Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical, Toxicological Chemistry
Pharmacognosy and Botany

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SPECIAL PHARMACEUTICAL CHEMISTRY

# Penicillins

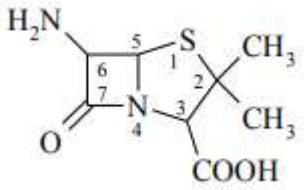
Lesson 10

IX term

Volgograd, 2024

## GENERAL CHARACTERISTIC

The structural basis of natural and semi-synthetic penicillins is 6-aminopenicillanic acid, which includes thiazolidine and lactam cycles:




*6-Aminopenicillanic acid*

The lactam cycle was first discovered in natural penicillins and is characterized by high lability to various factors.

The specificity of biological activity of penicillins is primarily due to the presence of thiazolidine and β- lactam cycles in the molecule. Cleavage of one of them leads to complete loss of activity.

The spatial configuration of penicillin molecules plays an important role in maintaining antibacterial activity.

The nature of the groups attached to the thiazolidine cycle at positions 2 and 3 has no appreciable effect on biological activity.

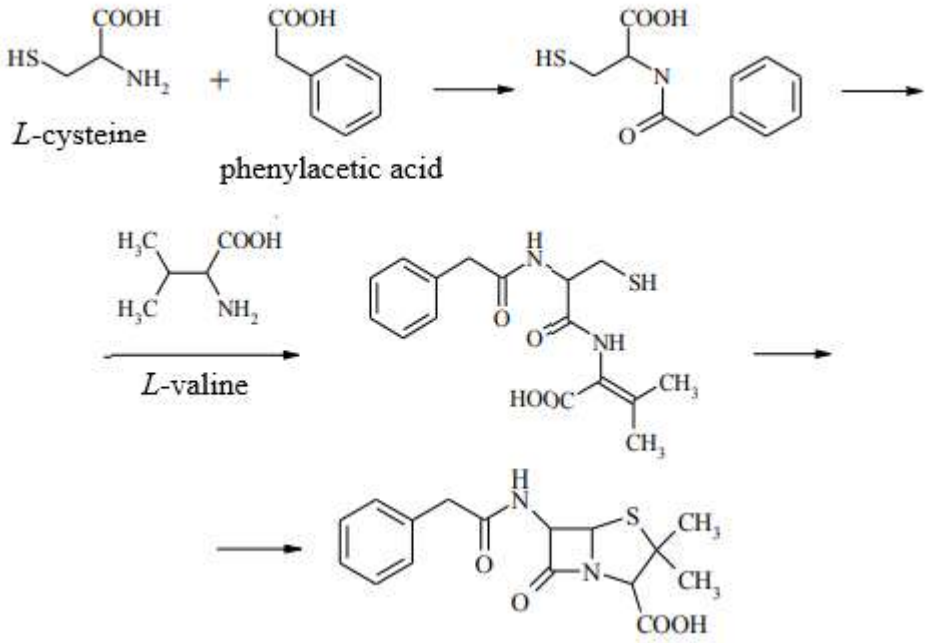
The substituent attached to the amino group at position 6 of the lactam cycle can have different chemical structures. This has led to a number of highly active semisynthetic analogues that are more stable than natural penicillin.

## OBTAINING PENICILLINS

Industrial mold strains selected for microbiological synthesis allow to obtain more than 3 mg/ml benzylpenicillin in 90-120 h of fermentation, which is hundreds of times more than with natural strains.

The composition of the nutrient medium usually includes 1-4% corn extract, 3-5 % lactose, 1-2 % glucose, 0.2-0.5% animal or vegetable fat, mineral salts. An obligatory component is a *precursor* whose chemical structure corresponds to the residue at position 6 of the antibiotic in question. In particular, for the production of benzylpenicillin, the precursor is phenylacetic acid, for the biosynthesis of phenoxymethylpenicillin - phenoxyacetic acid.

The study of penicillin biosynthesis with the help of labeled compounds made it possible to establish that the formation of the molecule is carried out on the basis of amino acids (*L*-cysteine and *L*-valine) and corresponding precursors contained in the nutrient medium. Scheme of the biosynthesis of the benzylpenicillin molecule:




The process of penicillin biosynthesis is carried out under aseptic conditions with continuous air aeration, temperature about 24 °C, pH 6.0-6.5 and constant stirring. The presence of fat in the nutrient medium has a foaming effect and at the same time stimulates the process of penicillin biosynthesis.

Penicillin is separated from the culture broth by filtration or centrifugation. First, mycelium and insoluble mineral salts are separated from the culture broth. Purification of the culture broth and isolation of penicillin from it is performed by solvent exchange in several steps (see Table 1).

*Table 1. Stages of penicillin isolation*

| № | Solvent | Media pH | Penicillin form |
|---|---|---|---|
| 1. | Culture liquid (solvent - water) | 6,0 - 6,5 | Penicillin-acid and ballast substances |
| 2. | Amyl acetate or butyl acetate | 6,0 - 6,5 | Penicillinic acid and ballast extract |
| 3. | Buffer solution (sodium bicarbonate) | 7,0 | Aqueous solution of sodium salt of penicillin with admixture of ballast substances |
| 4. | Chloroform and hydrochloric acid solution | 2,0 | Penicillin-acid extract |
| 5. | Buffer solution (sodium bicarbonate) | 7,0 | Aqueous solution of the sodium salt of penicillin free from ballast substances |

***Penicillins are separated*** by various methods:

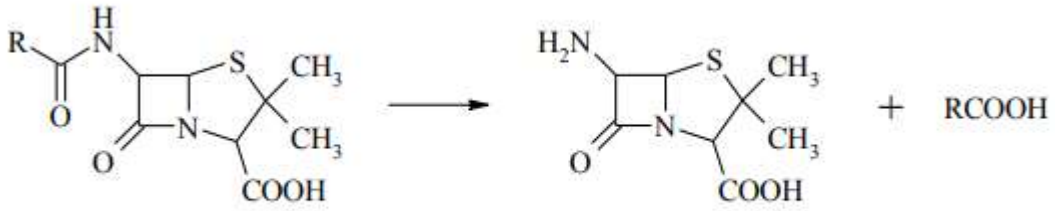
a) adsorption chromatography on activated carbon or alumina;
b) partition chromatography on silica gel;
c) countercurrent partition.

Water is removed from solutions by lyophilic drying at temperatures from - 30 to - 20°C in deep vacuum or by using a spray dryer, which allows to prevent decomposition. Crystalline salts of high purity are obtained by recrystallization from organic solvents.

The currently used natural penicillins are produced by *Penicillum chrysogenum*, *Penicillum notatum* or related microorganisms.

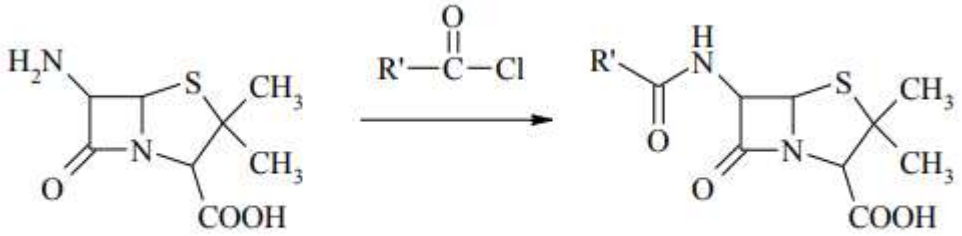
Benzylpenicillin and its numerous dosage forms are characterized by the highest chemotherapeutic efficacy and the lowest toxicity. However, the β-lactam cycle of benzylpenicillin is easily destroyed under the action of penicillase enzyme (β-lactamase) produced by many microorganisms. These circumstances served as prerequisites for the creation of semi-synthetic penicillins, the synthesis of which became possible after the isolation in 1957 of 6-aminopenicillanic acid (6-APA), which is the basis of penicillin antibiotics. 6-APA is obtained from benzylpenicillin or other penicillins by action of penicillin acylase. Chemical cleavage of penicillins to 6-APA is rarely used because of its high reactivity.

The process of 6-APA formation proceeds according to the scheme:



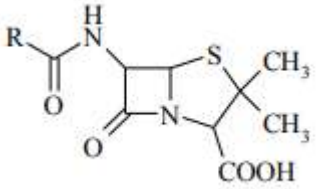

*Natural penicillins* — *6-Aminopenicillanic acid (6-APA)*

6-APA is recovered from the aqueous hydrolysate by extraction or ion exchange chromatography. A large number of semisynthetic penicillins, which are acyl derivatives of 6-APA, have been synthesized on the basis of 6-APA. The most commonly used acylating agents are carboxylic acid chlorohydrides:




*6-Aminopenicillanic acid (6-APA)* — *Semisynthetic penicillins*

Some of the synthesized semisynthetic penicillins, while retaining the high efficacy and low toxicity of benzylpenicillin, have acquired new properties, such as increased resistance and broader spectrum of action. General formula of natural and semisynthetic penicillins:



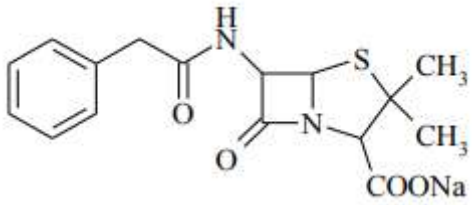
Of the many ***natural penicillins***, the *sodium, potassium, and novocaine salts of benzylpenicillin* (R = $CH_2C_6H_5$) and *phenoxymethylpenicillin* (R = $CH_2OC_6H_5$) are currently in medical use.

***Semisynthetic penicillins*** have an aromatic or heterocyclic substituent. Of these, *ampicillin, oxacillin, carbenicillin dynatrium salt, carfecillin sodium salt* are the most widely used.

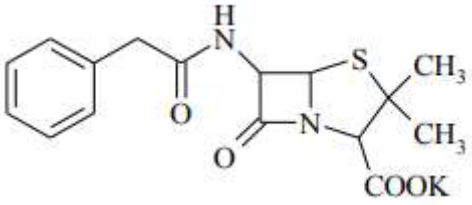
## BENZYLPENICILLIN SODIUM SALT

## BENZYLPENICILLIN POTASSIUM SALT

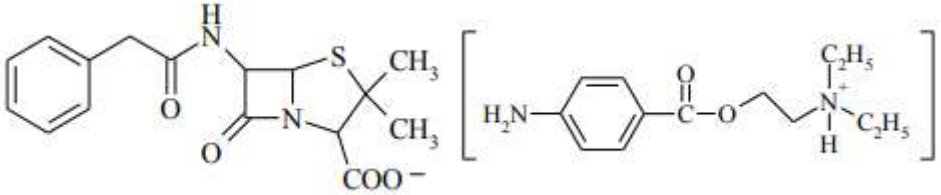
## BENZYLPENICILLIN NOVOCAINE SALT



benzylpenicillin sodium salt



benzylpenicillin potassium salt



benzylpenicillin novocaine salt

## PHYSICAL PROPERTIES

***Benzylpenicillin sodium salt*** **-** White fine crystalline powder, slightly hygroscopic. Specific rotation not less than +270° (2% aqueous solution). Easily soluble in water, soluble in ethyl alcohol, practically insoluble in chloroform and ether.

***Benzylpenicillin potassium salt*** **-** White fine crystalline powder, slightly hygroscopic. Specific rotation not less than +270° (2% aqueous solution). Easily soluble in water, soluble in ethyl alcohol, practically insoluble in chloroform and ether.

***Benzylpenicillin novocaine salt*** **-** White fine crystalline powder, odorless. Specific rotation from +165 to +185° (2% solution in methanol). It is slightly soluble in water and ethanol.

## IDENTIFICATION

### Spectral methods.

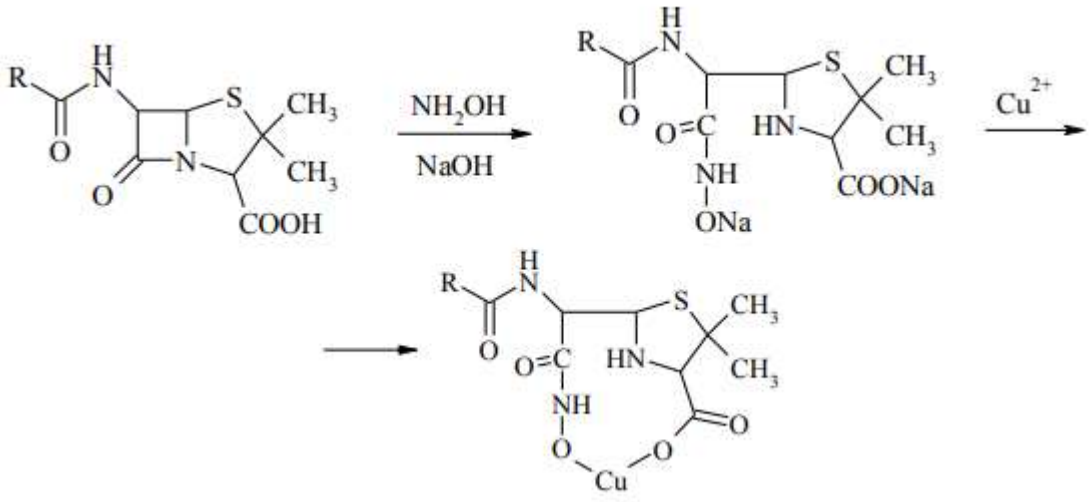
- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

### Chemical methods.

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

1. ***Hydroxamic reaction***

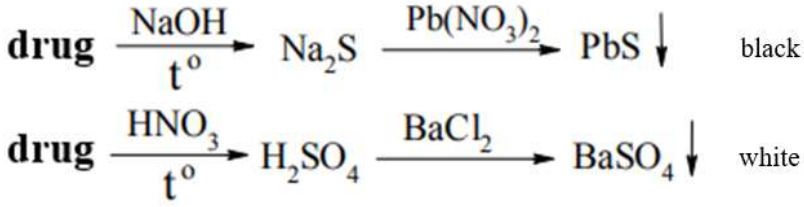
   To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:



With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

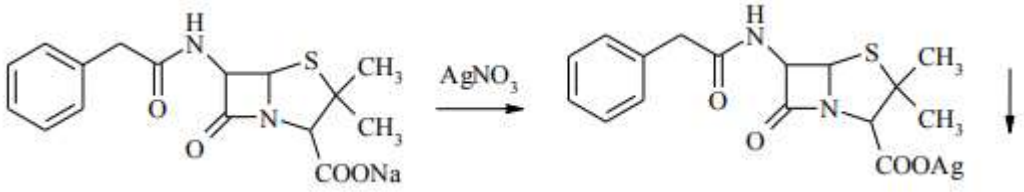
### 2. *The discovery of sulfur*

Sulphur can be detected in penicillin preparations after its transformation into sulphide ion by fusion with caustic alkali or after its transformation into sulphate anion after mineralization with concentrated nitric acid.




### 3. *Reaction with silver nitrate solution*

Interaction with silver nitrate produces a white precipitate that is soluble in excess ammonia.




### 4. *Reaction with ammonia solution of silver nitrate*

Penicillin preparations are capable of oxidation by ammonia solution of silver nitrate with formation of metallic silver precipitate when heated.

### 5. *Reaction with ammonia solution of copper nitrate*

The identity of penicillin antibiotics can be confirmed by oxidation with ammonia solution of copper nitrate on heating. A dark yellow coloration appears.

### 6. *Reaction with resorcinol in sulfuric acid*

In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

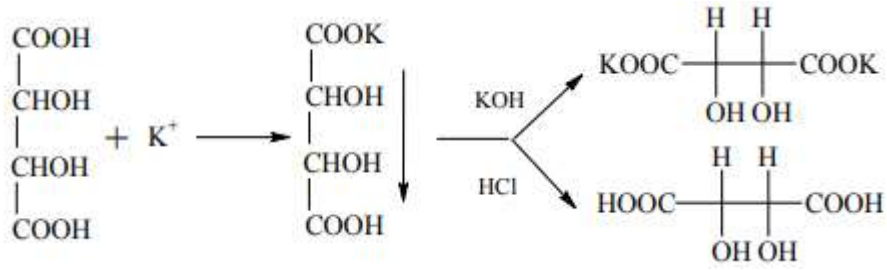
### 7. *Discovery of sodium or potassium*

Since benzylpenicillin salts dissociate very weakly in solutions, it is necessary to mineralize the preparation by burning in a crucible beforehand

to detect sodium or potassium ions. The residue is then dissolved in water and filtered. The filtrate is tested for sodium or potassium ion using the appropriate reactions

**Potassium**

***a) Potassium salts with tartaric acid solution*** give a white crystalline precipitate of acidic salt, soluble in solutions of mineral acids and alkalis:

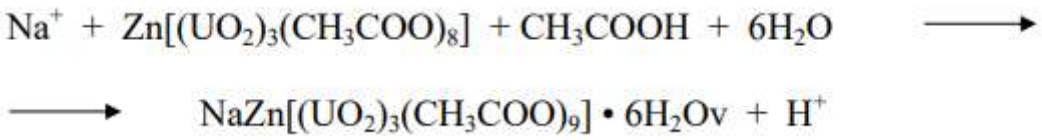


***b) interaction with sodium hexanitrocobaltate (III)*** leads to the formation of a yellow crystalline precipitate:

***c) Potassium salt introduced into a colorless flame*** colors it purple, and when viewed through a blue glass, purplish-red.

**Sodium**

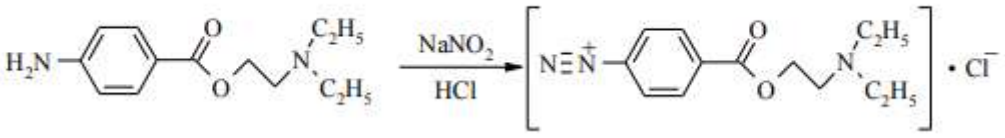
***a) Sodium salts*** form a yellow crystalline precipitate ***with zinc uranyl acetate***. The precipitate is insoluble in acetic acid.

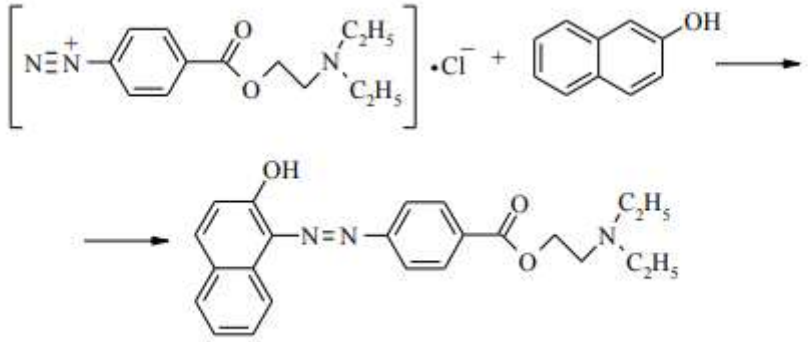


***b) sodium salt introduced into a colorless fla***me turns it yellow.

## *8. Formation of azo dye*

The novocaine salt of benzylpenicillin gives an azo dye formation reaction. A cherry-red coloration appears.





### 9. *Reaction with chromotropic acid*

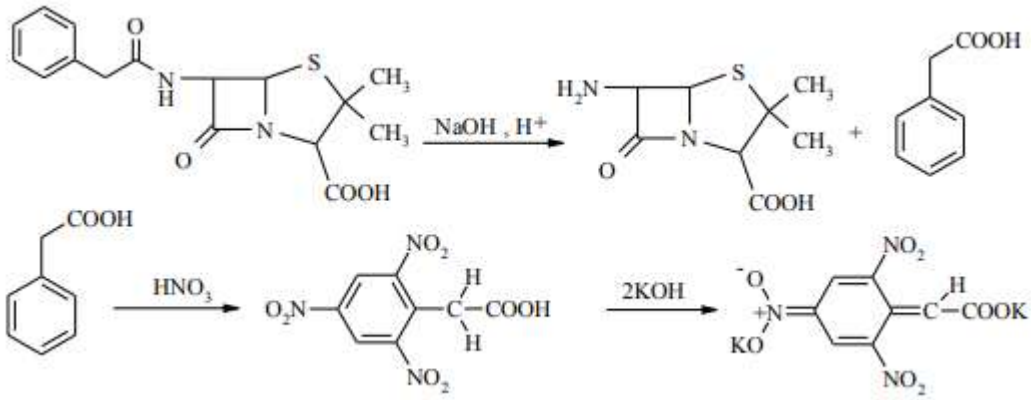
Yellow staining appears.

### 10. *Reaction with concentrated sulfuric acid*

Penicillin preparations form colored solutions after heating them with concentrated sulfuric acid.

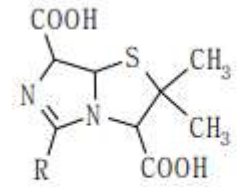
### 11. *Vitali-Morin reaction*

When a mixture of the drug and fuming nitric acid is evaporated, followed by the addition of alcoholic solution of potassium hydroxide and acetone, a violet coloration appears.

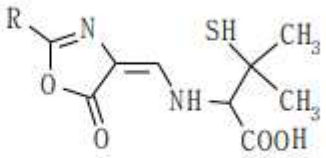


### 12. *Interaction with hydrochloric acid*

When drops of 25% hydrochloric acid are added to a solution of the sodium or potassium salt of benzylpenicillin, a precipitate of free benzylpenicillin is formed which is soluble in excess hydrochloric acid as well as in ethanol, chloroform and ether. The free penicillinic acid released by the reaction undergoes hydrolytic cleavage and isomerization in acidic medium. The isomerization products are penicillic and penicillenic acids:



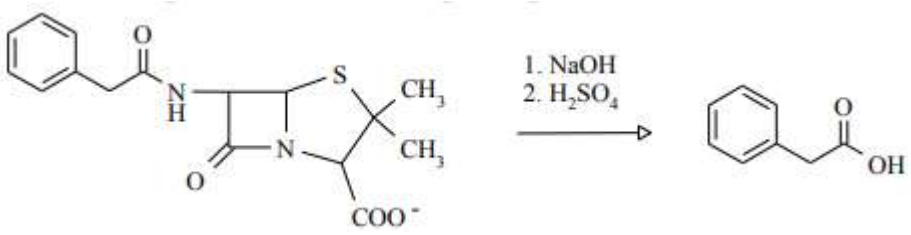

*Penylic acid*




*Penicillenic acid*

### *13.Alkaline hydrolysis*

Salts of benzylpenicillin are hydrolyzed by boiling in 4% solution of sodium hydroxide to the sodium salt of phenylacetic acid, which is detected by a characteristic odor after the addition of an excess of dilute sulfuric acid:



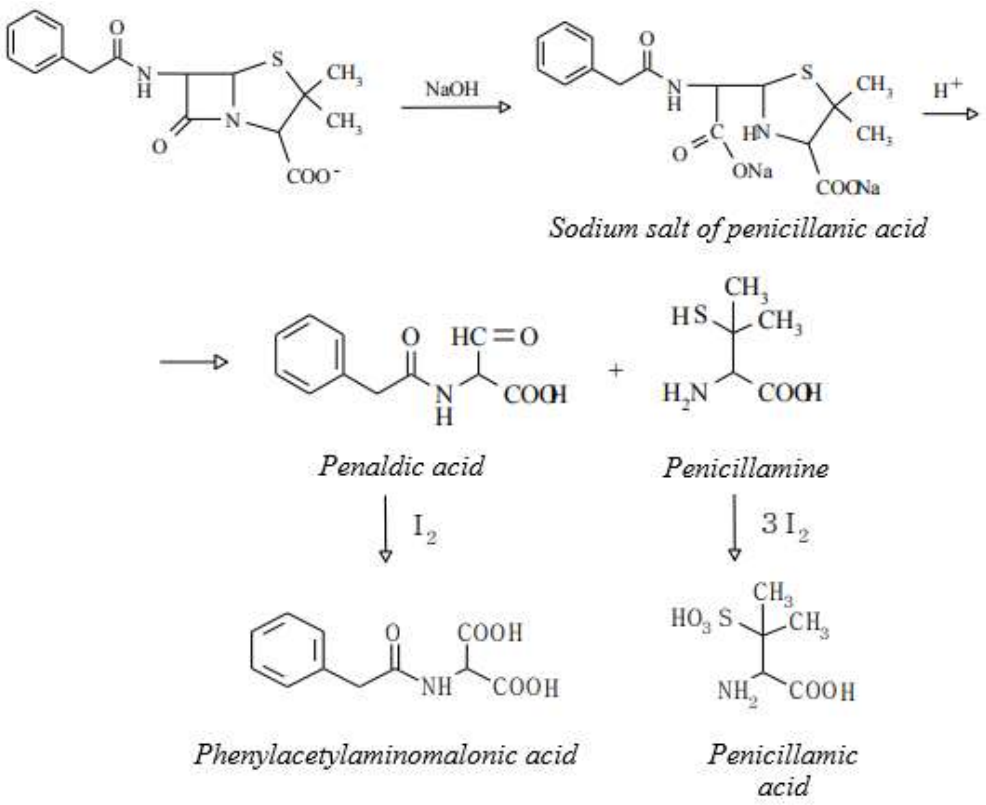

## PURITY TEST

Prepared 3% solutions of penicillin preparations in water for injection should be colorless and transparent for 24 h at a temperature not exceeding 10 oC. The pH of aqueous solutions of preparations should be in the range of 5.5 - 7.5. Weight loss during drying of an exact sample of the preparation at 100-105 oC to constant weight should not exceed 1%. In accordance with the requirements of regulatory documentation, penicillin preparations are tested for toxicity, pyrogenicity, sterility, and sodium and potassium salts of benzylpenicillin also for thermostability.

## ASSAY

### *1. Iodometric titration*

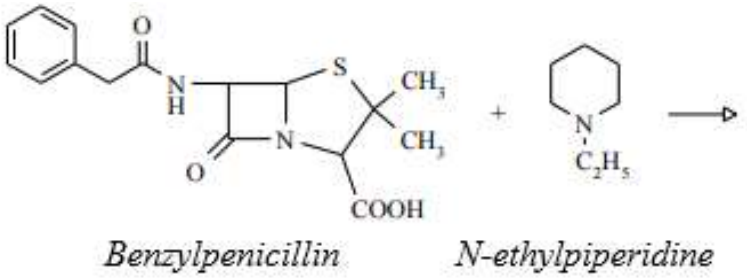
The product of penicillin inactivation - sodium salt of penicilloic acid, obtained by treatment of the drug with 1 M sodium hydroxide solution at room temperature, is oxidized with iodine. The oxidation process should be carried out at pH 4.5 in acetate buffer. The scheme of benzylpenicillin inactivation and oxidation with iodine is as follows:

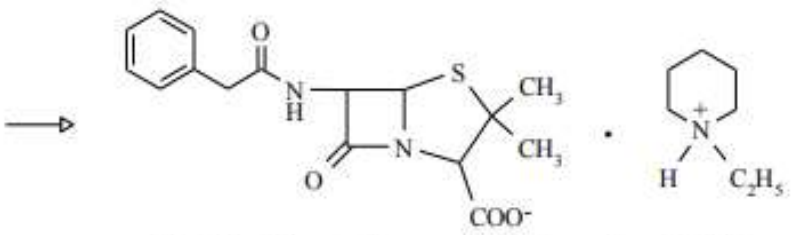



The sum of penicillins is determined by the reverse iodometric method: excess 0.01 M iodine solution is titrated with 0.01 M sodium thiosulfate solution. At the same time, a control experiment is performed with the same amount of drug not subjected to alkaline hydrolysis.

## *2. Gravimetric method*

The quantitative determination of the sodium, potassium and novocaine salts of benzylpenicillin is also performed by the gravimetric method. Benzylpenicillin is extracted with amyl acetate and quantitatively precipitated as N-ethylpiperidine salt:



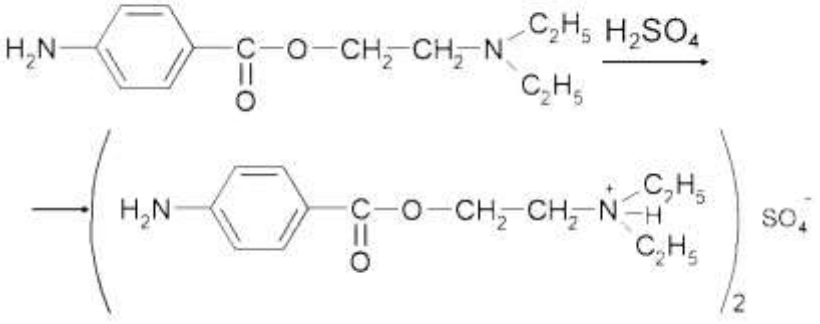


*N-ethylpiperidine salt of benzylpenicillin*

The precipitate of the N-ethylpiperidine salt of benzylpenicillin is washed, dried to a constant weight and weighed. Then the corresponding salt is converted to the corresponding salt.

### *3. Reverse acid-base titration of salts*

In novocaine salt of benzylpenicillin determine the content of novocaine, which is extracted with chloroform in the form of base, washed thoroughly with water, then add 0.01 M solution of sulfuric acid, excess of which is titrated with 0.01 M solution of sodium hydroxide. Novocaine content should be not less than 37.5 % and not more than 40.5 %.



$$H_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O$$

### *4. Microbiological determination*

The activity of penicillin preparations is established by microbiological method by antibacterial action on a certain strain of Staphylococcus aureus. One unit of action corresponds to the activity of 0.5988 μg of chemically pure sodium salt of benzylpenicillin (1670 units in 1 mg). The microbiological method for the determination of penicillins gives highly reproducible results.

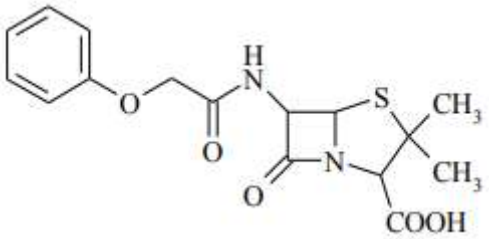
### *5. Spectrophotometric determination*

It is based on the interaction of the drug solution with imidazole and mercury (II) chloride when heated in a water bath. The light absorbance of the resulting solution is measured at 325 nm.

## STORAGE

Benzylpenicillin sodium should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 25°C.

# PHENOXYMETHYLPENICILLIN




## PHYSICAL PROPERTIES

A white, fine crystalline powder. Even in the absence of light, Phenoxymethylpenicillin is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Soluble in 1700 parts of water and in 7 parts of ethanol.

## IDENTIFICATION

**Spectral methods.**

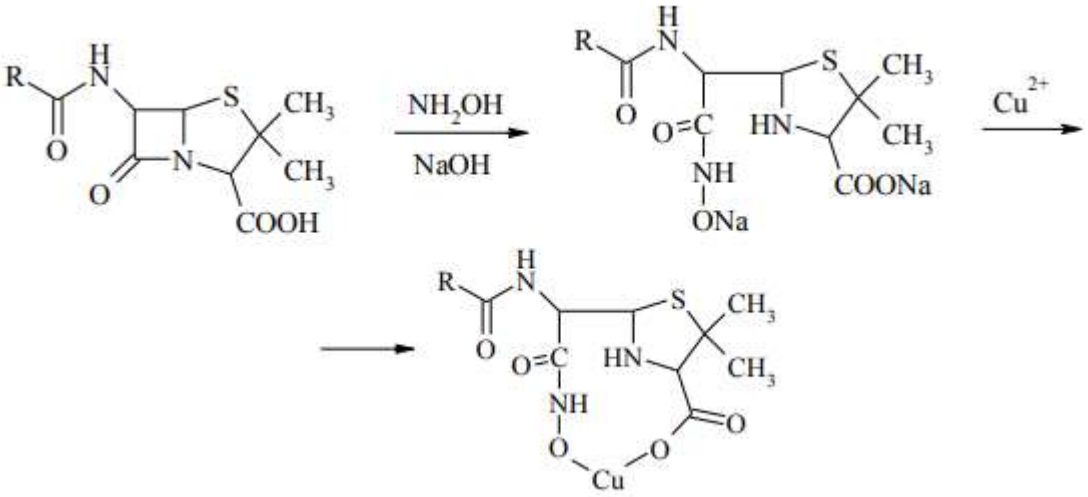
- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

**Chemical methods.**

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

1. ***Hydroxamic reaction***

   To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:



With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

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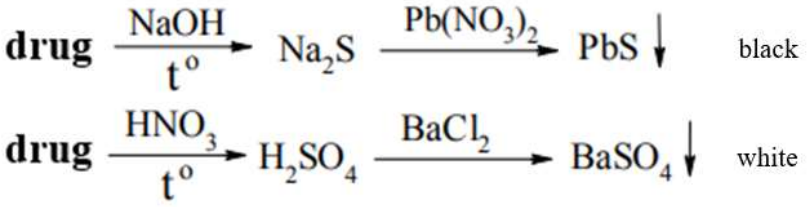

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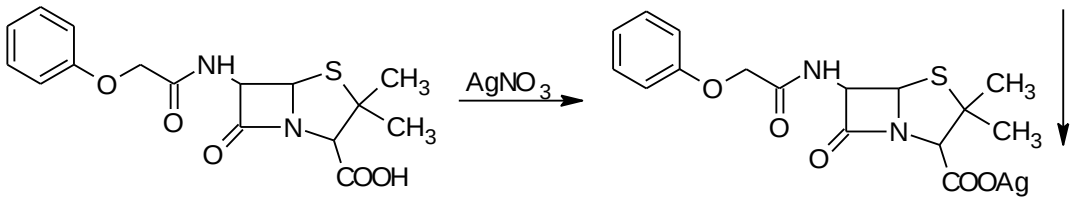

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***6. Reaction with resorcinol in sulfuric acid***

In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

***7. Reaction with chromotropic acid***

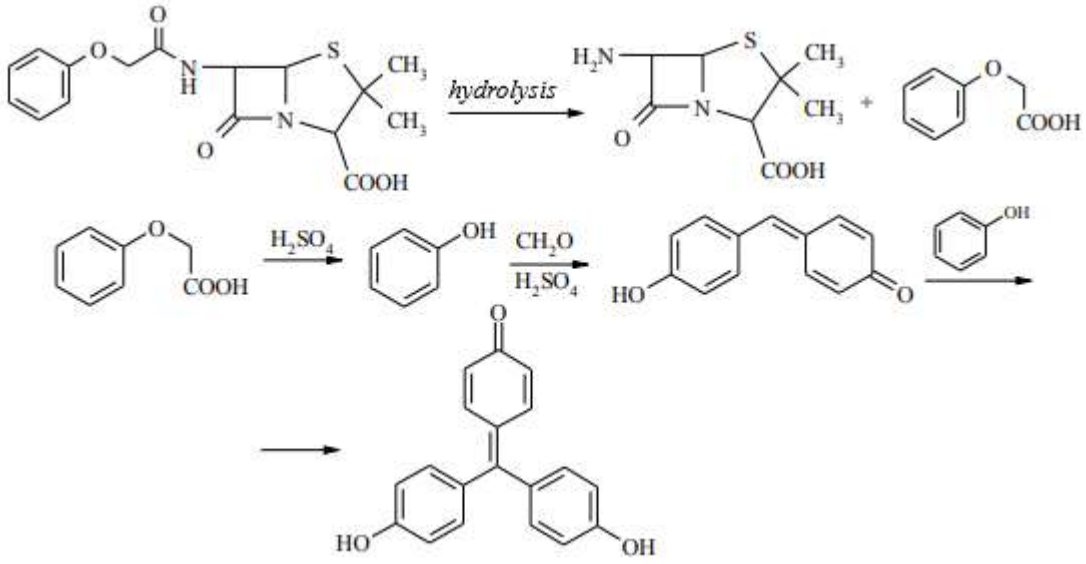
When interacting with chromotropic acid in the presence of sulfuric acid, an orange coloration is observed, turning to red.

***8. Reaction with concentrated sulfuric acid***

Phenoxymethylpenicillin differs from other penicillins in its negative reaction with concentrated sulfuric acid: the solution remains colorless after heating on a water bath.

***9. Reaction with Marquis reagent***

Phenoxymethylpenicillin is distinguished by its ability to react with Marquis reagent. The presence of phenoxyacetic acid residue causes the formation of red coloring. When heated in a water bath, an increase in the intensity of coloration is observed:

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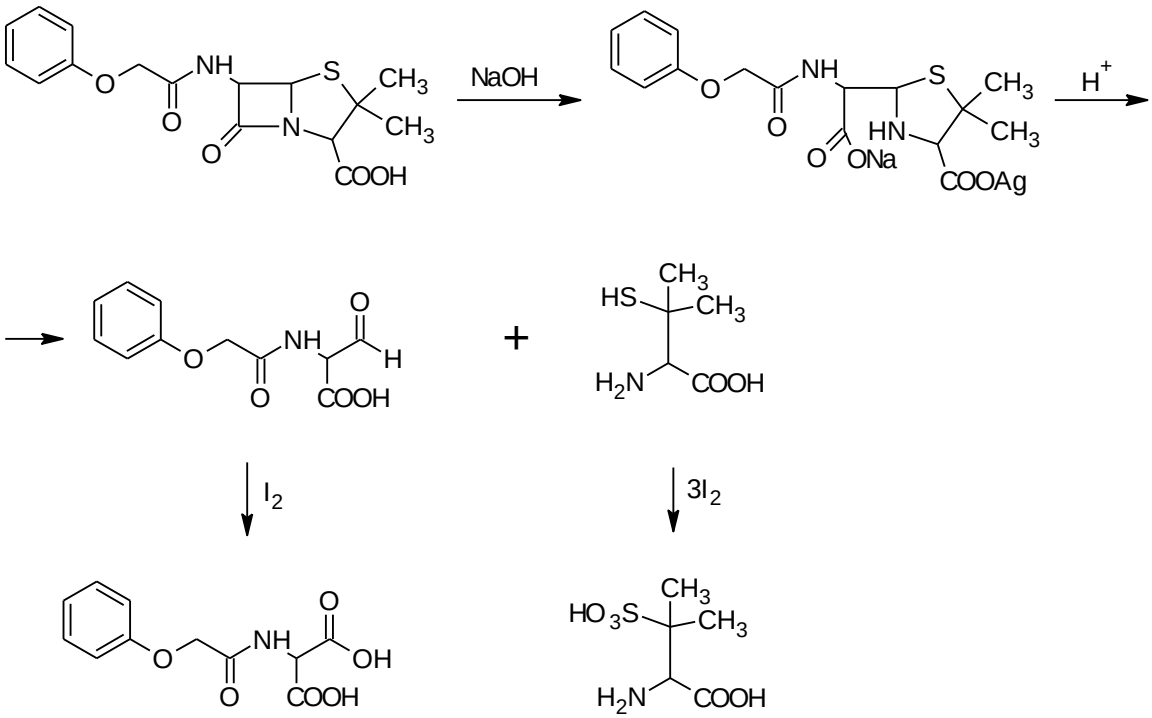


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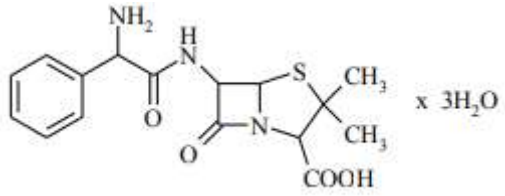
It is based on the interaction of the drug solution with imidazole and mercury (II) chloride when heated in a water bath. The light absorbance of the resulting solution is measured at 325 nm.

**STORAGE**

Phenoxymethylpenicillin should be kept in a tightly closed container, protected from light.

## SEMISYNTHETIC PENICILLINS

## AMPICILLIN




**PHYSICAL PROPERTIES**

A white or almost white, crystalline powder; odourless or almost odourless. Slightly soluble in water; practically insoluble in ethanol and ether.

**IDENTIFICATION**

**Spectral methods.**

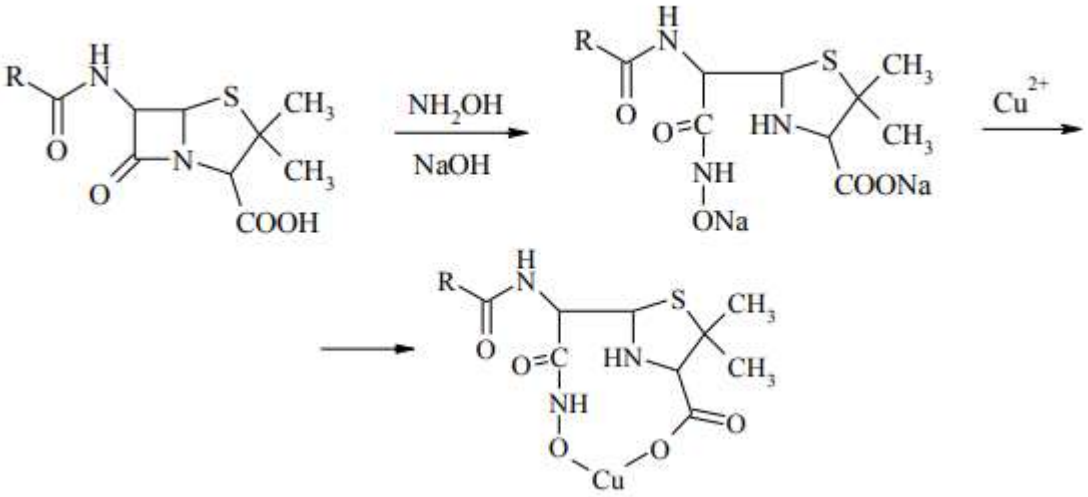
- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

**Chemical methods.**

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

1. ***Hydroxamic reaction***

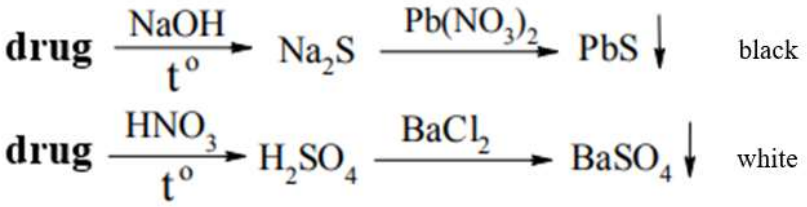
   To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:



With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

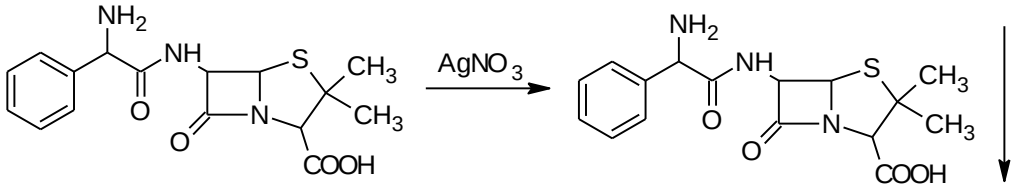
2. ***The discovery of sulfur***

Sulphur can be detected in penicillin preparations after its transformation into sulphide ion by fusion with caustic alkali or after its transformation into sulphate anion after mineralization with concentrated nitric acid.



3. ***Reaction with silver nitrate solution***

Interaction with silver nitrate produces a white precipitate that is soluble in excess ammonia.



4. ***Reaction with ammonia solution of silver nitrate***

Penicillin preparations are capable of oxidation by ammonia solution of silver nitrate with formation of metallic silver precipitate when heated.

5. ***Reaction with ammonia solution of copper nitrate***

The identity of penicillin antibiotics can be confirmed by oxidation with ammonia solution of copper nitrate on heating. A dark yellow coloration appears.

### 6. *Reaction with resorcinol in sulfuric acid*

In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

### 7. *Reaction with chromotropic acid*

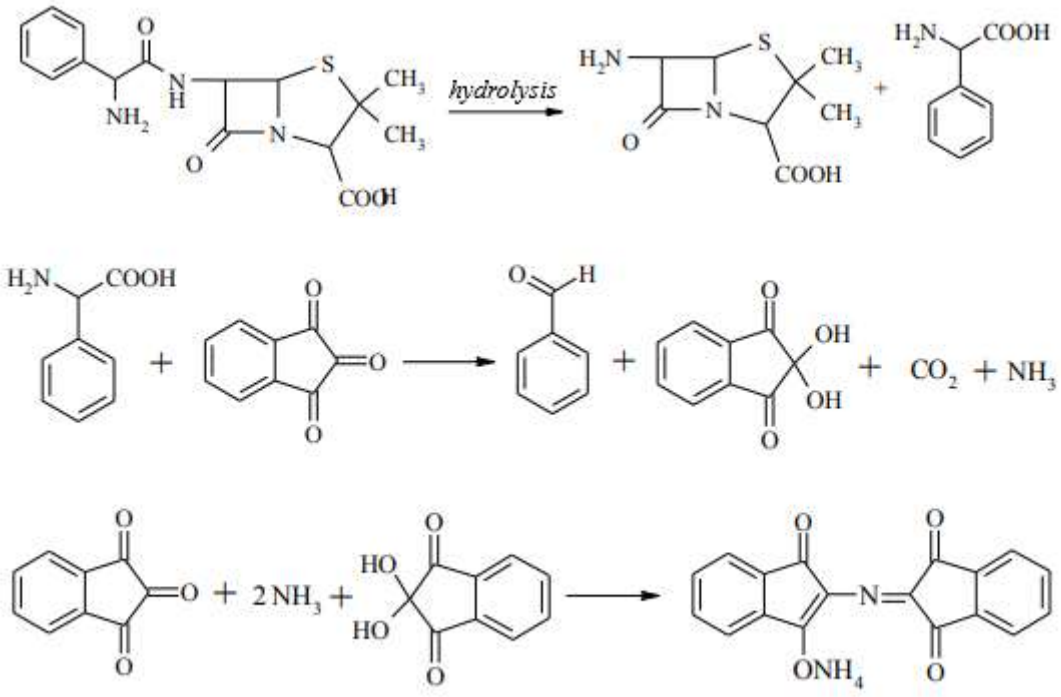
When interacting with chromotropic acid in the presence of sulfuric acid. A dark red coloration appears, changing to purple coloration.

### 8. *Reaction with concentrated sulfuric acid*

Penicillin preparations form colored solutions after heating them with concentrated sulfuric acid.

### 9. *Reaction with ninhydrine*

Ampicillin, due to the presence of a phenylaminoacetic acid residue in its molecule, reacts positively with ninhydrin like the amino acids:




### 10. *Reaction with Fehling's reagent*

In addition, ampicillin, due to the presence of a phenylamino-acetic acid residue in its molecule, acquires a violet coloration when interacting with Fehling's reagent**.**

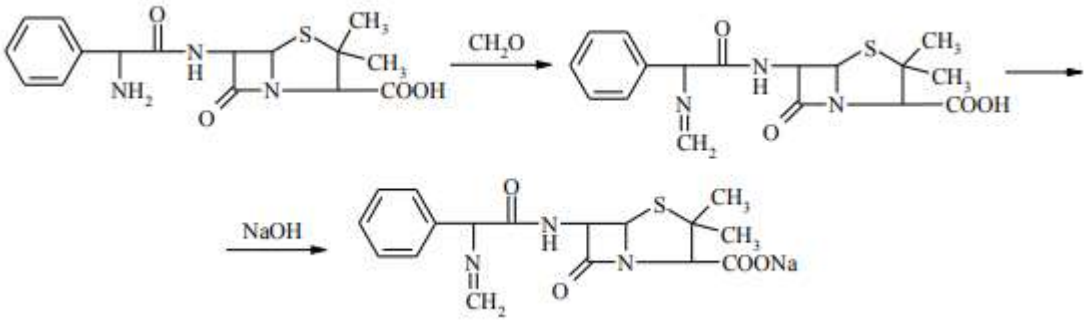
**PURITY TEST**

Prepared 3% solutions of penicillin preparations in water for injection should be colorless and transparent for 24 h at a temperature not exceeding 10 oC. The pH of aqueous solutions of preparations should be in the range of 5.5 - 7.5. Weight loss during drying of an exact sample of the preparation at 100-105 oC to constant weight should not exceed 1%. In accordance with the requirements of regulatory documentation, penicillin preparations are tested for toxicity, pyrogenicity, sterility.

**ASSAY**

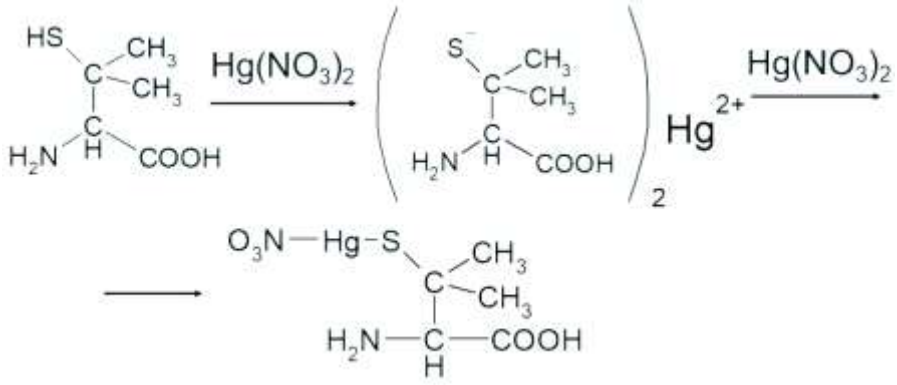
1. ***Formyl titration***

   Ampicillin is quantitatively determined by formol titration as amino acid preparations. A suspension of the preparation is dissolved in water, dilute formaldehyde solution is added and after 2 min titrated with sodium hydroxide solution (indicator phenolphthalein).




2. ***Mercurimetric method***

   The mercurimetric method for the determination of ampicillin using mercury (II) nitrate as a titrant is known. After successive alkaline and acid hydrolysis, titrated with mercury (II) nitrate:




3. ***Microbiological determination***

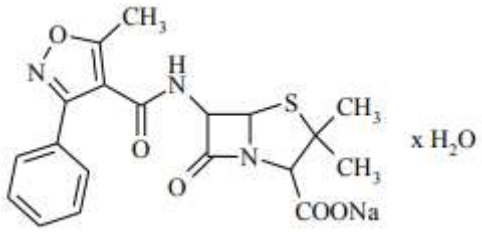
The activity of penicillin preparations is established by microbiological method by antibacterial action on a certain strain of Staphylococcus aureus. One unit of action corresponds to the activity of 0.5988 μg of chemically pure sodium salt of benzylpenicillin (1670 units in 1 mg). The microbiological method for the determination of penicillins gives highly reproducible results.

4. ***Spectrophotometric determination***

**STORAGE**

Ampicillin should be kept in a tightly closed container, protected from light.

## OXACILLIN SODIUM SALT




**PHYSICAL PROPERTIES**

White fine crystalline powder. Easily soluble in water, hardly soluble in ethanol, practically insoluble in chloroform and ether.

**IDENTIFICATION**

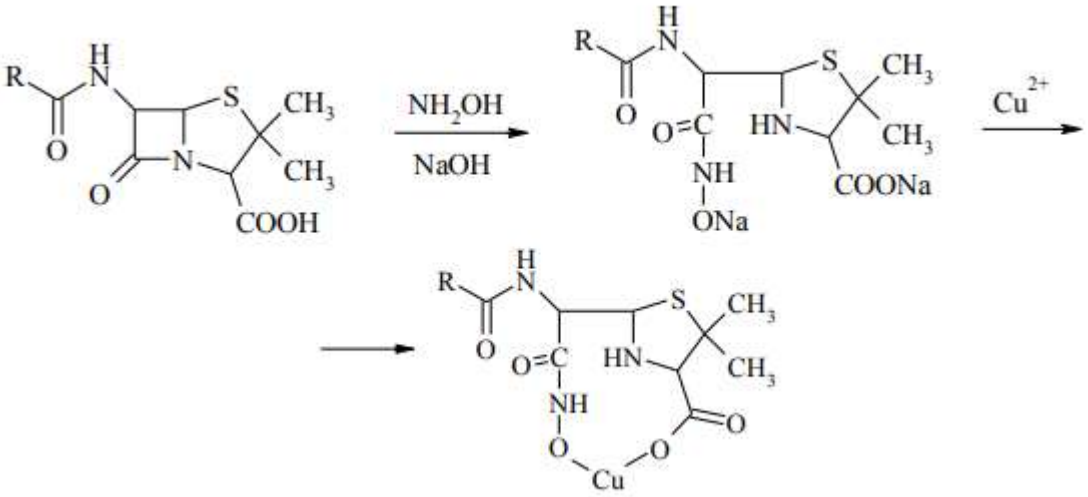
**Spectral methods.**

- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

**Chemical methods.**

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

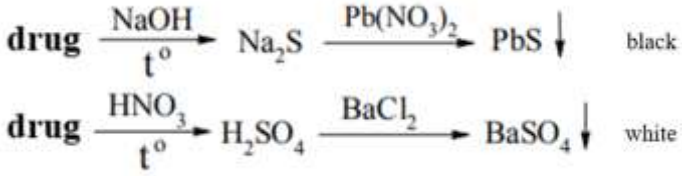
1. ***Hydroxamic reaction***
   To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:



With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

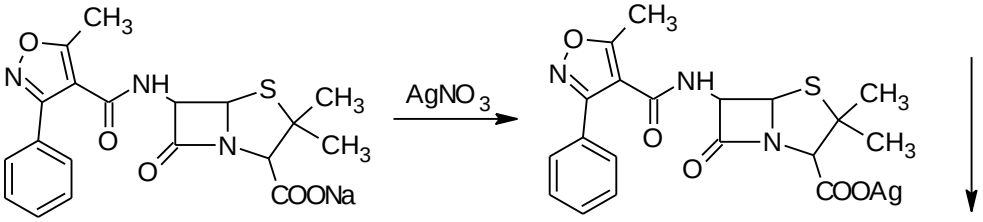
***2. The discovery of sulfur***

Sulphur can be detected in penicillin preparations after its transformation into sulphide ion by fusion with caustic alkali or after its transformation into sulphate anion after mineralization with concentrated nitric acid.



***3. Reaction with silver nitrate solution***

Interaction with silver nitrate produces a white precipitate that is soluble in excess ammonia.



***4. Reaction with ammonia solution of silver nitrate***

Penicillin preparations are capable of oxidation by ammonia solution of silver nitrate with formation of metallic silver precipitate when heated.

***5. Reaction with ammonia solution of copper nitrate***

The identity of penicillin antibiotics can be confirmed by oxidation with ammonia solution of copper nitrate on heating. A dark yellow coloration appears.

6. ***Reaction with resorcinol in sulfuric acid***
   In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

7. ***Discovery of sodium***
   ***a) Sodium salts*** form a yellow crystalline precipitate ***with zinc uranyl acetate***. The precipitate is insoluble in acetic acid.

$$Na^+ + Zn[(UO_2)_3(CH_3COO)_8] + CH_3COOH + 6H_2O \longrightarrow$$

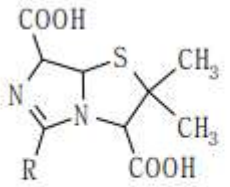
$$\longrightarrow NaZn[(UO_2)_3(CH_3COO)_9] \cdot 6H_2Ov + H^+$$

   ***b) sodium salt introduced into a colorless fla***me turns it yellow.

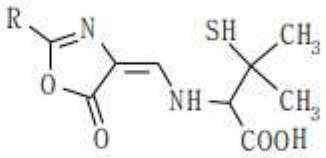
8. ***Reaction with chromotropic acid***
   Purple staining appears.

9. ***Reaction with concentrated sulfuric acid***
   Penicillin preparations form colored solutions after heating them with concentrated sulfuric acid.

10. ***Interaction with hydrochloric acid***
    When adding 25% hydrochloric acid dropwise to a solution of the sodium salt of oxacillin, a precipitate of free oxacillin is formed, which is soluble in excess hydrochloric acid, as well as in ethanol, chloroform and ether. The free penicillinic acid released as a result of the reaction undergoes hydrolytic cleavage and isomerization in acidic medium. The products of isomerization are penicillic and penicillenic acids:




*Penylic acid*




*Penicillenic acid*

**PURITY TEST**

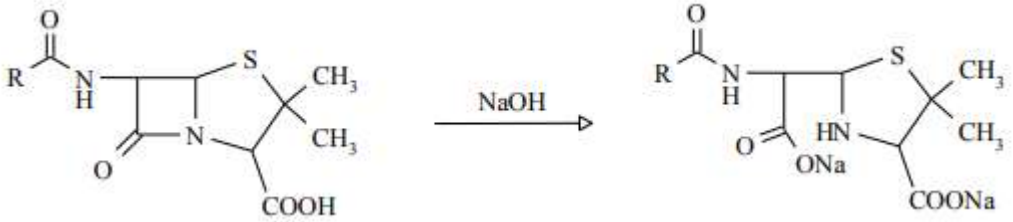
Prepared 3% solutions of penicillin preparations in water for injection should be colorless and transparent for 24 h at a temperature not exceeding 10 oC. The pH of aqueous solutions of preparations should be in the range of 5.5 - 7.5. Weight loss during drying of an exact sample of the preparation at 100-105 oC to constant

weight should not exceed 1%. In accordance with the requirements of regulatory documentation, penicillin preparations are tested for toxicity, pyrogenicity, sterility.

## ASSAY

1. ***Neutralization method (reverse titration)***

   The amount of oxacillin sodium salt is determined by the reverse neutralization method. It is based on the quantitative process of hydrolysis with 0.1 M sodium hydroxide solution to form penicillin derivatives when heated on a water bath:




   Excess sodium hydroxide is titrated with 0.1 M hydrochloric acid solution (indicator phenolphthalein).

2. ***Microbiological determination***

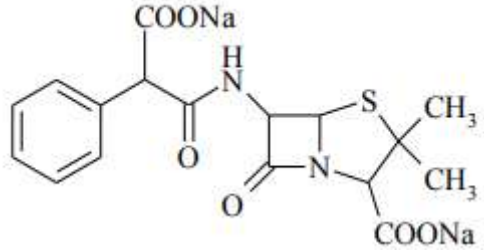
   The activity of penicillin preparations is established by microbiological method by antibacterial action on a certain strain of Staphylococcus aureus. One unit of action corresponds to the activity of 0.5988 μg of chemically pure sodium salt of benzylpenicillin (1670 units in 1 mg). The microbiological method for the determination of penicillins gives highly reproducible results.

3. ***Spectrophotometric determination***

## STORAGE

Stored in a dry place at room temperature. Oxacillin sodium salt is packed in glass jars or polyethylene bags.

# CARBENICILLIN DYNATRIUM SALT



## PHYSICAL PROPERTIES

White or almost white fine crystalline powder, odorless. Readily soluble in water, slowly soluble in ethanol, practically insoluble in chloroform and ether

## IDENTIFICATION

### Spectral methods.

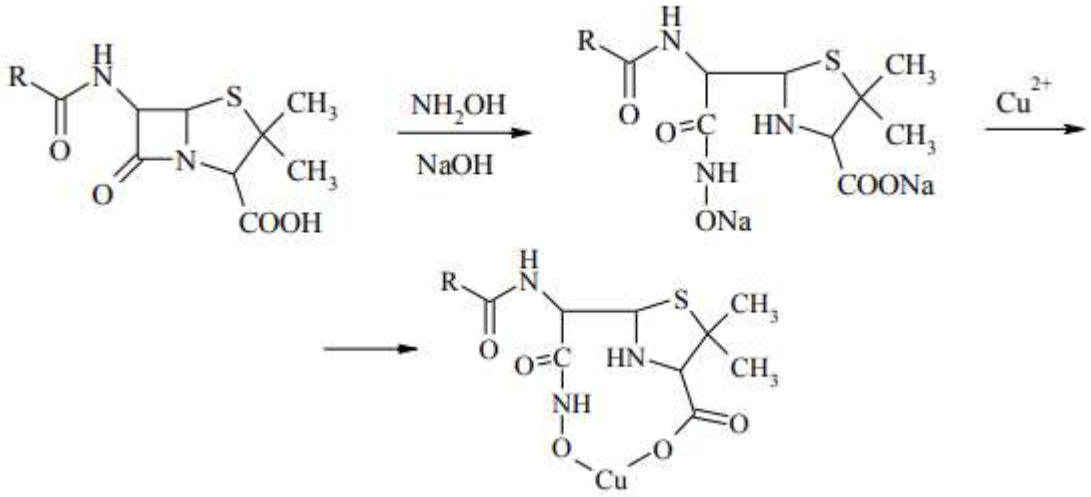
- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

### Chemical methods.

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

1. ***Hydroxamic reaction***

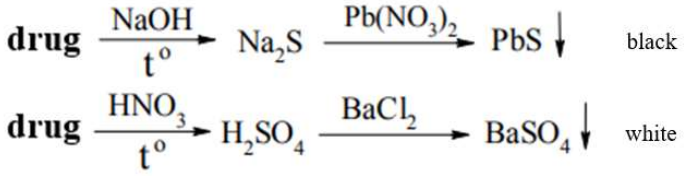
   To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:




   With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

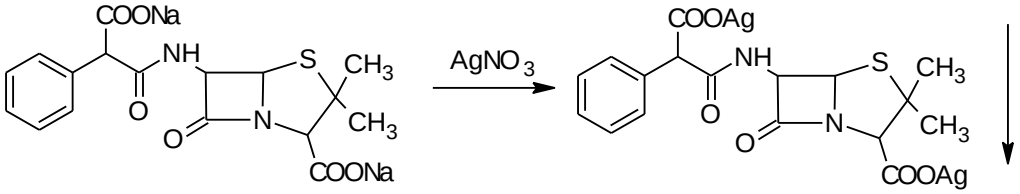
2. ***The discovery of sulfur***

   Sulphur can be detected in penicillin preparations after its transformation into sulphide ion by fusion with caustic alkali or after its transformation into sulphate anion after mineralization with concentrated nitric acid.




### 3. *Reaction with silver nitrate solution*

Interaction with silver nitrate produces a white precipitate that is soluble in excess ammonia.




### 4. *Reaction with ammonia solution of silver nitrate*

Penicillin preparations are capable of oxidation by ammonia solution of silver nitrate with formation of metallic silver precipitate when heated.

### 5. *Reaction with ammonia solution of copper nitrate*

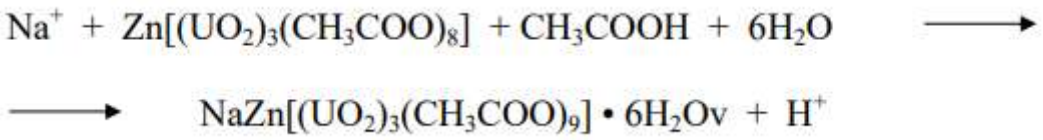
The identity of penicillin antibiotics can be confirmed by oxidation with ammonia solution of copper nitrate on heating. A dark yellow coloration appears.

### 6. *Reaction with resorcinol in sulfuric acid*

In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

### 7. *Discovery of sodium*

***a) Sodium salts*** form a yellow crystalline precipitate ***with zinc uranyl acetate***. The precipitate is insoluble in acetic acid.



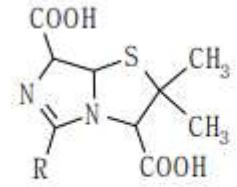

***b) sodium salt introduced into a colorless fla***me turns it yellow.

### 8. *Reaction with concentrated sulfuric acid*

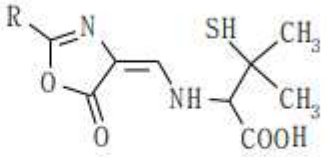
Penicillin preparations form colored solutions after heating them with concentrated sulfuric acid.

### *9. Interaction with hydrochloric acid*

When adding 25% hydrochloric acid dropwise to a solution of the sodium salt of carbenicillin, a precipitate of free carbenicillin is formed, which is soluble in excess hydrochloric acid, as well as in ethanol, chloroform and ether. The free penicillinic acid released as a result of the reaction undergoes hydrolytic cleavage and isomerization in acidic medium. The products of isomerization are penicillic and penicillenic acids:




*Penylic acid*




*Penicillenic acid*

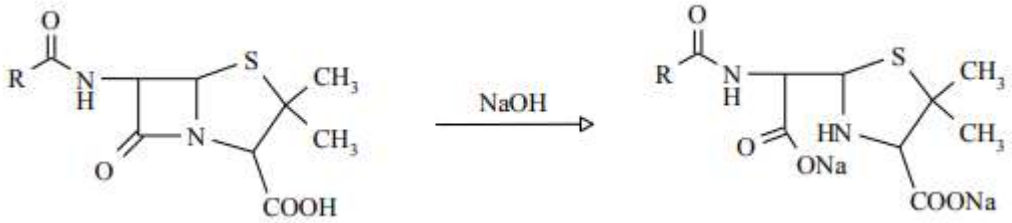
## PURITY TEST

Prepared 3% solutions of penicillin preparations in water for injection should be colorless and transparent for 24 h at a temperature not exceeding 10 oC. The pH of aqueous solutions of preparations should be in the range of 5.5 - 7.5. Weight loss during drying of an exact sample of the preparation at 100-105 oC to constant weight should not exceed 1%. In accordance with the requirements of regulatory documentation, penicillin preparations are tested for toxicity, pyrogenicity, sterility.

## ASSAY

### *1. Neutralization method (reverse titration)*

The method is based on the quantitative process of hydrolysis by 0.1 M sodium hydroxide solution upon heating in a water bath to form penicillic acid derivatives:




Excess sodium hydroxide is titrated with 0.1 M hydrochloric acid solution (indicator phenolphthalein).

2. ***Microbiological determination***

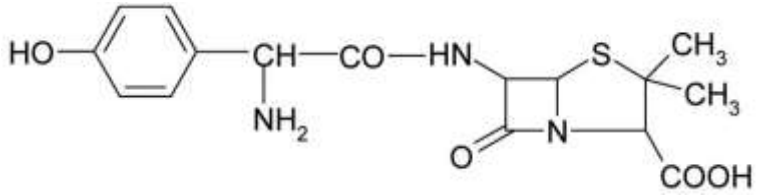
   The activity of penicillin preparations is established by microbiological method by antibacterial action on a certain strain of Staphylococcus aureus. One unit of action corresponds to the activity of 0.5988 μg of chemically pure sodium salt of benzylpenicillin (1670 units in 1 mg). The microbiological method for the determination of penicillins gives highly reproducible results.

3. ***Spectrophotometric determination***

**STORAGE**

Carbenicillin dinatrium salt is stored at a temperature not higher than +5 °C in a dry place protected from light.

## AMOXICILLIN




**PHYSICAL PROPERTIES**

A white or almost white, crystalline powder. Slightly soluble in water and methanol; very slightly soluble in ethanol, practically insoluble in fatty oils.

**IDENTIFICATION**

**Spectral methods.**

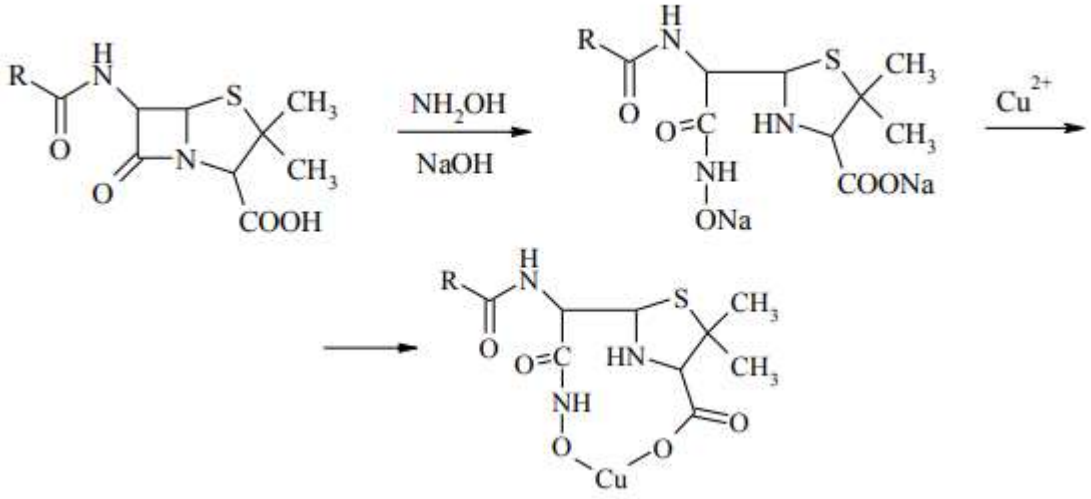
- The identity of penicillin preparations is confirmed by UV and IR spectrophotometry.
- An important physical constant of penicillin preparations is the specific rotation of aqueous or alcoholic solutions. All of them rotate the plane of the polarized beam to the right

**Chemical methods.**

General chemical properties are due to the presence of sulfur atoms in the dihydrothiazine ring (oxidation ability), carboxyl group (salt formation) and β-lactam ring (hydroxamic reaction).

1. ***Hydroxamic reaction***

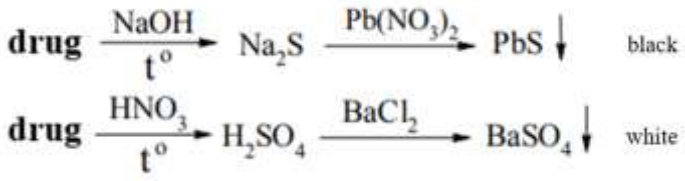
To test the identity of penicillin preparations, use a color reaction based on the rupture of the β-lactam cycle and the formation of the copper salt of hydroxamic acid in the form of a green precipitate:




With iron (III) salts, hydroxamic acid gives red-colored intra-complex salts.

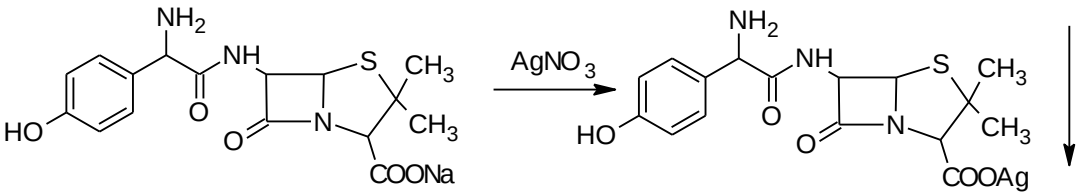
2. ***The discovery of sulfur***

Sulphur can be detected in penicillin preparations after its transformation into sulphide ion by fusion with caustic alkali or after its transformation into sulphate anion after mineralization with concentrated nitric acid.




3. ***Reaction with silver nitrate solution***

Interaction with silver nitrate produces a white precipitate that is soluble in excess ammonia.




4. ***Reaction with ammonia solution of silver nitrate***

Penicillin preparations are capable of oxidation by ammonia solution of silver nitrate with formation of metallic silver precipitate when heated.

5. ***Reaction with ammonia solution of copper nitrate***
   The identity of penicillin antibiotics can be confirmed by oxidation with ammonia solution of copper nitrate on heating. A dark yellow coloration appears.

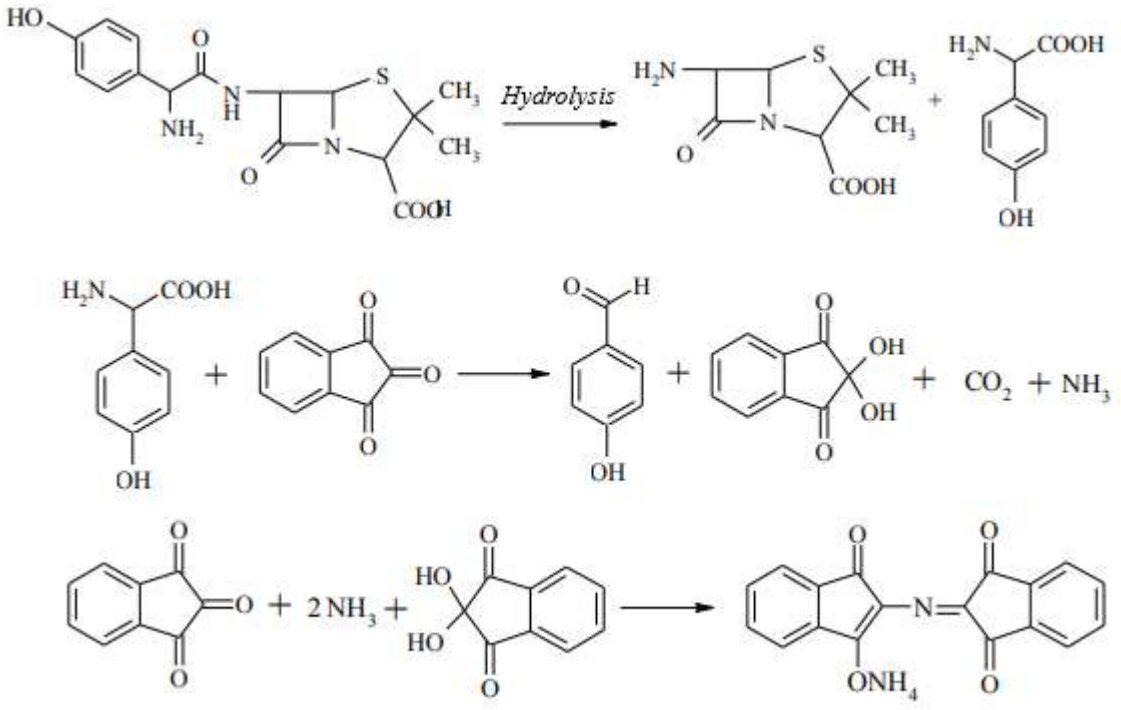
6. ***Reaction with resorcinol in sulfuric acid***
   In the presence of sulfuric acid, penicillin drugs are capable of condensation reactions with phenolic compounds such as resorcinol or β-naphthol. A yellow-green coloration appears.

7. ***Reaction with chromotropic acid***
   Red staining appears.

8. ***Reaction with concentrated sulfuric acid***
   Penicillin preparations form colored solutions after heating them with concentrated sulfuric acid.

9. ***Reaction with ninhydrine***

   Blue staining appears.




**PURITY TEST**

Prepared 3% solutions of penicillin preparations in water for injection should be colorless and transparent for 24 h at a temperature not exceeding 10 oC. The pH of

aqueous solutions of preparations should be in the range of 5.5 - 7.5. Weight loss during drying of an exact sample of the preparation at 100-105 oC to constant weight should not exceed 1%. In accordance with the requirements of regulatory documentation, penicillin preparations are tested for toxicity, pyrogenicity, sterility.

## ASSAY

1. ***HPLC***
2. ***Microbiological determination***

   The activity of penicillin preparations is established by microbiological method by antibacterial action on a certain strain of Staphylococcus aureus. One unit of action corresponds to the activity of 0.5988 μg of chemically pure sodium salt of benzylpenicillin (1670 units in 1 mg). The microbiological method for the determination of penicillins gives highly reproducible results.

3. ***Spectrophotometric determination***

## STORAGE

Amoxicillin trihydrate should be kept in a tightly closed container.