Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical, Toxicological Chemistry
Pharmacognosy and Botany

SPECIAL PHARMACEUTICAL CHEMISTRY

Steroids. Classification. Cardiac glycosides

Lesson 4

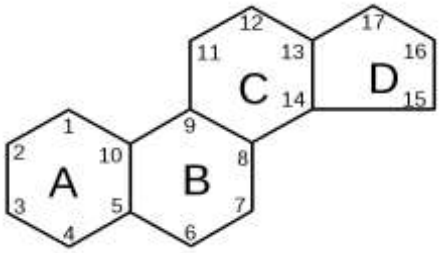
IX term

Volgograd, 2024

STEROIDS

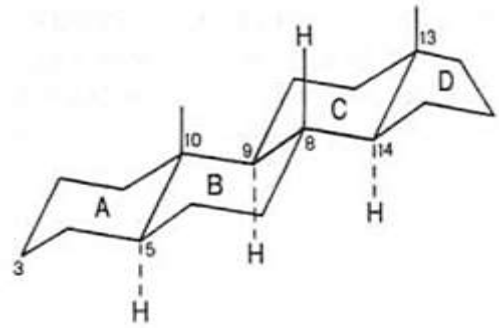
Features of structure

Steroids are a group of natural and synthesised chemical compounds - derivatives of partially or fully hydrogenated 1,2-cyclopentenophenanthrene, in the molecular skeleton of which 17 carbon atoms form 4 articulated rings A, B, C, D.



Due to their complex structure and asymmetry, steroid molecules have many potential stereoisomers. Each of the six-carbon rings (A, B, and C rings) of the steroid core can adopt two different spatial conformations - the ‘’armchair‘’ or ‘’boat‘’ conformation.

In natural steroids, including cholesterol, all rings are in the form of ‘armchair’, which is a more stable conformation. In turn, in relation to each other, the rings can be in cis- or trans-positions.



The methyl groups attached to the steroid cycle at positions 10 and 13 are called angular. The substituent and hydrogen atoms (at position 8, 9, 14) are orientated in space in the cis- or trans-position. It is conventionally assumed that the angular methyl groups are located above the drawing plane (this is indicated by a solid line). If other substituents are in the cis-position, i.e. in the same plane as the angular groups (β-configuration), they are also indicated by a solid line, and if in the trans-position (α-configuration), by a dashed line.

Biochemical role of steroids

Steroids are widely distributed in nature and are involved in a wide variety of biological functions. Steroid synthesis is carried out in the organism of plants and higher animals and humans.

Steroidal in nature are sex hormones, vitamin D, adrenal hormones, bile acids, arthropod moulting and metamorphosis hormones, insect repellents to deter predators, and poisons in the skin of toads. Steroids play an important role in the composition of protoplasm, forming complexes with proteins and participating in the construction of intracellular membranes.

Both natural and synthetic steroids with a similar structure have very different physiological effects, which is why they are widely used in medicine as anti-inflammatory, cardiac, contraceptive and other agents.

Steroids occupy an important place among the drugs used for the treatment and prevention of various groups of diseases in various fields of medicine: endocrinology, oncology, rheumatology, ophthalmology, dermatology, haematology, intensive care medicine, gynaecology, etc. Steroids are used in the treatment and prevention of various groups of diseases. At the same time, a number of drugs used, including for vital indications, do not have non-steroidal analogues.

Classification of steroids

A peculiarity of steroids is the presence of hydroxyl or keto groups in the third position of the cycle. Depending on the structure of the A and B cycles, the placement and nature of the substituents in the molecule and the nature of the side chain, as well as the properties and biological action, steroids are divided into

- Sterols,
- D-vitamins,
- bile acids and alcohols,
- Hormones,
- Steroidal saponins,
- Steroidal glycoalkaloids and alkaloids,
- Cardiac glycosides.

Methods for the analysis of steroid structure compounds

1. In identification tests, the reaction of the formation of coloured and fluorescent substances by the action of *concentrated sulphuric acid* is widely used to confirm the steroid cycle in molecules.
2. *The α-ketol group* is discovered by showing reducing properties.
3. *The keto group* is discovered by the reaction of the formation of ketoximes by interaction with hydroxylamine, and of hydrazones with phenylhydrazine and other hydrazines and hydrazides.

4. *The presence of alcohol and phenolic hydroxyls* in the molecules is confirmed by the esterification reaction followed by the determination of the melting point of the esters formed.
5. *The presence of ester groups* (in acetates, propionates, enanthates, etc.) is confirmed either by the formation of coloured salts of hydroxamic acids or by hydrolysis reactions in alkaline or acid medium.
6. *In the presence of phenolic hydroxyl* in the molecule (natural and synthetic oestrogens), halogenation reactions and formation of azo compounds are also used.
7. Identity tests are also carried out using *UV and IR spectrophotometry.*

For quantitative analysis, the above chemical reactions are used by titrimetric or photocolorimetric methods. UV spectrophotometry is also used.

CARDIAC GLYCOSIDES

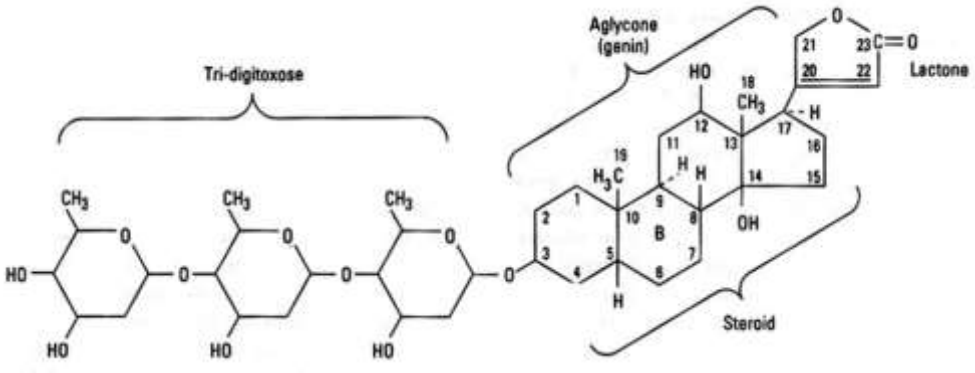
Glycosides are widespread in the plant world.

Glycosides are substances in which the glycosyl part of the molecule (the cyclic form of sugars) is linked via an oxygen, sulphur or nitrogen atom to a radical of an organic compound that is not a sugar. The latter is called an aglycone or a genyne.

According to the nature of the sugar part of the molecule, glycosides are divided into two large groups: pyranosides (glycosides with a six-membered sugar cycle) and furanosides (glycosides with a five-membered sugar cycle).

The process of hydrolysis of most glycosides occurs very easily under the action of enzymes called glucosidases. The hydrolytic cleavage of most glycosides also occurs under the influence of acids, alkalis, heat, etc. The hydrolytic cleavage of glycosides is very easy under the action of enzymes called glucosidases. This is important for the isolation of glycosides from plants, preparation of medicinal substances, their analysis and use in medicine.

Cardiac glycosides are biologically active substances contained in some plant species and have the ability in very small doses to have a specific effect on the heart muscle.

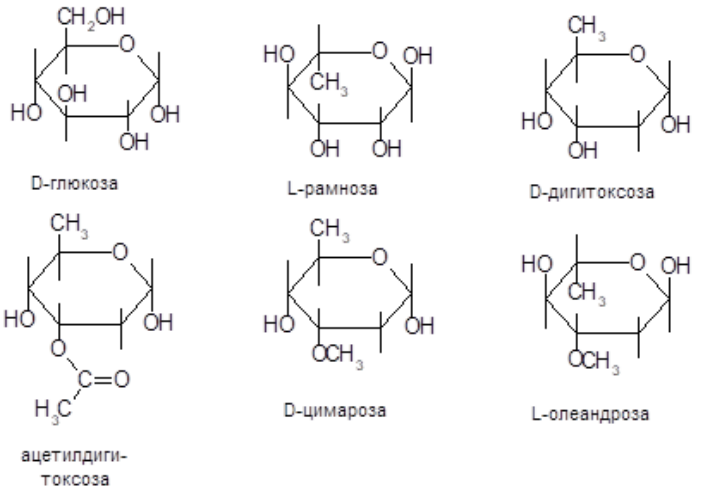


Sources of cardiac glycosides are various species of foxglove (foxglove large-flowered, foxglove purple, foxglove rusty, foxglove woolly), spring gentian, oleander, lily of the valley, obovonik, various species of jaundice, strophanthus, Helléborus and other plants. The use of cardiac glycosides has a long history. The "Papyrus of Ebers" from the XVI century BC mentions the treatment of heart diseases with these plants.

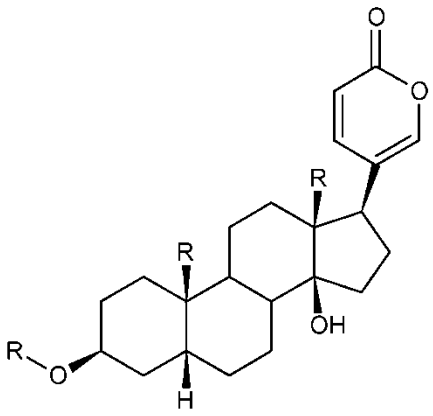
Plants usually contain *primary (genoin) glycosides*. These are very labile substances, easily decomposed (under the influence of enzymes, acids, alkalis, heating) with the formation of secondary glycosides.

The latter can also be easily hydrolysed into aglycones and a sugar component. In 1875, a secondary glycoside, digitoxin, was isolated from purple foxglove, and in the 40s of the 20th century, digoxin was isolated from woolly foxglove.

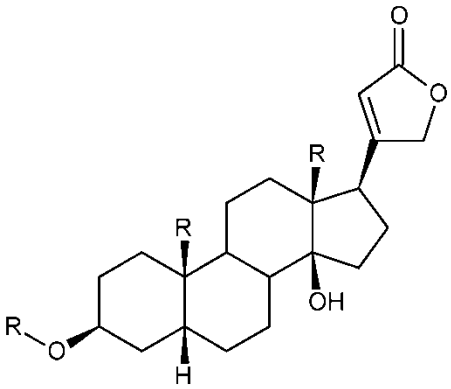
In accordance with their chemical structure, cardiac glycosides are esters in which the aglycone and *mono-, di-, tri- or tetrasaccharide residues* are linked by a glycosidic bond. Some primary glycosides have an acetic acid residue attached to the sugar moiety. The sugars in cardiac glycosides, with the exception of glucose and rhamnose, are specific to this group of substances and are 6-deoxyhexoses or their 3-O-methyl esters. More than 50 carbohydrates have been isolated from cardiac glycosides. The most important monosaccharides found in cardiac glycosides are:



The aglycones (genines) of cardiac glycosides have a steroidal structure, i.e. they are derivatives of cyclopentaneperhydrophenanthrene. They are unsaturated steroidal lactones. According to their chemical structure, the aglycones can be divided into two groups, differing in the structure of the lactone cycle attached at position 17, which usually occupies the β-configuration. The five-membered lactone cycle is part of the structure of cardenolide aglycones, and the six-membered lactone cycle is part of the structure of bufadienolides. The general formulae of these groups of glycosides:



Bufadienolide



Cardenolide

Cardenolides are found in various species of foxglove, strophanthus, lily of the valley, oleander, etc.

Bufadienolides are found in bryophytes, sea bulbs and have also been found in animals (toads).

Relationship between structure and pharmacological action

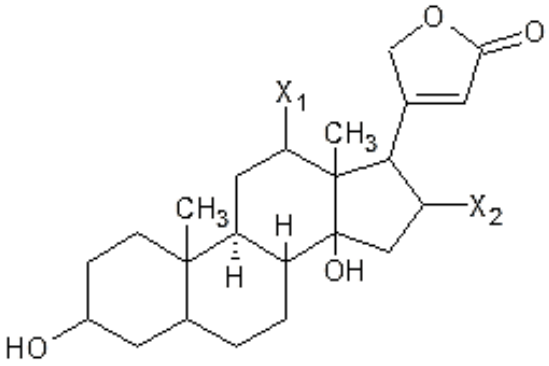
There is a clear relationship between the chemical structure and pharmacological action of cardiac glycosides. The carrier of the biological activity is the aglycone. The sugar moiety attached to the aglycone at position 3 influences the rate of absorption and therefore the duration of action. The more monosaccharide residues in the glycoside molecule, the more active it is.

The specific action of the glycoside on the heart (slowing of the heart rate and strengthening of cardiac contractions) is due to the presence in the aglycone molecule of a five- or six-membered lactone cycle attached at position 17 and hydroxyl at position 14. The cardiotonic effect is strongly influenced by the substituent at position 10. Most aglycones at this position have a methyl or aldehyde group. Oxidation of the aldehyde group to a carboxyl group significantly weakens the effect on the myocardium. Replacement of the steroid cycle of aglycones by benzene, naphthalene derivatives, as well as replacement of the

lactone cycle by other radicals, and even changing the nature of the bond between the steroid nucleus and the lactone, leads to a loss of pharmacological activity.

Glycosides of the foxglove subgroup: digitoxin, acetyl digitoxin, digoxin

Foxglove glycosides belong to the group of cardenolides. They are slowly absorbed and slowly eliminated from the body, have a strong cumulative effect. Their aglycones have the general formula:

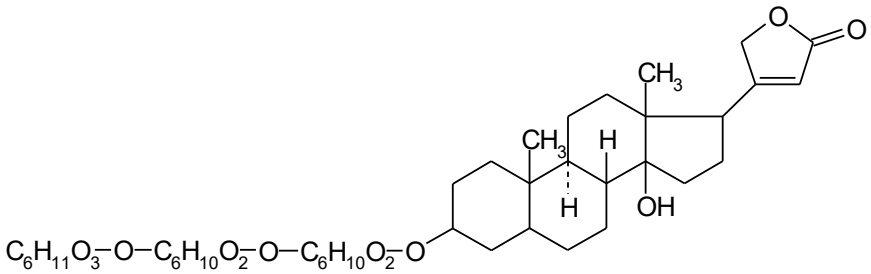


and differ from each other by the radicals X1, X2,

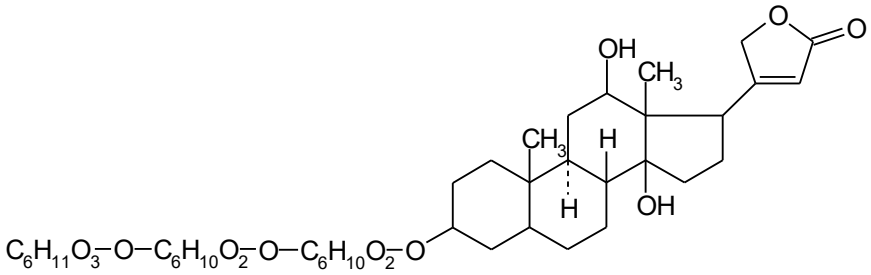
Aglycones	Radicals	
	X_2	X_1
Digitoxigenin	–	–
Hytoxigenin	–OH –	–
Digoxigenin		–OH –

Foxglove purple and woolly foxglove contain primary glycosides. During hydrolytic cleavage, as well as during storage and drying of raw materials under the action of enzymes, primary glycosides are destroyed with the formation of secondary glycosides and other hydrolysis products.

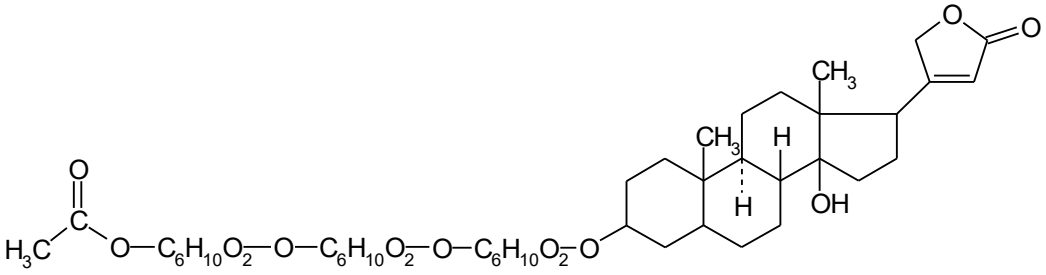
Digitoxin is a white crystalline powder. Practically insoluble in water, slightly soluble in ethanol, difficult in chloroform. Specific rotation from +16 to +19° (1% solution in chloroform).



Digoxin is a white crystalline powder. It is practically insoluble in water, very insoluble in ethanol and chloroform, and soluble in methanol.



Acetyldigitoxin - white lamellar crystals or white crystalline powder. Melting point is 218-227°C. Specific rotation from +24.6° (1% solution in methanol).



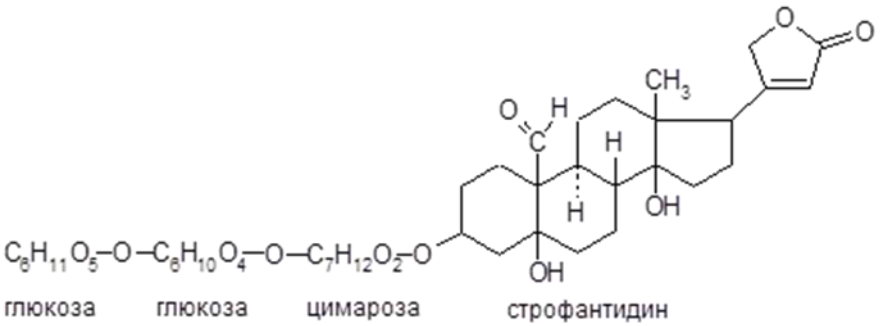
Glycosides of the subgroup Strophanthus

This subgroup includes cardenolides whose aglycones have an aldehyde group (-CHO) at position 10, e.g. strophanthidin, or an oxymethyl group (-CH_2OH), e.g. strophanthidol. The glycosides of this subgroup are rapidly absorbed and excreted from the body, have virtually no cumulative effect, and therefore act quickly.
The preparations Strophanthin K and Corglycone are used in medical practice.

Strophanthin K is a mixture of cardiac glycosides extracted from the seeds of Strophanthus combe and contains mainly K-strophanthin-β and K-strophantoside. K-strophanthin-β consists of a strophanthidin aglycone and a sugar residue (glucose and cimarose); K-strophanthoside has an additional one part α-D-glucose.

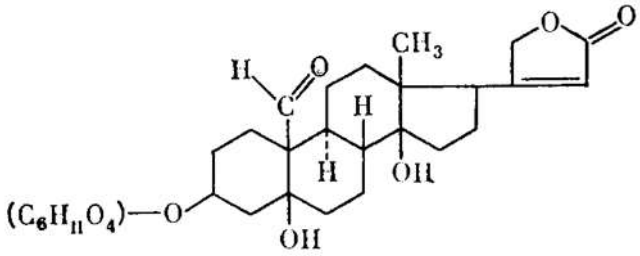
K-strophanthin-β may be regarded as a secondary glycoside of K-strophanthoside. The latter, losing a glucose molecule, is converted into K-strophanthin-β.

Strophanthin K (Strophanthinum K) is a white or white powder with slightly yellowish tinge. Hardly soluble in water and alcohol, practically insoluble in chloroform and ether.



Corglycone as required by the pharmacopoeial monograph is also a sum of at least five glycosides obtained from the leaves of lily of May lily of the valley and used for the preparation of corglycone solution for injection. The major glycoside contained in the leaves of May lily of the valley is convallatoxin. It includes convallatoxigenin (identical to strophanthidin) and the sugar component L-rhamnose.

Corglycon (Corglyconum) is a slightly yellowish amorphous powder, odourless, bitter tasting, easily soluble in alcohol, difficult in water.



Obtaining cardiac glycosides

The synthesis of some cardiac glycosides has been carried out, but has not been used in practice due to the multi-step process and low yields. Therefore, the only industrial source of cardiac glycosides, which has a history of more than a century, is plant material. The extraction process is very complicated because the plant contains enzymes that can irreversibly change the chemical structure of the glycosides. Such changes can also occur under the influence of light, temperature, etc. There are many different methods of extraction.

The plant usually contains several cardiac glycosides and a number of related substances.

The general scheme for obtaining cardiac glycosides consists of preliminary *degreasing* of the plant raw materials with ether or naphtha.

Depending on their solubility, acetone, alcohols, ethyl acetate, often with the addition of water, are used to *extract* the glycosides from the plants.

The raw material is then *infused* with 70% ethyl alcohol to remove chlorophyll. The alcohol is distilled off under vacuum and the primary glycosides are extracted from the residue with warm water, with a secondary insistence for several days.

From the resulting mixture of crude glycosides, resins (with ether) and saponins (with lead acetate solution) are removed. *Adsorption* of impurities on aluminium oxide is also used for this purpose.

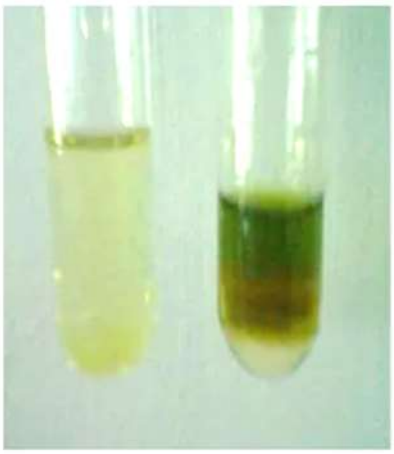
Glycosides are precipitated by saturating the mixture with an aqueous solution of ammonium sulphate.

The separation of the mixture of glycosides is based on the difference in their solubility in organic solvents. Chromatographic methods or countercurrent distribution of substances in specially selected solvent systems are used for separation.

Identification

1. Reactions for the detection of the steroid cycle:

(a) Liebermann-Burchardt reaction. The process is dependent upon the ability of steroids to undergo dehydration when subjected to the action of acetic anhydride and concentrated sulfuric acid. The reaction results in the formation of a pink-coloured layer of acetic anhydride, which gradually undergoes a change in colouration to green or blue. The colouring is dependent on the structure of the genin. In the presence of acetic anhydride and concentrated sulfuric acid, strophanthin and its glycosides undergo a colour change from olive green to yellow.



(b) Fluorimetry. A mixture of phosphoric and sulphuric acids with iron (III) chloride, a solution of iron perchlorate in sulphuric acid, and so forth, is employed as a reagent. Reactions are deemed acceptable when the glycoside to be analysed forms coloured mono- or dianhydride derivatives as a consequence of dehydrogenation.

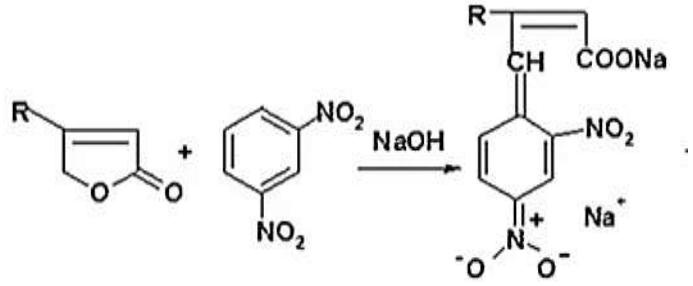
(c) Reaction with concentrated sulphuric acid. A green colouration is observed.

(d) Heating a solution of glycoside in acetic anhydride with a 20-25% solution of antimony (III) chloride to 100°C results in purple colouring.

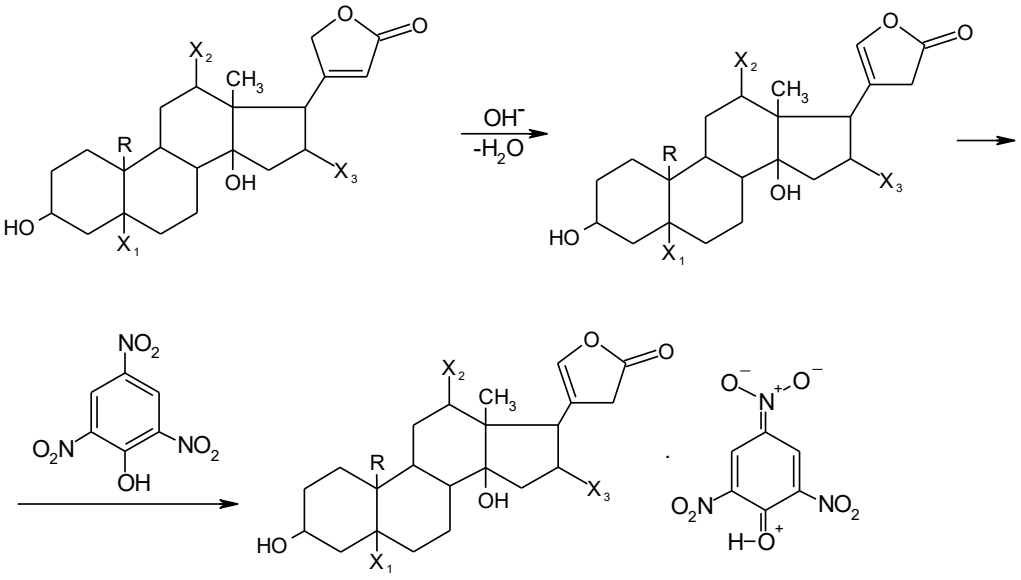
2. Reactions on the detection of a five-membered lactone cycle with a double bond at the α,β-position in the cardenolide molecule.

(a) The Legall reaction, which consists of the formation of a red-coloured product when a cardiac glycoside interacts with a solution of sodium nitroprusside in an alkaline medium.

(*b) The Raymond reaction* is based on the formation of red-purple coloured products of interaction with nitro derivatives of the aromatic series in an alkaline medium, e.g. with m-dinitrobenzene.

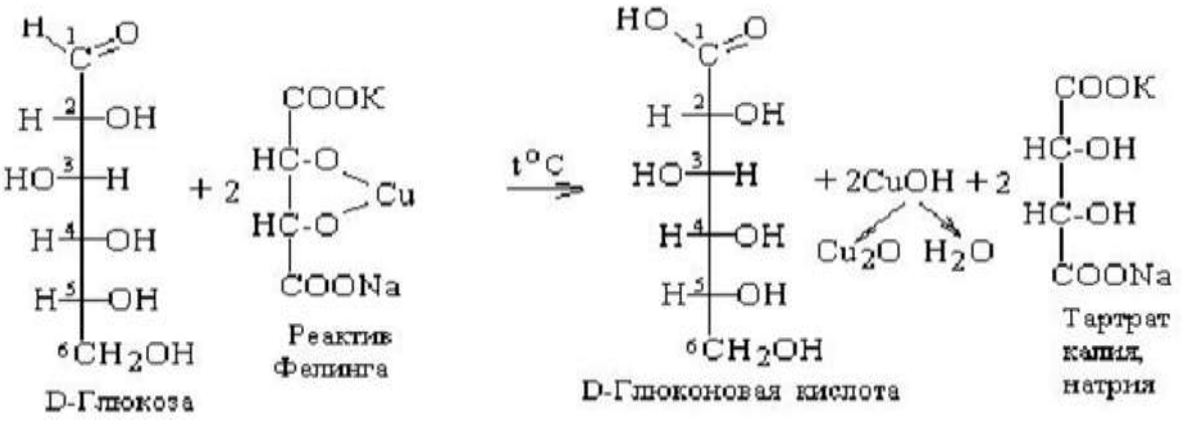


(c) Balier reaction. In the presence of alkali, cardiac glycosides produce an orange colour reaction with picric acid.



3. Reactions to detect the sugar component in cardiac glycosides.

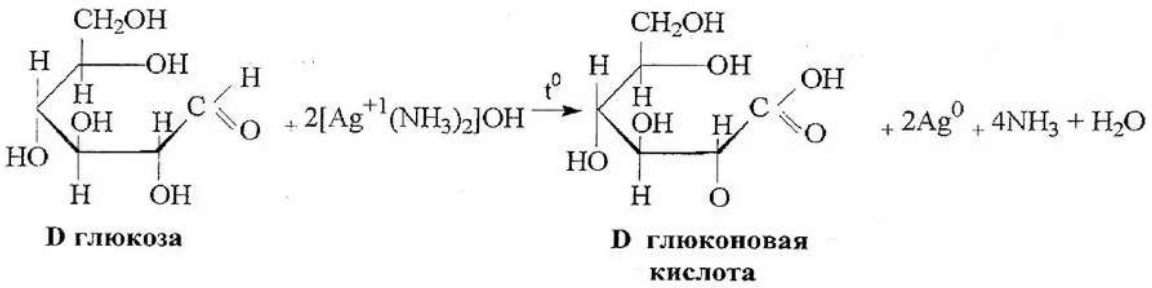
(a) Reaction with Fehling's reagent. Recovery of Fehling's reagent and formation of brick-red precipitate Cu_2O.



(b) Silver mirror reaction. When interacting with ammonia solution of silver nitrate, silver is reduced.

$$2AgNO_3 + 2NaOH \rightarrow Ag_2O\downarrow + 2NaNO_3 + H_2O$$

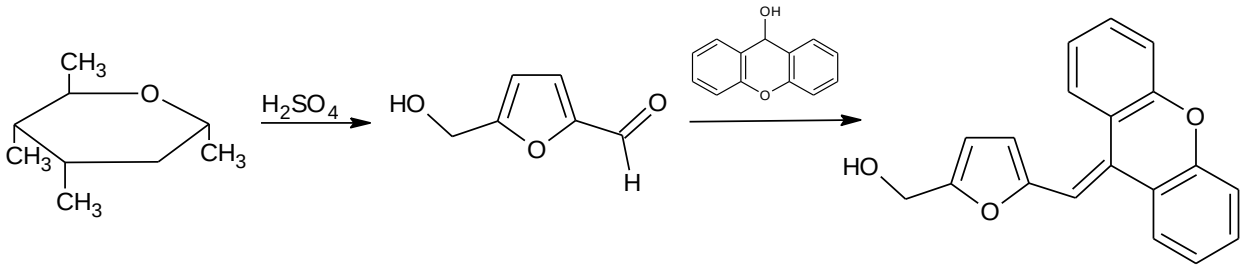
$$Ag_2O + 4NH_3 + H_2O \rightarrow 2[Ag(NH_3)_2]OH$$



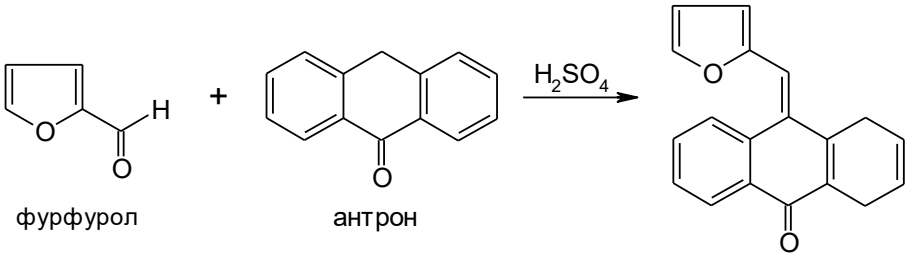
c) Keller-Kiliani reaction. This reaction is specific for 2-deoxysaccharides, which are contained in the molecules of the majority of cardiac glycosides. Of the various methods, the pharmacopoeial monograph recommends a technique consisting of pre-dissolving 1-2 mg of glycoside in glacial acetic acid containing 0.05% iron (III) chloride. The solution is then carefully poured into a test tube containing concentrated sulfuric acid, and the colouring of the upper layer is observed. This confirms the presence of sugar in the molecule, specifically digitoxose, which produces a blue-green colouration. Additionally, the presence of the aglycone, di-hitoxigenin, is indicated by the colouration observed at the border between the two layers,

which may appear purple-red or brown. This method serves to authenticate the presence of digitoxin.

(d) Pezetz reaction. Deoxysaccharides can be identified through the use of xanthidrol. When xanthidrol (dibenzo-γ-pyranol) is heated with the tested glycoside in the presence of glacial acetic acid and a few drops of sulphuric or phosphoric acid are added, a red colouration is observed.



A comparable reaction is elicited by anthrone. The technique is based on the formation of furfurole or its derivatives from sugar components under the action of concentrated sulfuric acid. The condensation product formed between furfurole and anthrone exhibits a green or blue-green colouration.



4. **Infrared spectrometry.** The spectrum should be identical to that of a standard digoxin sample taken under the same conditions.
5. **UV spectrometry** is possible due to the selective absorption in the UV region of the spectrum (215-220 nm) due to the presence of α,β-unsaturated lactone cycle in the aglycones.
6. The identity of cardiac glycosides can be confirmed by **specific rotation.**
7. The identity of the corglycone is established by **TLC.**
8. **The HPLC method** is highly sensitive and allows the determination of not only the major glycosides but also the associated glycosides.

Purity test

When testing the purity of pharmaceutical preparations of cardiac glycosides determine the loss in weight on drying, sulphate ash and heavy metals, transparency and colour of solutions, but special attention should be paid to the presence of impurities of extraneous glycosides.

Quantification

1. ***UV spectrophotometry.*** Digitoxin is quantified at wavelengths of 215 and 219 nm.
2. ***Photometry*** in alkaline medium of coloured products of interaction of cardiac glycosides with nitro derivatives of the aromatic series. The most widely used reagent is picric acid or sodium picrate (Balier reaction) for the determination of digitoxin, digoxin, strophanthin G, etc.

3. HPLC method.

4. ***By biological method activity*** is established by comparison with standard drugs and expressed in FUA (frog), CUA (cat) or PUA (pigeon) units of action. In the biological control method, the lowest doses of the standard and test drug that cause systolic cardiac arrest in experimental animals are determined. Then the content of units of action (UA) in 1.0 g of the test drug, in one tablet or in 1 ml of solution is calculated.

   The disadvantage of biological control is labour-intensive, time-consuming and low accuracy. It is often combined with the use of physicochemical methods.

5. A number of cardiac glycosides and their dosage forms can be determined by ***polarographic method***.

Storage

Medicinal preparations of cardiac glycosides are stored in well-sealed containers to protect them from light and moisture (corglycone at a temperature not exceeding +5°C). Such conditions permit the prevention of their hydrolytic cleavage.

The stability of glycosides is significantly influenced by enzymes, particularly in plant raw materials. Accordingly, it is essential to inactivate enzymes during the storage and preparation of medicinal substances. This is accomplished through the drying of the raw material at temperatures between 40 and 60 degrees Celsius, or through the treatment of the material with ethanol, ether, or chloroform vapours. Subsequently, the stability of the glycosides is markedly enhanced.

Medical use

Cardiac glycosides are employed as cardiotonic agents in the treatment of acute and chronic circulatory or cardiovascular insufficiency. They differ in terms of their potency, duration of action, and the speed of their effect, as well as their impact on the central nervous system.

An overdose results in a pronounced disruption to cardiac function. It is important to consider the capacity of cardiac glycosides to gradually accumulate in the body, a process known as degree of accumulation.