**Pharmaceetical cheistry IV**

**Lecture 5**

**Topic:** Purine derivatives. Xanthine derivatives. Purine nucleosides. Antimetabolit preparations as purine derivatives.

PURINE ALKALOIDS (XANTHINES)

The naturally occurring xanthines are caffeine, theophylline, and theobromine.

Caffeine enjoys wide use as a CNS stimulant. Theophylline has sum» use as a CNS stimulant (as will be discussed later); its CNS-stimulant properties are encountered more often as side effects, sometimes severe and potentially life-threatening, of its use in bronchial asthma thcrapy. Theobromine has very little CNS activity and will not be discussed further as a CNS stimulant. Its l-[5-oxohexyl] derivative pentoxifyline (Trental) is useful in intermittent claudication, presumably in part by improving red blood cell deformability. Its potential use in other occlusive disorders, such as acute stroke, is under investigation.

Caffeine is used often as it occurs in brewed coffee (~85 mg/cup), brewed tea (~ 60 mg/cup), and cola beverages (~50 mg/12 fl oz). In most subjects 85 to 250 mg of caffeine acts as a cortical stimulant am facilitates clear thinking and wakefulness, promotes an ability to concentrate on the task at hand, and lessens fatigue. As the dose is increased, side effects indicative of excessive stimulation, such asrestlessness, anxiety, nervousness, and tremulousness, become more marked. With further increases in dosage, convulsions can occur.

Theophylline’s CNS effects at lower dose levels have been studied little. At high doses, the tendency to produce convulsions is greater for theophylline than for caffeine.

In addition to being cortical stimulants, theophylline and caffeine are medullary stimulants, and both are used in treating sleep apnea in un infants. Caffeine (as caffeine and sodium benzoate) may used rarely in treating poisoning from CNS-depressant drugs, though not a preferred choice.

Xanthines can exist in two forms - enol-form (1) and keto-form (2):

 

**Caffeine**

(Coffeinum), Ph. Eur.



1.3.7-Trimethyl-3,7-dihydro-l/f-purine-2,6-dione

**Properties.** A white, crystalline powder or silky, white crystals, sublimes readily, sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol. It dissolves in concentrated solutions of alkali benzoates or salicylates.

**Identification.** 1. The melting point is 234-239 °C.

2. Examine by infrared absorption spectrophotometry.

3. With iodinated potassium iodide solution and dilute hydrochloric acid-a brown precipitate is formed. Neutralise with dilute sodium hydroxide solution -the precipitate (caffeine $∙$ I4 $∙$ HI) dissolves.

4. With acetylacetone, dilute sodium hydroxide solution and dimethylaminobenzaldehyde solution-an intense blue colour develops:

C

H

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N

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H

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C

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H

3

O

C

H

3

N

N

O

4

H

N

5. It complies with the test for loss on drying.

6. It gives the reaction of xanthines with strong hydrogen peroxide solution and dilute hydrochloric acid. Heat to dryness on a water-bath until a yellowish-red residue is obtained. Add dilute ammonia-the polour of the residue changes to violet-red.

Non-Pharmacopoeial reactions: a) the reaction with tannin - a white precipitate is formed, which is soluble in the excess of reagent; b) reactions with general precipitative reagents.

**Assay.** 1. Non-aqueous titration; medium of glacial acetic acid, determining the end-point potentiometrically; s=1.

2. Iodometry, back titration, the indicator is the starch solution:

s=1/2:



 kofein kofein-peryodid

I2 + 2Na2S2O3 $\rightarrow $ 2NaI + Na2S4O6

**Usage.** Stimulant of the CNS and a weak diuretic.

**Storage.** Store protected from light.

Theobromine

(Theobrominum), Ph. Eur.



3,7-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

ethanol, slightly soluble in ammonia, practically insoluble in ether. It dissolves in dilute solutions of alkali hydroxides and in mineral acids.

Identification. 1. Infrared absorption spectrophotometry.

2. With dilute ammonia and silver nitrate solution the solution remains clear. Boil the solution for a few minutes - a white, line precipitate is formed.

1. It gives the reaction of xanthines (see Caffeine).

Non-Pharmacopoeial reactions: a) after interaction with NaOH with

the excess of the substance and cobalt (II) chloride - an intense colour is formed, which disappears forming a bluish precipitat:

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b) reactions with general precipitative reagents.

**Assay.** Indirect alkalimetry: add to the substance solution 0.1 M silver nitrate and shake. Titrate with 0.1 M sodium hydroxide using bromothymol blue solution as an indicator; s=1:



**Usage.** Antispasmodic, a weak diuretic.

**Storage.** Store protected from light.

Theophylline

(Theophyllinum), Ph. Eur. **O**

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O

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H

C

O

N

C

H

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N

N

H

2

O

H

M.k. 198,18

1,3-Dimethyl-3,7-dihydro- 1H-purine-2,6-dionc

**Properties.** A white, crystalline powder, slightly soluble in water, sparingly soluble in ethanol. It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

Identification. 1. The melting point.

 2. Infrared absorption spectrophotometry.

3. Heat the substance with of potassium hydroxide solution in a water-bath, then add diazotised sulphanilic acid solution - a red colour slowly develops. Carry out a blank test.

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4. It complies with the test for loss on drying.

5. It gives the reaction of xanthines (see Caffeine).

Non-Pharmacopoeial reactions: a) after interaction NaOH with the excess of the substance and cobalt (II) chloride solution - an in­tense violet colour is formed, which disappears forming a bluish pre­cipitate (see Theobromine);

1. with sodium nitroprusside solution - a green colour is formed, which disappears after addition the excess of acid;
2. reactions with general precipitative reagents.

**Assay.** Indirect alkalimetry: add to the substance solution 0.1 M sil­ver nitrate and shake. Titrate with 0.1 M sodium hydroxide using bromothymol blue solution as an indicator; s=1:

O

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C

O

N

C

H

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H

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A

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O

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O

N

3

H

C

O

N

C

H

3

N

N

+

H

N

O

3

A

g

HNO3 + NaOH → NaNO3 + H2O

**Usage.** Antispasmodic, a weak diuretic.

Storage. Store protected from light.

Caffeine-Sodium Benzoate

(Coffeinum-natrii benzoas)

C8H10N4O2⋅ C6H5COONa və ya Kofein $∙$ C6H5COONa

Properties. A white, crystalline powder, freely soluble in water, slightly soluble in ethanol.

Identification. 1. Caffeine: a) the melting point is 234-239 °C.

1. the reaction of xanthines;
2. the reaction with tannin;
3. the reaction with iodine solution.

2. Sodium benzoate-, a) the reaction with FeCl3;

b) reactions of sodium.

Assay. 1 .Caffeine is determined by the iodometry method (see Caf­feine). Calculate on a dry substance (38.0-40.0 %).

2. Sodium benzoate is determined by the acidimetry method (the indicator is the solution of methyl orange and methyl blue (1:1)) in the presence of ether; s = 1.

C6H5COONa + HCl → C6H5COOH + NaCl

Calculate on a dry substance (58.0-62.0 %).

Usage. Stimulant of the CNS.

Storage. Store protected from light.

Theophylline-ethylenediamine, Ph. Eur. Aminophylline

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Theophylline and 1,2-ethylenediamine

Theophylline-ethylenediamine contains 84.0-87.4 % of theophyl­line and 13.5-15.0% of ethylenediamine, both calculated with refe­rence to the anhydrous substance.

Properties. A white or yellowish crystalline powder, sometimes granular, with the weak odour of ammonia. Freely soluble in water (the solution becomes cloudy through absorption of carbon dioxide), practically insoluble in ethanol.

**Identification**. Theophylline: 1. After interaction with hydrochloric acid the precipitate obtained melts at 270 to 274 0C.

1. Examine the precipitate by IR-spectrophotometry.
2. The precipitate gives test of xanthines (see Caffeine).
3. Heat the substance with of potassium hydroxide solution in a water-bath, then add diazotised sulphanilic acid solution - a red colour slowly develops. Carry out a blank test (see Theophyl­line).
4. The precipitate gives the reaction of xanthines (see Caffeine).

Ethylene diamine. To the filtrate (see the first reaction) add benzoyl chloride, make alkaline with dilute sodium hydroxide solution, shake. Filter the precipitate dissolve it in alcohol and add water. The preci­pitate formed melts at 248 to 252 °C.

The non-Pharmacopoeial reaction: with copper (II) sulphate - a bright-violet colour appears:

C

H

2

C

H

2

N

H

2

N

H

2

3

+

C

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S

O

4

C

H

2

N

H

2

C

H

2

N

H

2

C

u

3

S

O

4

**Assay.** 1. Ethylene diamine is determined by the acidimetry me­thod using bromocresol green solution as an indicator; s = 1/2:

C

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C

H

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N

H

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N

H

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+

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H

C

l

C

H

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C

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1. Theophylline - see Theophylline (it is determined after drying at the temperature of 135 °C); s - 1.

**Usage.** Antispasmodic (bronchodilator).

**Storage.** Store protected from light.

**Pentoxifylline**

(Pentoxifyllinum), Ph. Eur.

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**Properties.** A white or almost white, crystalline powder, soluble in water, freely soluble in methylene chloride, sparingly soluble in alcohol very slightly soluble in ether.

Identification. 1. The melting point is 103-107 °C.

1. Examine by infrared absorption spectrophotometry.
2. Thin-layer chromatography.
3. It gives the reaction of xanthines (see Caffeine).

Non-Pharmacopoeial reactions- with general precipitative regents.

**Assay.** Non-aqueous titration in the mixture of anhydrous aced and acetic anhydride. The end-point is stated potentiometrically: s=1.

Usage. Vasodilator.

**Storage.** Store protected from light.

**DERIVATES OF PURINE**

Purine is a condensed heterocyclic system, it consists of two cycles: pyrimidine and imidazole:



**Aciclovir**

**(Aciclovirum), Ph. Eur.**

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R

N

N

2

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N

H

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4

5

6

7

8

9

2-Amino-9-[(2-hydroxyethoxyl)methyl]-l,9-dihydro-6H-purin-6-one

Preparation

Properties. A white or almost white, crystalline powder, slightly soluble in water, freely soluble in dimethyl sulphoxide, very' slightly soluble in alcohol. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

**Identification.** IR-spectrum.

Purity. The related substances are determined by thin-layer chro­matography and by liquid chromatography.

**Assay.** Non-aqueous titration in the medium of anhydrous acetic - acid. Titrate with 0.1 M perchloric acid determining the end-point potentiometrically. Carry out a blank titration; s=1.

Usage. Antiviral.

Storage. Protected from light.

**Mercaptopurine**

**(Mercaptopurinum), Ph. Eur.**



7*H*-Purine-6-thiol

Preparation



Properties. A yellow, crystalline powder, practically insoluble n water and in ether, slightly soluble in alcohol. It dissolves in soluluui of alkali hydroxides.

**Identification.** 1. UV spectrum.

2. Dissolve the substance in alcohol, heat and add mercuric acetate in alcohol. A white precipitate is formed.

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N

a

+

N

a

2

S

+

2

H

2

O

Na2[Fe(CN)5NO] + Na2S ⟶ Na4[Fe(CN)5NOS]

3. Dissolve the substance in alcohol, heat and add solution of lc.ni acetate in alcohol. A yellow precipitate is formed.

**Non-Pharmacopoeial reactions**: a) the alkaline solution of the sub­stance after the interaction with sodium nitroprusside solution gets a yellow-green colouring, which becomes dark-green in the acidic medium;

b) the alkaline solution of the substance with copper (Il).chloride solution and hydroxylamine hydrochloride solution forms orange- yellow precipitates.

**Assay.** 1. Alkalimetry in the medium of dimethylformamide deter­mining the end-point potentiometrically. Titrate with 0:l M tetrabutylammonium hydroxide solution, s=1.

AgNO3 + NH4SCN ⟶ AgSCN↓ + NH4NO3

3NH4SCN + FeNH4(SO4)2 ⟶ Fe(SCN)3 + 2(NH4)2SO4

2. Argentometry by Volgard; s = 1/2.

3. Mercurimetry, back titration. The excess of 0.05 M mercury (II) nitrate precipitates mercury mercaptopurinate. After the filtration the excess is titrated with ammonium thyocyanate solution using ferric ammonia sulphate as an indicator.

**Usage.** For treating oncology diseases.

**Storage**. Store protected from light

**Adenosinetriphosphoric Acid**

**(Acidum adenosintriphosphoricum)**

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R

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ATPA is a natural part of human and animal tissues. It is formed during the reactions of oxidation and during the process of carbo­hydrates glycolysis. It takes part in various processes of metabolism.

Properties. A white, crystalline powder, hygroscopic. In medicine 1 % solution of sodium adenosinetriphosphate for injections is 'used (Solutio Natrii adenosintriphosphatis 1 % pro injectionibus) - trans­parent or a slightly yellowish liquid with pH = 7.0—7.3.

Identification. 1. Ribose is determined with the solution of FeCl3 in concentrated HC1 and the orcinol solution. After heating on a boil­ing water-bath for 20 min the mixture turns green:

2. Reactions of sodium.

3. Reactions of phosphates after hydrolysis:

Assay. Spectrophotometry after chromatographic separating the impurities of adenosine, adenosinemonophosphate and adenosine- diphosphate.

**Usage.** It is used for myodistrophy and myoatrophy and for treat­ment some cardiac diseases

Storage. Protected from light.

**Riboxinum**

O

N

N

8

N

9

N

H

1

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6

7

O

C

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H

O

H

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H

9-β-D-ribofuranosylhypoxanthine

**Properties.** A white or slightly yellowish, crystalline powder. Slightly soluble in water, very slightly soluble in ethanol, practically insoluble in ether and in chloroform.

**Identification.** 1. IR-spectrum.

2. Determine ribose (see Adenosinetriphosphoric Acid).

**Assay.** Spectrophotometry.

**Usage.** It is used-for the treating of some cardiac diseases (infarct, myocardial dystrophy) and liver diseases.

**Storage**. Protected from light.