarpus), anabasine from Anabasis aphylla.

Free alkaloids (bases) are readily soluble in organic solvents and insoluble or poorly soluble in water, produce salts with acids. But caffeine, ephedrine, pylocarpine, codein are soluble in water. Most of them are soluble in alcohol, some (papverine-hydrochlrode, cocaine hydrochlrode) in chloroform. Alkaloid salts are readily soluble in water, but insoluble in organic solvent. Water-soluble alkaloid salts are usually used in medicine. For example, atropine-sulfate, strycnine-nitrate and oth.

Alkaloids are optically active substances. Some of them fluoresce under UV-light. Alkaloids with a small degree of dissociation (caffeine, colchicine) don’t produce salts. Codeine has the strongest basic properties, caffeine is the weakest.

рН of water –alcohol solutions of alkaloids doesn’t exceed 8-8,5.

Alkalines, ammonium solution, carbonates and magnesium oxide decompose the alkaloid salts to free bases.

Alkaloids are weak bases, their salts possess varying degrees of strength. Caffeine has the weakest basic properties ( dissociation constant K = 10-14 , codeine is the strongest (K = 10-7). 9

Free alkaloids (bases) are soluble in organic solvents (alcohol, chloroform, diethyl ether, bezene and oth.) and insoluble or poorly soluble in water. However there are alkaloids soluble in water (for example, caffeine, ephedrine, codeine and oth.).

Alkaloid salts are soluble in water, practically insoluble or poorly soluble in organic oslvents (except alcohol). Some alkaloid salts (for example papaverine hydrochloride) are soluble in chloroform.

Alkaloids are often present in the salt form and accumulated in cell sap in plants. Alkaloids often occur in plants as salts of organic or inorganic acids (citric, oxalic, succinic, acetic, sulfuric, phosphoric and oth. acids). Medicinal preparations of alkaloids are based on chlorides, nitrates, phosphates, sometimes tartrates or salicylates of them. In some plants alkaloids bind with specific organic acids that are characteristic for a certain family or even individual plant. For example, meconic acid (β-hydroxy-γ-pyrone--α:, a'-dicaroboxylic) acid is characteristic for papaver somniferum, quinic acid(cyclohexane-1,3,4,5,-tetrahydroxycarboxylic) – for cinchona tree.

Alkaloids produce salts with acids which are similar ammonium salts produced by ammonium and hydrochloric acid.

Ammonia solution, magnesium oxide, carbonate magnesium decompose alkaloid salts into free alkaloids (bases). Alkalines destruct alkaloids.

Alkaloids containing a phenol hydroxyl group produce phenolates with alkalines and react with iron (III) salts. Morphine is precipitated by alkaline, then it is dissolved in excess that gives a possibility to determine it among other alkaloids. Esters of alkaloids (atropine, cocaine) are saponified by alkalines.

*Spectral analysis*

In addition to qualitative reacitons, chromatographic analysis, determination of melting point, specific rotation, molecular weight, UV-, IR-, proton NMR, mass-spectroscopy are widely used for identification of alkaloids. In this case, it is not necessary to take the spectra of a sample, since it can be taken from the literature.

UV-, IR-, proton NMR-, mass-spectroscopy are widely used to establish the structure of the alkaloid. Since the interpretation of the spectra enables to establish the presence or absence of conjugated double bonds and various functional groups (carbonyl, N-methyl, hydroxyl, etc.), an aromatic ring, etc.

In IR spectra absoprtion bands at 1740 sm -1 indicates the presence of ester bond; 2940 sm-1 – alcohol hydroxyl. In UV spectra of atropine λπ,βχ = 252, 258, 262 nm are noted which are characteristic for conjugated bonds in aromatic cycle.

In IR spectra of morphine absoprtion bands at 3220-3480 sm-1 are typical for phenolic and alcohol hydroxyl groups. In UV spectra of morphine λmax=284 nm indicates the presence of aromatic cycle.

**Distribution and role of alkaloids in plants**

Today alkaloids comprise 10% of world flora.

Alkaloids are rarely in lower plants (fungi, Claviceps, Penicillium) and gymnosperms (Ephedra and Taxas genus). The plants of Berberidaceae, Ranunculaceae, Papaveraceae, Fumariaceae, Apocynaceae, Gentianaceae, Asclopediaceae, Solanaceae, Lobeliaceae, Fabaceae, Rutaceae, Equisetaceae family are rich in alkaloids.

Alkaloids are localized in different organs of plants,for example cinchona tree bark, aconite tuber, leaves of cocaine shrub, fruits of anisetree, seed of physostigmine.

In plants alkaloids are accumulated in the form of salts of citric, succinic, tartaric, malonic, acetic and oth. acids. Each plant contains as a rule the mixture of several alkaloids, sometimes they contain about 50 alkaloids and they are similar for their structure (for example, claviceps purpurea, Catharanthus roseus). However, there are some plants that have only one alkaloid (ricinine in ricin).

The content of alkaloids in plants is hundredth of a percent to 1%, rarely – 10-15% (cinchona tree bark).

Alkaloids are involved in major life process of plant organism. They play a role as hormones and catalysators in plant respiration oxidizing until peroxides, they are converted into N-xoides, oxygen thus obtained is used for other biochemical transformations by plants.

Alkaloids are regulators of metabolism and growth of the root system.

It is established that they mostly protect plants from feeding by insects (antifeedants). Alkaloids have sensitizing properties, due to which the sensitivity of plant cells to light increases, as a result, it enhances the formation of generative organs and the development phase

**Pharmacological effects and applicaiton of alkaloids**

The classificaiton of natural alkaloids demonstrates that their chemical structures are various, so a wide range of pharmacological activity of alkaloids is manifested.  Effects of some alkaloids on the human body are studied well. These substances effect on specific receptors or influence enzyme activity.

Stimulation or blockade of receptors by natural alkaloids or their derivatives leads to the treatment or prevention of pathological conditions. Some alkaloids have influence on enzyme activiity, which effect is associated with induction or reduction of enzyme acitivity.

Alkaloid-analeptics directly or by means of refluxes stimulate vitally active centres of medulla. . They are used in inhibition of Central Nervous System, asphyxia, collapse, cardiac insufficiency.

Alkaloids exert a direct or reflex effect on CNS, on enzyme acitivity or specific receptors. Receptors are named due to the sensitivity to natural mediators and their antagonists. For example, m-cholinoreceptors (sensitive to muscarine), n-cholinoreceptors (sensitive to nicotine), H1 and H2 –histamine, dopamine, serotonine, opioid and oth. Stimulation or blockade of receptors by natural alkaloids or their derivatives leads to the treatment or prevention of pathological conditions. Alkaloids have strong influence on enzyme activity. Some of them are connected with induction or reduction of enzyme activity. .

For example physostigmine, neostigmine and other anticholinesterase remedies decrease acetylcholine. Alkaloid-analeptics directly or by means of refluxes stimulate vitally active centres of medulla. They are used in inhibition of Central Nervous System, asphyxia, collapse, cardiac insufficiency.

Some of them are directly used in chemist’s shops for manufacturing of extemporal medicines (infusions, decoctions). Most of alkaloid-containing plant species is used for extraction of more than 100 individual alkaloids and production of different total preparations (tinctures, extracts, concentrates and oth.). Various dosage forms (solutions, ampoules, tablets, dragee) are produced on the basis of individual alkaloids.

Thus when the first alkaloids were synthesized in the 19th century, they immediately found application in clinical practice. Alkaloids are used in the form of salts in medical practice.

Table. Application of alkaloids in medicine

|  |  |
| --- | --- |
| Alkaloid | Pharmacological effect |
| Atropine, hyoscyamine, scopolamine | Anticholinesterase |
| Aymalin | Anti-arrhythmic |
| Emetine | Antiprotozoal, emetic |
| Ergoalkaloids | sympathomimetic, vasodilation, antihypertensive |
| Physostigmine | acetylcholinesterase inhibitor |
| quinine | Antipyretic, antimalarials |
| quinidine | Anti-arrhythmic |
| Codeine | Emetic |
| cocaine | Anesthetic |
| colchicine | Arthrifuge |
| morphine | Narcotic analgetic |
| reserpine | Antihypertensive |
| tubocurarine | Miorelaxant |
| vinblastine, vincristine | Antitumour |
| vincamine | Vasodilation, antihypertensive |

At present many synthetic and semisynthetic drugs are structural modifications of the alkaloids which were designed to enhance or change the primary effect of the drug and reduce unwanted side effects. For example, naloxone, an opioid receptor antagonist, is a derivative of thebaine which is present in opium.

Most alkaloids are psychoactive substances. Cocaine and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as ibigonine, dimethyltryptamine, psilocybin and oth.) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers.

There are alkaloids that do not have strong psychoactive effect themselves, but are precursors for semi-synthetic psychoactive drugs. For example, ephedrine and pseudoephedrine are used to produce methcathinone (ephedrine) and methamphetamine.

Alkaloid

N

Pyridine

N

H

Piperidine

N

Chinolizin

9

8

7

6

10

5

4

1

2

3

N

Chinolizidin

N

N

C

H

3

**DEMO**

Nicotine

N

H

N

Anabasine

O

N

N

\_ \_

-

H

**DEMO**

Cytisine

N

N

\*

H

I

N

N

\*

H

I

**DEMO**

or

Pachycarpine hydriodide

nortropaneeee

8

N

-

H

H

2

C

C

H

C

H

2

C

H

2

C

H

2

C

H

H

2

C

7

6

1

5

4

3

2

nortropan

N

-

C

H

3

**DEMO**

Tropane

N

-

C

H

3

H

O

H

Tropine

N

-

C

H

3

H

O

H

C

O

O

H

Ecgonine

N

-

C

H

3

O

-

C

-

C

6

H

5

C

-

O

H

O

O

**DEMO**

Cocaine

N

7

6

5

8

4

3

2

1

Pyrrolizidine

C

H

3

N

1-methylpyrrolizidine

C

H

23

N

O

O

=

C

-

C

-

C

H

2

-

C

H

-

C

-

C

=

O

C

H

O

H

O

C

O

O

H

C

H

3

C

H

3

C

H

3

C

H

O

H

C

H

O

H

C

O

O

H

**DEMO**

\*

Platiphylline-hydrotartrate

N

2

3

4

5

6

7

8

1

**DEMO**

Quinoline

N

7

6

1

5

4

3

2

N

C

H

2

C

H

2

C

H

H

2

C

H

2

C

C

H

2

C

H

2

8

or

Quinuclidine

N

C

H

3

O

\*

H

N

O

**DEMO**

Echinopsine-nitrate

C

O

O

H

O

H

O

H

O

H

H

O

Quinic acid derivative

1

N

N

8

2

3

4

5

6

7

8

2

7

6

1

5

4

3

C

H

3

O

C

O

H

H

C

H

=

C

H

2

1

1

x

1

0

9

Quinine

1

2

3

4

5

6

7

8

N

**DEMO**

Isoquinoline

N

H

1,2,3,4- Tetrahydroisoquinoline

N

C

H

2

1-benzilisoquinoline

N

-

C

H

3

**DEMO**

Aporphine

1

2

3

4

5

6

7

8

1

0

1

1

N

H

1

2

1

3

1

4

1

5

1

6

9

Morphinane

N

O

C

3

H

O

C

3

H

O

C

3

H

O

C

3

H

Papaverine

O

H

O

R

C

H

3

N

2

1

3

4

1

2

1

1

1

0

9

1

3

1

6

1

4

1

5

5

6

7

8

H

O

H

H

R = H morphine

R = CH3 codeine

O

O

3

H

C

O

3

H

C

C

H

3

N

Tebaine

The indole nucleus can be viewed as a condensation of the pyrrole and benzene ring

1

2

3

4

5

6

7

N

H

**DEMO**

indole

8

N

1

2

3

4

5

6

7

indolizin (piperolidin)

8

2

7

6

1

5

4

3

1

0

1

1

9

1

2

1

3

N

N

H

β-carboline

1

2

3

4

5

R

N

-

H

N

**DEMO**

imidazole

-

R

N

N

R1

O

O

C

H

2

 pilocarpine – R=CH3; R1=C2H5

pilocarpidine – R=H; R1=C2H5

pilosine – R=CH3; R1=C6H5 – CHOH

O

H

O

H

C

H

3

N

2

1

3

4

1

2

1

1

1

0

9

1

3

1

6

1

4

1

5

5

6

7

8

H

O

H

H

Morphine

O

H

O

H3C

C

H

3

N

2

1

3

4

1

2

1

1

1

0

9

1

3

1

6

1

4

1

5

5

6

7

8

H

O

H

H

Codeine

-

CH3

N

N

C2H5

O

O

C

H

2

Pilocarpine

-

H

N

N

C2H5

O

O

C

H

2

Pilocarpidine

-

CH3

N

N

C6H5 – CHOH

O

O

C

H

2

Pilosine

C

C

N

C

H

3

H

O

H

H

H

C

H

3

Ephedrine

C

C

N

C

H

3

H

O

H

H

H

C

H

3

Pseudoephedrine

C

H

2

N

H

C

O

(

C

H

2

)

4

C

H

C

H

C

H

C

H

3

C

H

3

O

3

H

C

O

H

Capsaicine

C

3

H

C

3

H

C

C

H

2

H

C

N

H

C

N

H

2

N

H

Qalegine

N

H

O

O

C

H

3

O

H

3

C

O

3

H

C

O

3

H

C

C

O

C

H

3

A

B

C

Colchicine

N

H

O

O

C

H

3

O

C

O

3

H

C

O

3

H

C

C

H

3

C

H

3

A

B

Colchamine

Colchicine: R = OCH3;

Colchicoside: R = glycosyl

C

H

C

O

O

C

C

H

2

C

C

C

H

2

O

O

H

O

C

H

3

C

H

3

C

H

3

H

C

N

Platyphylline

C

C

O

O

C

C

H

2

C

C

C

H

2

O

O

H

O

C

H

3

C

H

2

C

H

3

H

C

N

Seneciphylline

C

H

2

O

C

O

O

C

O

C

C

2

H

O

H

C

H

C

H

3

C

C

H

3

C

H

C

3

H

N

Sarracine

N

N

H

Anabasine hydrochloride

O

O

G

l

c

O

H

O

H

O

O

Harpagoside

*Lobelia inflata chemycal composition*

H

2

H

2

C

C

O

C

H

O

H

(-)-Lobeline

N

O

N

Aphylline

C

H

2

O

H

N

N

Lupinine

N

C

H

3

O

C

C

H

C

6

H

5

C

H

2

O

H

O

Hyoscyamine

N

C

H

3

O

C

C

H

C

6

H

5

C

H

2

O

H

O

O

Scopolamine

C

O

C

H

C

H

2

O

H

O

N

C

H

3

H

\*

L-hyocyiamine

C

O

C

H

C

H

2

O

H

O

N

C

H

3

H

\*

Tropic acid

D-Hyoscyamine

C

O

C

H

C

H

2

O

H

O

N

C

H

3

H

O

\*

L-Scopolamine

C

H

3

N

O

H

H

Tropine

(tropan-3-α-ol)

O

N

C

H

3

O

H

Scopine

N

C

H

3

O

H

C

O

O

H

Ecgonine

N

C

H

3

O

C

O

C

6

H

5

C

O

O

C

H

3

Cckaine (ether of methylbenzoylecgonine )

N

N

O

Thermopsine

N

N

O

R

Cytisine R=H

Methylcytisine R=CH3

N

N

O

H

Cytisine

N

N

O

CH3

Methylcytisine

O

O

N

Securinine

C

H

3

N

O

C

3

H

O

Nufaridine

O

C

H

3

N

O

H

S

O

H

N

C

H

3

O

Nuphleine

R

O

H

N

4

5

6

3

2

1

9

8

4

7

N

5

6

7

8

1

2

3

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/

R [α]D alcohol

Quinine OCH3 -1580 stereoisomers

Quinidine OCH3 +3340

Cinchonine H +2230 stereoisomers

Cinchonidine H -1780

H3CO

O

H

N

4

5

6

3

2

1

9

8

4

7

N

5

6

7

8

1

2

3

/

/

/

/

/

/

/

/

[α]D alcohol

Quinine OCH3 -1580 stereoisomers

H3CO

O

H

N

4

5

6

3

2

1

9

8

4

7

N

5

6

7

8

1

2

3

/

/

/

/

/

/

/

/

[α]D alcohol

Quinidine OCH3 +3340

H

O

H

N

4

5

6

3

2

1

9

8

4

7

N

5

6

7

8

1

2

3

/

/

/

/

/

/

/

/

[α]D alcohol

Cinchonine H +2230 stereoisomers

H

O

H

N

4

5

6

3

2

1

9

8

4

7

N

5

6

7

8

1

2

3

/

/

/

/

/

/

/

/

[α]D alcohol

Cinchonidine H -1780

O

H

N

H

2

N

C

H

3

Native alkaloid

N

C

H

3

O

Echinopsine (1-methyl-quinolone-4)

N

H

N

C

H

3

Echinopsidine

C

H

3

N

O

H

N

H

2

Echinorine

C

H

3

N

C

H

3

N

N

C

H

3

O

Echinopsine

***chemical composition of Plaun***

O

N

Licopodine

N

O

H

C

H

3

O

O

Annotinine

O

O

O

H

3

C

N

C

H

3

O

O

O

C

H

3

O

C

H

3

Narcotine

***chemical composition of*** *Ungeria*

O

H

O

O

3

H

C

N

C

H

3

Galantamine

N

O

H

O

H

O

O

Licorine

***chemical composition of*** *Cephaelis ipecacuanha*

O

C

H

3

O

C

H

3

H

H

N

H

C

H

2

5

H

2

C

H

N

O

C

3

H

O

C

3

H

(-)-Emetine

O

C

H

3

O

C

H

3

H

H

N

H

C

H

2

5

H

2

C

H

N

O

H

O

C

3

H

(-)-Cephaleine

O

C

3

H

O

C

3

H

N

C

H

3

O

C

H

3

O

C

3

H

Qlaucine

O

3

H

C

O

3

H

C

N

C

H

3

N

3

H

C

O

H

O

C

H

3

O

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1

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Talmine

N

O

C

H

3

O

C

H

3

O

O

Canadine

O

3

H

C

O

3

H

C

N

C

H

3

N

3

H

C

O

CH3

Прог

O

C

H

3

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4

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H

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C

H

3

O

C

H

3

O

Fetidine

O

3

H

C

O

H

O

H

O

3

H

C

C

H

3

C

H

3

N

+

Maqnoflorin (thalictrine)

O

O

O

C

H

3

O

C

H

3

N

8

5

H

2

C

6

1

3

1

2

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1

0

9

4

3

2

1

A

B

D

C

O

H

-

Berberine

N

O

O

O

H

O

C

H

3

O

C

H

3

C

H

2

Berberine

N

H

C

H

3

N

Harmane

N

H

C

H

3

N

O

C

3

H

Harmine

N

N

O

H

Peganine (vasicine)

N

N

O

H

O

Vasicinone

Deoxypeganine hydrochloride

O

C

3

H

N

H

N

C

H

3

Harmaline

O

O

C

H

3

C

H

3

Maltol

O

H

N

C

H

3

O

O

O

O

Chelidonine

N

C

H

3

O

O

O

O

O

Protopine

N

C

H

3

O

O

O

O

C

H

3

O

C

H

3

Allocryptopine

O

O

N

C

H

3

O

C

H

3

O

3

H

C

+

Chelerythrine

O

O

O

O

N

+

Coptisine

N

C

H

3

+

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O

Sanguinarine

N

O

C

H

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C

Hyndarine

N

O

C

H

3

O

C

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3

C

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C

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H

Rotundine

O

O

3

H

C

O

3

H

C

N

H

Stephanine

N

N

C

H

3

H

Harmane

N

N

C

H

3

H

O

C

3

H

Harmine

R

1

2

R

N

C

H

3

N

H

Lysergic acid

Lysergic acid R1- H; R2- COOH

Isolysergic acid R1- COOH ; R2 -H

Ergobasine (ergometrine)

R1 – H; R2 – CO – NH – CH – CH2O

|

CH3

H

HOOC

N

C

H

3

N

H

Lysergic acid

COOH

H

N

C

H

3

N

H

Isolysergic acid

H

N

C

H

3

N

H

CO – NH – CH – CH2O

Ergobasine (ergometrine)

C

H

O

O

R

3

N

N

O

H

R

2

1

R

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergoalkaloids:

R1 R2 R3

Ergotamine - H - H - CH2C6H5

Ergosine - H - H - CH2CH(CH3)2

Ergostine - CH2CH3 - H - CH2C6H5

Ergocristine - CH3 - CH3 - CH2C6H5

Ergocriptine - CH3 - CH3 - CH2CH(CH3)2

Ergocornine - CH3 - CH3 - CH(CH3)2

C

H

O

O

CH2C6H5

N

N

O

H

H

H

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergotamine

C

H

O

O

CH2CH(CH3)2

N

N

O

H

H

H

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergosine

C

H

O

O

CH2C6H5

N

N

O

H

H

CH2CH3

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergostine

C

H

O

O

CH2C6H5

N

N

O

H

CH3

CH3

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergocristine

C

H

O

O

CH2CH(CH3)2

N

N

O

H

CH3

CH3

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergocriptine

C

H

O

O

CH(CH3)2

N

N

O

H

CH3

CH3

N

H

H

N

H

C

O

N

C

H

3

H

H

Ergocornine

H

N

H

C

O

N

C

H

3

N

H

C

H

C

H

2

O

H

C

H

3

H

Ergobasine

N

H

C

H

3

N

H

1

2

3

4

6

7

8

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2

1

3

1

4

1

5

5

8

Ergoline

N

H

C

H

3

N

H

H

C

O

O

H

5

8

D-Lysergic acid

N

H

C

H

3

N

H

C

O

O

H

H

5

8

D-Isolysergic acid

H

N

N

H

2

C

O

O

H

A

B

H

N

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C

R

C

H

3

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R

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D

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C

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3

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C

H

A

B

N

H

R

8

H

Tryptophan Clavines Ergoalkaloides

N

C

3

H

C

H

3

N

N

H

Brevicolline

O

C

3

H

H

N

H

N

H

H

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C

H

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C

H

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C

H

3

Reserpine

O

O

C

3

H

C

N

N

A

B

D

C

E

O

-

+

Serpentine

O

H

C

H

3

N

N

O

H

C

2

H

5

Ajmaline

1

2

İochimbane: R1 = R2 = H

β-İochimbine: R1 = CH3OOC; R2 = OH

N

H

N

1

R

2

Reserpine: R1 = OCH3;

R2 = 3,4,5- trimethoxybenzoyl

Rescinnamine: R1 = OCH3;

R2 = 3,4,5- trimethoxycinnamoyl

N

H

N

H

H

C

O

O

C

3

H

C

H

3

O

H

Raubsine

H

N

H

H

N

H

C

H

2

O

H

O

H

Sarpagine

1

R

2

R

N

O

O

N

R1 R2

Strychnine H H

Brucine OCH3 OCH3

H

H

N

O

O

N

Strychnin

H3CO

H3CO

N

O

O

N

Brusine

N

N

H

C

O

O

3

H

C

Catharanthine

N

N

H

C

O

O

3

H

C

O

H

N

N

CH3

O

C

3

H

O

H

C

O

C

H

3

O

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C

O

C

H

3

Vincaleukoblastine (vinblastine)

N

N

H

C

O

O

3

H

C

O

H

N

N

CHO

O

C

3

H

O

H

C

O

C

H

3

O

O

C

O

C

H

3

Leucocristine (vincristine)

N

N

H

C

O

O

3

H

C

O

H

N

N

R

O

C

3

H

O

H

C

O

C

H

3

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C

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C

H

3

Vincaleukoblastine (vinblastine)

V

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a

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Vincaleukoblastine (vinblastine)

Leucocristine (vincristine)

C

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3

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H

C

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3

Vindaline

N

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C

3

H

H

N

H

H

O

H

C

O

O

C

H

3

O

C

O

C

H

3

Vindoline

Chemical composition of Vinca minor

N

N

O

H

C

H

3

C

O

O

Vincamine

N

N

O

H

C

H

3

C

O

O

O

C

3

H

Vincine

O

N

C

3

H

O

C

H

3

N

N

N

C

H

3

1

2

3

4

5

6

7

8

9

Caffeine

O

N

C

3

H

O

C

H

3

N

N

N

H

Theothylline

O

O

H

O

H

O

H

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H

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H

O

H

O

H

8

6

1

Catechin-61,8-dimer

O

O

H

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H

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H

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H

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H

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H

O

H

8

4

Catechin-4,8-dimer

N

N

C

H

3

O

N

H

O

N

C

H

3

Theobromine

O

H

C

H

3

C

H

3

C

3

H

N

H

C

H

3

O

Solasodine

O

O

A

D

B

C

1

7

Progesterone

O

N

O

O

H

H

Ervine

N

C

H

3

C

H

3

O

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H

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C

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C

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Protoveratrine A

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H

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C

H

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C

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C

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C

H

3

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C

H

3

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C

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H

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H

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C

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H

N

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C

H

3

Aconitine

O

H

2

C

5

H

O

C

H

3

N

O

O

N

H

C

O

C

H

3

O

C

H

3

O

C

H

3

O

H

Lappaconitine

**Glycoalkaloids (steroidal alkaloids).**

Glycoalkaloids are the derivatives of cyclopentanoperhydrophenanthrene with nitorgen having the properties of steroid saponins and alkaloids.

Glycoalkaloids are often used for the synthesis of hormonal preparations of cortisone type.

The structure of steroidal alkaloids is based on cyclopentanoperhydrophenanthrene, which is associated with a heterocyclic system. In position 3, there is an OH group through which the carbohydrate moiety of the molecule is attached; in positions 10, 13, 18 - methyl groups. Most steroidal alkaloids have a double bond in position 5,6. The carbohydrate part of molecule is presented as D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, L-fructose and D-glucuronic and D-galacturonic acids.

Steroidal alkaloids are divided into 2 groups: 1) nitrogen-containing analogues of saponins. They are often found in genus Solanum. Alkaloids of this group form normal (solasodin) and isoseries (tomatidine). 2) nitrogen-containing steroidal compounds, in which rings E and F are condensed. These compounds are often found in genus solanum and veratrum. This group is subdivided into 2 groups: а) yer-veratrum steroidal glycoalkaloids. Their molecule contains about 3 atoms of oxygen, yervina, rubiyervin, isorubiyervin, veramarine, verticine and oth. are typical examples of this group.

b) ceveratrum steroidal alkaloids. Their molecule contains more than 3 atoms of oxygen. The main examples of this group are sabine, veracivine, germine.

*Physico-chemical properties*

Steroidal alkaloids mainly are present, which crystallize from 80% ethanol well. Amorhpous glycoalkaloids are found, for example solanocapsidine. Glycoalklaoids are optically active compounds with specific angle of rotation. They almost don’t dissolve in water, ethyl ether and chlorofrom, dissolve in warm ethanol.

Steroidal alkaloids have basic properties and can produce salts due to the presence of nitrogen. The salts of most glycoalkaloids are amorphous substances (except crystalline solanine hydrochloride), its melting point is 212 C (with decomposition). Glycoalkaloids salts are soluble in water as other alkaloid salts.

Steroidal alkaloids are hydrolysed by enzymes and acids. Alkaline hydrolysis is carried out rarely. Because some glycoalkaloids are resistant to alkaline.

*Isolation from plant material*

One of the distributed isolation method of glycoalkaloids is acid extraction with dilute acids, after extraction they are precipitated by ammonia. Dilute sulfuric acid, 0,5-2% solution of nitric and phosphoric acids, 2% solution of cold metaphosphoric acid, 5% acetic acid and oth. are used for this purpose. The deficiency of acid extraction is poor filtration of fluffy precipitate of “raw” glycoalkaloids, that can be partially avoided by using lime milk for alkalization instead of ammonia solution.

The extraction with acidified alcohol leads to the contamination of extracts with a large amount of accompanying substances, that make it difficult the further analysis and preparative isolation of glycoalkaloids and their aglycones.

Preparation of extract

Crushed plant material is poured with 5% solution of acetic acid at a dilution 1:10, shaken up for 40 min, then it is filtered through paper filter. Several drops of 1% solution of cholesterol in ethanol is added to 1 ml of extract. A precipitate is formed.

Neutral aluminium oxide of (II) and (III) degree of activity according Brockmann is used as sorbent for separation of glycoalkaloids by column chromatography, but the elution is carried out with benzene and chloroform.

Free aglycones are separated well on in-active neutral aluminium oxide. Elution is carried out with the mixture of ethyl acetate and hexane (at dilution 7:3).

UV-, IR- and proton NMR-spectroscopy are widely used for identification and establishement of glycoalkaloids structure.

*Qualitative reactions*

Precipitation and colour reactions are used for detection of steroidal alkaloids and their aglycones in plant material.  Paper, thin layer chromatography are also used.

Glycoalkaloids are precipitated with cholesterol, digitonin, give colour reactions with n-hydroxybensaldehyde, anisaldehyde, resorcinol, formaldehyde. Albert’s reaction (formaldehyde in strongly acidic media) is most often used – crimson-red colour is produced in presence of glycoalkaloids. .

Different mixtures of solvents: methylenethylketone sautrated with water – n-butanol-water (10:2:5) are used for the detection of steroidal alkaloids by paper chromatography. Dragendorff’s regaent or chloroform solution of antimony trichloride with further short-term heating at 105 are used for the treatment of chromatogram. Glycoalkaloids gradually produce brick-red colour.

Silica-gel is used as a sorbent in thin layer chromatography. N-butanol-methanol-diethylamine (17:1:2); chloroform-methanol-acetic acid (18:1:1); chloform-25% ammonia solution (1000:1); hexane-acetone (4:1) and oth. are used as a mixture of solvents. Chromatograms are treated with iodine vapour.

*Quantitative determination*

Methods of quantitative determination of steroidal alkaloids can be divided into the following groups:

1. Titrimetric methods. Glycoalkaloids are well titrated with hydrochloric acid and dimethyl yellow. The deficiency of titrimetric methods is a duration of washing of glycoalkaloids from ammonia.

2. Bromination method.  Glycoalkaloids with double bond in the 5:6 position can be brominated with pyridine-sulfate-bromide. This method is suitable for the analysis of solasodine preparations, not for plant material and intermediate.

3. “Sugar” methods. The detached sugars are titrated with 0,1 n potassium permanganate after acid hydrolysis and precipitation of aglycones. The deficiency of “sugar” methods is some sugars can be precipitated with “raw” glycoalkaloids and thus they can overestimate the results.

4. Gravimetric method. The method is: glycoalkaloids are extracted from plant material with dilute solution of H2SO4 and further precipiation them with ammonia solution. This residue of glycoalkaloids is treated with boiling ethanol and hydrolysed with dilute HCL upon boiling. After alkalization aglycones are exhaustively extracted with benzene. The solvent is removed, the residue is dried to constant mass at 120 C and weighed.

5. Colorimetric methods. Although steroidal alkaloids give colour reactions with most alkaloids, only the reaction with formmaldehyde in strongly acidic media (raspberry red colour) is used for quantitative determination. Coloring with other aldehydes doesn’t follow Beer- Lambert’s law.

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