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Unit II

Chemical Properties of Proteins

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Chemical Properties of Amino Acid

Chemical reactions of amino acids due to carboxyl and amino groups:

- **Reaction due to carboxyl groups**
- **Reaction due to amino groups**
- **Reaction due to carboxyl and amino groups**

Chemical Properties of Amino Acid

Chemical reactions of amino acids due to carboxyl and amino groups:

Reaction due to carboxyl groups

a) Decarboxylation:

b) Reaction with Alkalies (Salt formation):

c) Reaction with Alcohols (Esterification) :

d) Reaction with Amines:

Reaction due to amino groups

a) Reaction with Formaldehyde

b) Reaction with Benzaldehyde

c) Reaction with Nitrous acid
(Van slyke reaction):

d) Reaction with Sanger's Reagent

e) Reaction with DANSYL Chloride

f) Reaction with acylating agents (Acylation):

CHEMICAL PROPERTIES

A. HYDROLYSIS

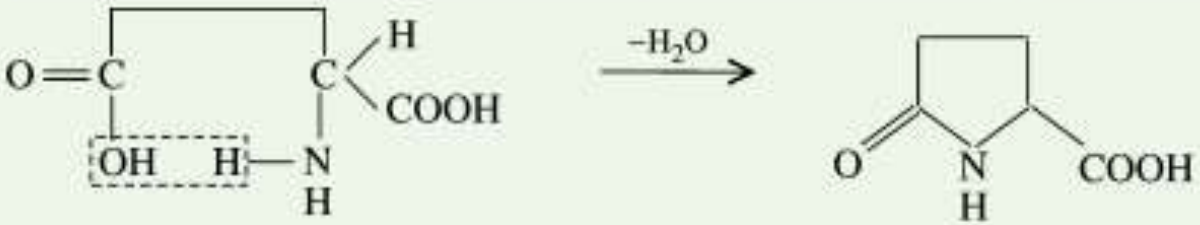
Proteins are hydrolyzed by a variety of hydrolytic agents.

1. By acidic agents. Proteins, upon hydrolysis with conc. HCl (6–12N) at 100–110°C for 6 to 20 hrs, yield amino acids in the form of their hydrochlorides. Undesirable side-effects of acid hydrolysis include the following:

(*a*) Tryptophan, serine and threonine are destroyed during acid hydrolysis and as such this reaction is not used for their isolation.

(*b*) Asparagine and glutamine are deamidated to aspartate and glutamate respectively.

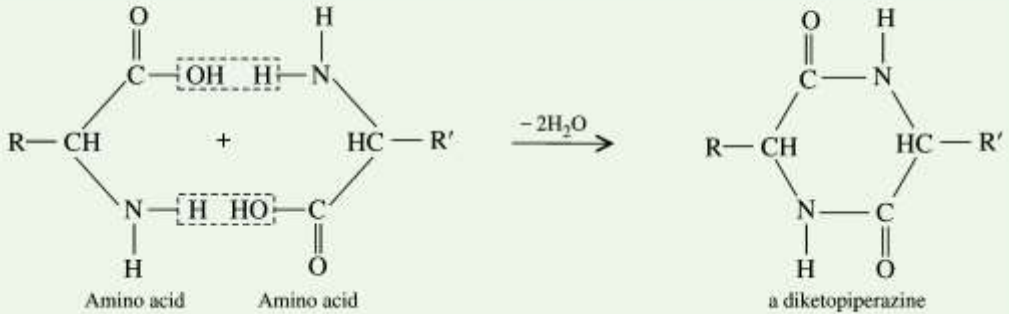
(*c*) Glutamic acid undergoes *intramolecular dehydration* to pyrollidone 5-carboxylic acid.



Glutamic acid

Pyrollidone 5-carboxylic acid

(*d*) Other amino acids may undergo *intermolecular dehydration* forming cyclic anhydrides or diketopiperazines.



2. By alkaline agents. Proteins may also be hydrolyzed with 2N NaOH. Alkaline hydrolysis is, however, less used as it is highly disadvantageous:

(*a*) It leads to the destruction of certain amino acids like arginine, cysteine, cystine, serine, threonine etc.

(*b*) It also causes loss of optical activity (or racemization) of the amino acids.

3. By proteolytic enzymes. Under relatively mild conditions of temperature and acidity, certain proteolytic enzymes like pepsin and trypsin hydrolyze the proteins. Enzyme hydrolysis is used for the isolation of certain amino acids like tryptophan. Two important drawbacks with this type of hydrolysis are:

(*a*) It requires prolonged incubation.

(*b*) Hydrolysis is incomplete.

Reaction due to carboxyl groups

a) Decarboxylation:

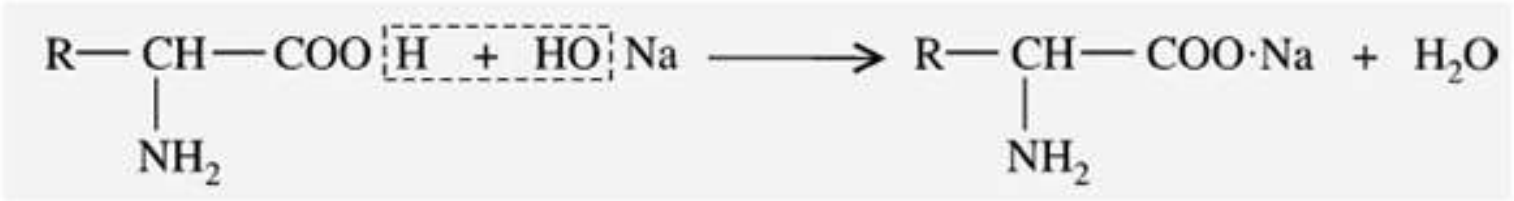
The amino acids will undergo alpha decarboxylation to form the corresponding "amines". Thus important amines are produced from amino acids.

- **Histidine** → Histamine + CO_2
- **Tyrosine** →Tyramine + CO_2
- **Tryptophan** →Tryptamine + CO_2
- **Lysine** →Cadaverine + CO_2
- **Glutamic acid** → Gamma Amino Butyric Acid (GABA) + CO_2

Reaction due to carboxyl groups

b) Reaction with Alkalies (Salt formation):

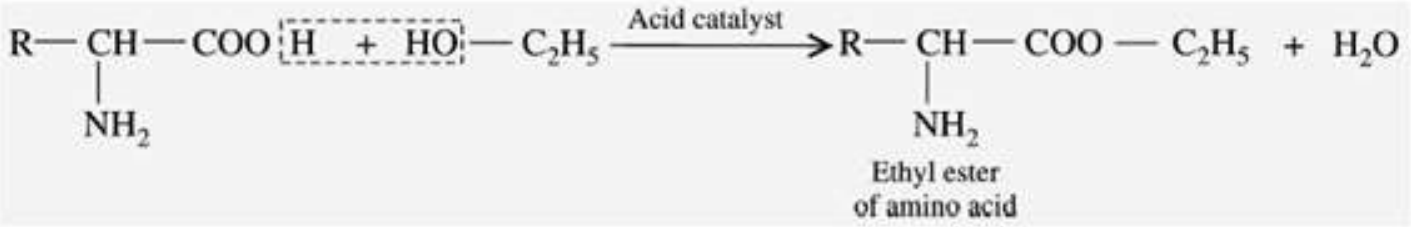
The carboxyl group of amino acids can release a H^+ ion with the formation of Carboxylate (COO^-) ions. These may be neutralized by cations like Na^+ and Ca^{+2} to form Salts. Thus amino acids react with alkalies to form "Salts".



Reaction due to carboxyl groups

c) Reaction with Alcohols (Esterification) :

When the amino acids is reacted with alcohol to form, "Ester". The esters are volatile in contrast to the form amino acids.



Reaction due to carboxyl groups

d) Reaction with Amines:

Amino acid reacts with Amines to form "Amides".

Reaction due to amino groups

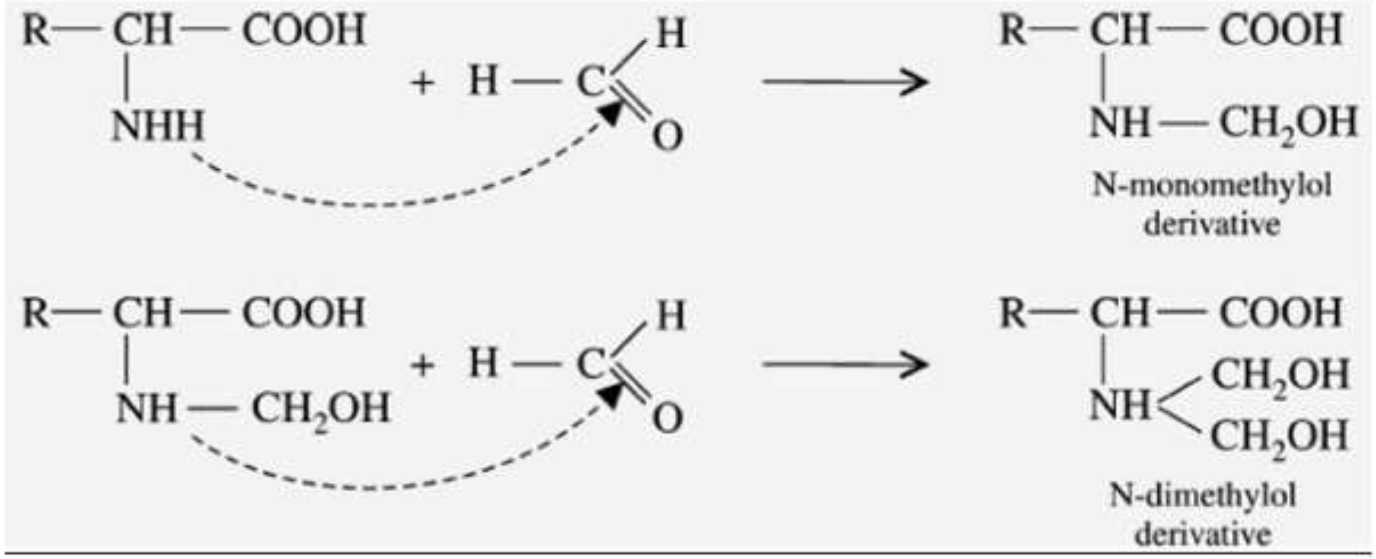
a) Reaction with Mineral acids (Salt formation)

When the amino acids are treated with mineral acids (like HCl), it forms “Acid Salts”.

Reaction due to amino groups

b) Reaction with Formaldehyde:

When the amino acid reacts with two molecules of Formaldehyde it forms "N-dimethylol derivative" (Hydroxy-methyl derivative). This reaction is done in two steps. These derivatives are insoluble in water and resistant to attack by microorganisms.



Reaction due to amino groups

c) Reaction with Benzaldehyde:

When the amino acid reacts with Benzaldehyde, it gives "Schiff's base".

Reaction due to amino groups

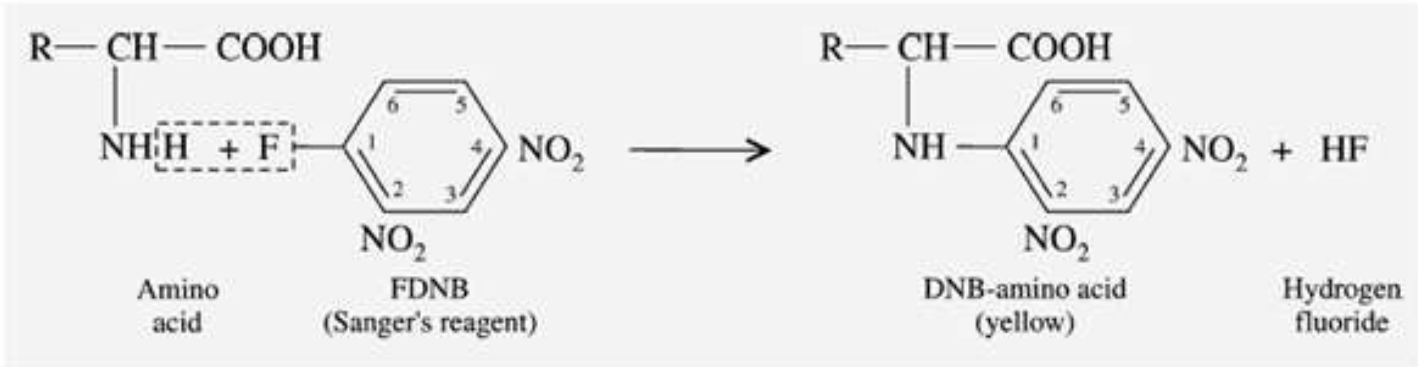
d) Reaction with Nitrous acid (Van slyke reaction):

When the amino acids react with Nitrous acid (HNO2) to liberate N2 gas and to produce the corresponding "α-hydroxyl acid". The imino acids Proline and Hydroxyproline do not respond to this reaction.

Reaction due to amino groups

e) Reaction with Sanger's reagent:

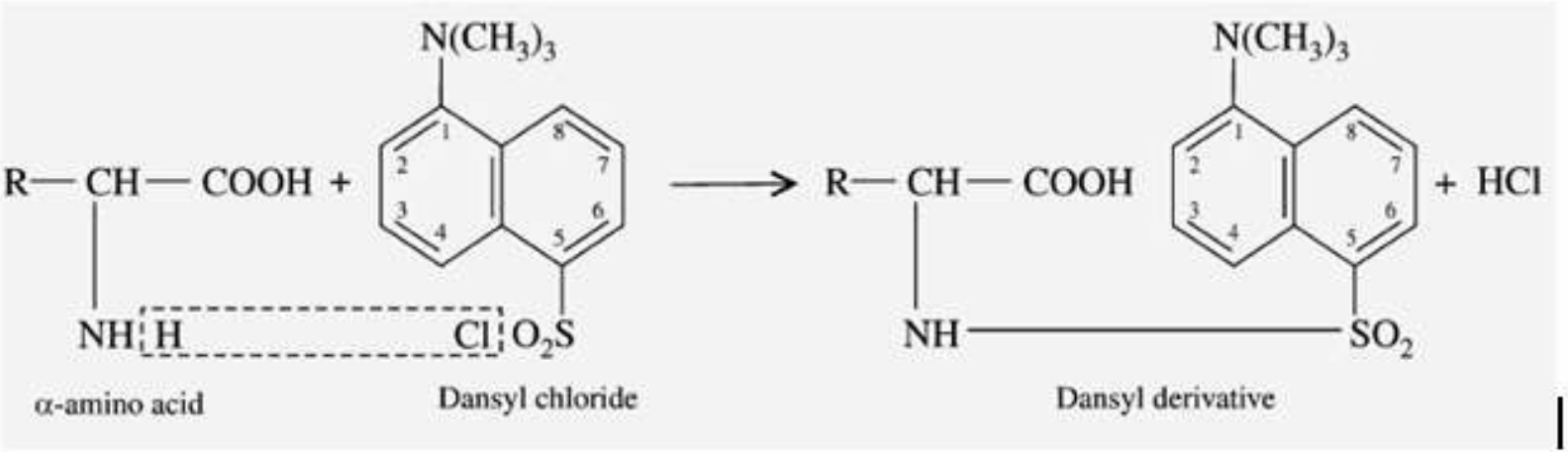
"1-flouro-2,4-dinitrobenzene" is called Sanger's reagent (FDNB). In mildly alkaline solution, sanger's reagent reacts with α-amino acid to produce Yellow colored derivative, DNB-amino acid.



Reaction due to amino groups

f) Reaction with DANSYl Chloride:

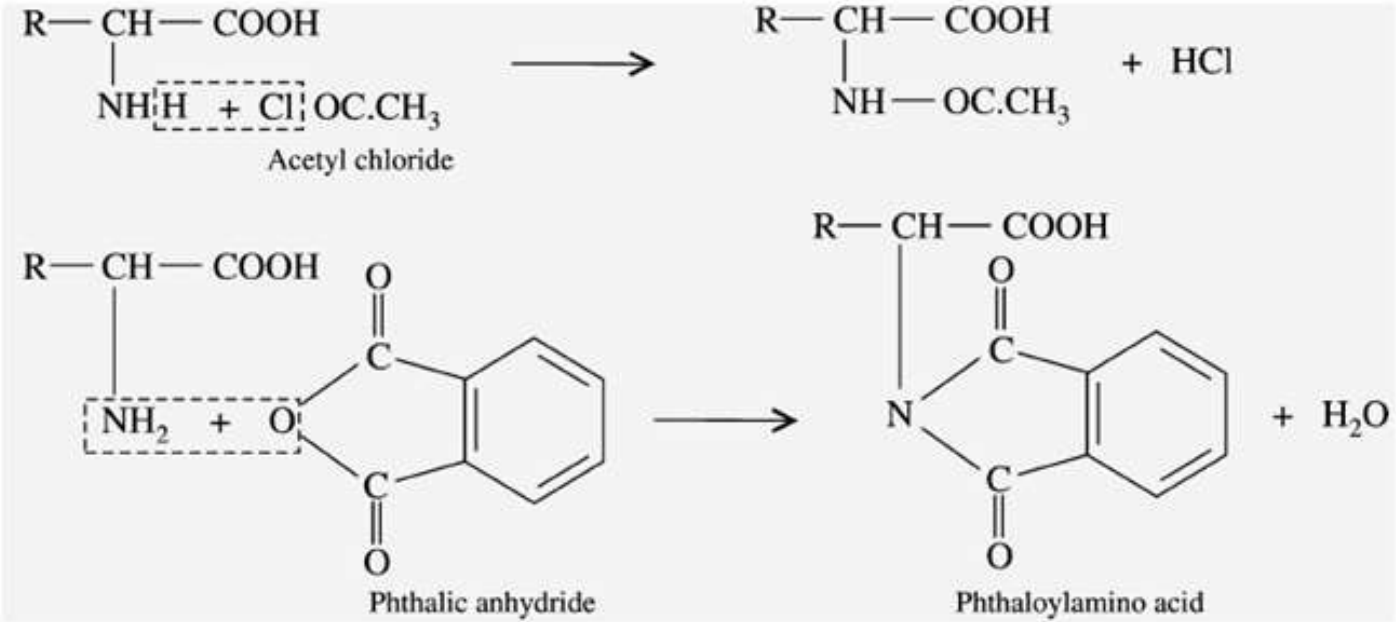
DANSYl chloride means "Dimethyl Amino Naptha Sulphonyl Chloride". When the amino acid reacts with DANSYl chloride reagent, it gives a "Flourescent DANSYl derivative".



Reaction due to amino groups

g) Reaction with acylating agents (Acylation):

When the amino acids react with "Acid chloride" and acid anhydride (Pthalic anhydride) in alkaline medium it gives "pthaloyl amino acid".

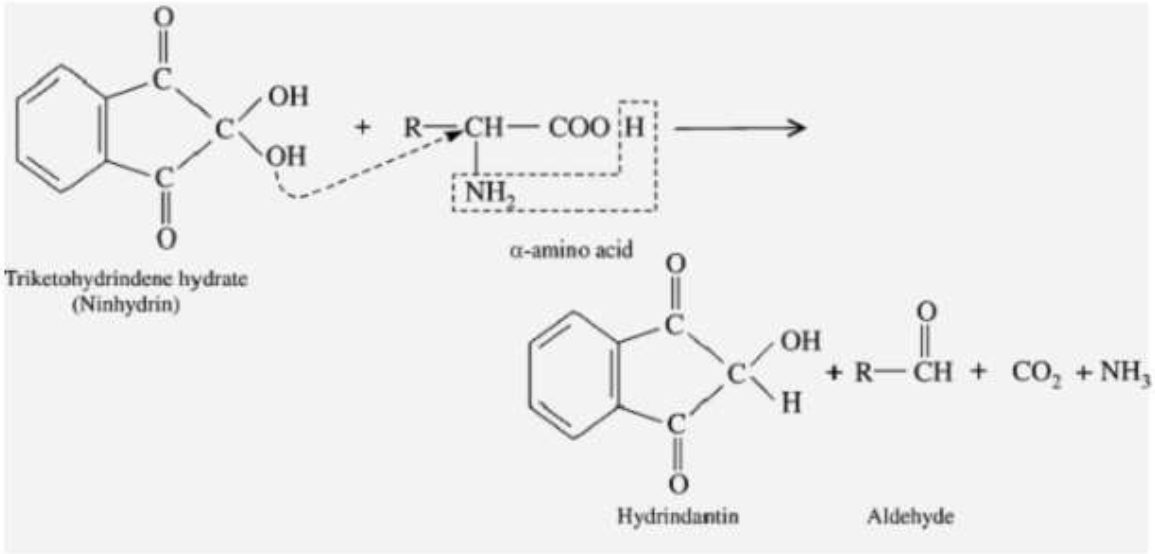


Reaction due to carboxyl and amino groups

Ninhydrin reaction:

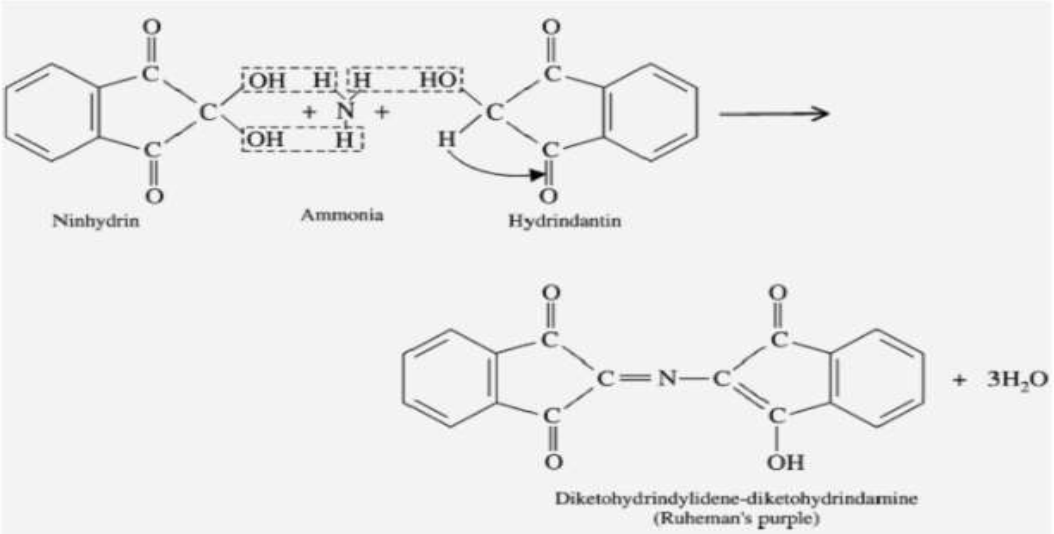
Step1:

Ninhydrin (=indane 1,2,3-trione hydrate) is a powerful oxidizing agent and causes oxidative decarboxylation of α-amino acids producing CO_2, NH_3 and an aldehyde with one less carbon atom than the parent amino acid.



Step2:

The reduced ninhydrin then reacts with the liberated NH3 and a mole of ninhydrin, forming Blue-colored Rhumann's complex.

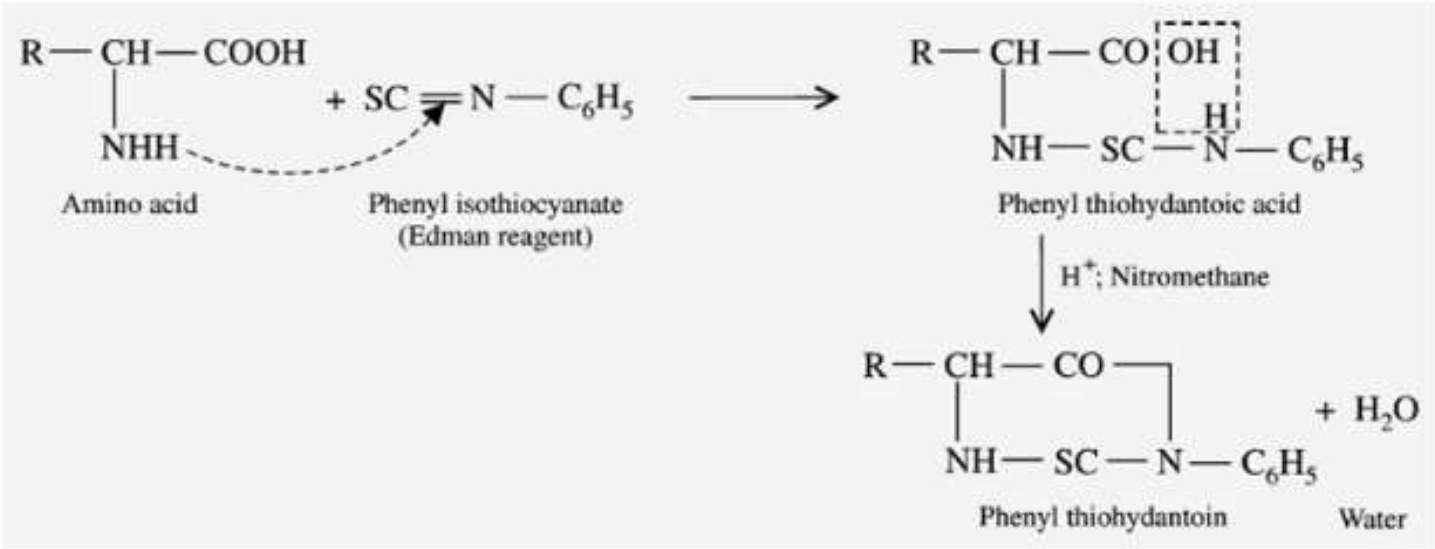


This reaction is very sensitive reaction and it is used for amino acid and imino acid identification.

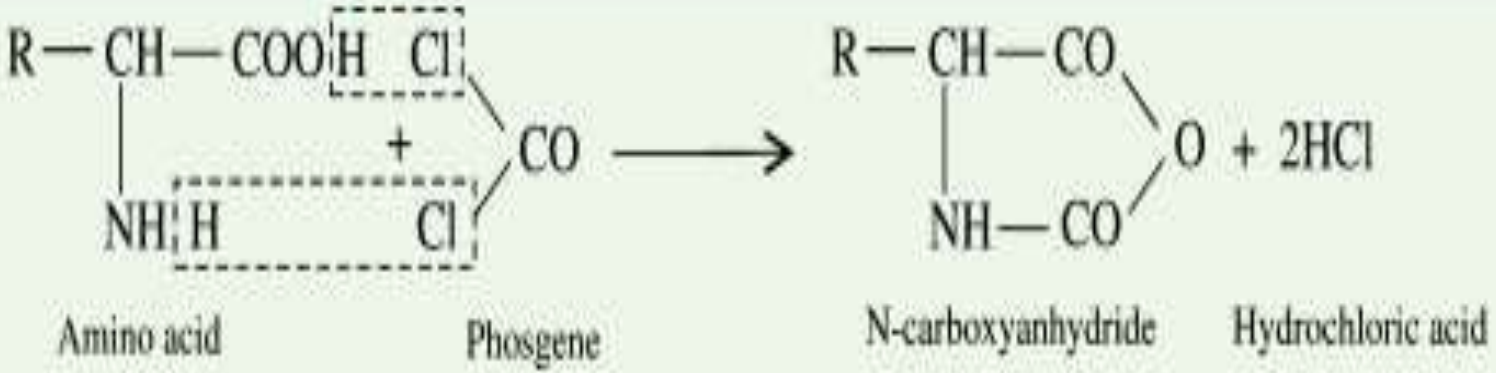
When Amino acids (or) Imino acid reacts with Ninhydrin molecule it gives Color. When it gives **Purple color (Rhumann's Complex)** –the Unknown sample is Amino acids (Which have primary amine $-NH_2$) or it is gives Yellow color – the Unknown sample is Imino acid (**-NH-**).

Reaction with Edmann's degradation:

Edmann's reagent is **"phenylisothiocyanate"**. When amino acids react with Edmann's reagent it gives "*phenyl thiohydantoic acid*" finally it turns into cyclized form "*Phenyl thiohydantoin*" (Edmann's derivative).

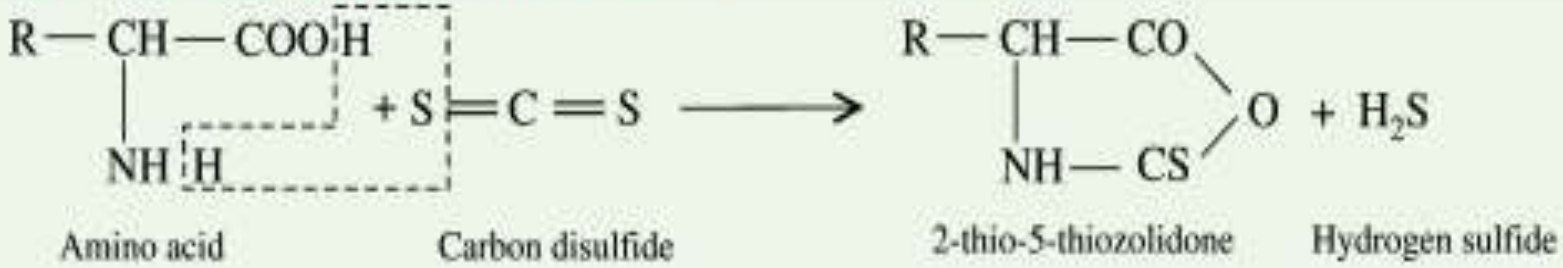


4. Reaction with phosgene. With phosgene, *N-carboxyanhydride is formed.*



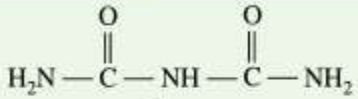
Reaction with carbon disulfide

5. Reaction with carbon disulfide. With carbon disulfide, *2-thio-5-thiozolidone* is produced.



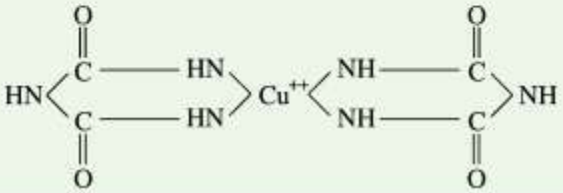
Reactions with R Group or Side chain

1. Biuret test. Compounds containing peptide bonds produce a characteristic purple colour when treated with an alkaline 0.2% copper sulfate solution (or biuret reagent). This reaction is termed as 'biuret reaction' since it is also given by the substance biuret.



Biuret

The colour deepens as the number of peptide bonds is increased and the proteins produce a deep blue-violet colour due to the probable formation of a coordination complex whose structure is given below:



2. Xanthoproteic test. Yellow colour develops on boiling proteins with conc. HNO_3 due to the presence of benzene ring. This reaction is due to the nitration of the phenyl rings (of *tyrosine, tryptophan* and *phenylalanine*) to yield yellow substitution products, which turn orange upon addition of alkali.

$$\text{Protein} + HNO_3 \xrightarrow{\text{Xanthoproteic Test}} \underset{(= \text{Picric acid})}{\text{Trinitrophenol}} + NO\uparrow$$

3. Millon's test. Red colour develops when proteins are heated with $Hg.NO_3$ in HNO_2. The reaction is specific for *tyrosine* and takes place between mercuric and mercurous nitrates and tyrosine residues of the protein. *Tryptophan* also responds to this reaction.

4. Hopkins–Cole test or Glyoxylic acid test. Violet ring develops on addition of conc. H_2SO_4 (36 N) at the junction of protein and glyoxylic acid solutions. The test is specific for *tryptophan*.

5. Folin's test. Blue colour develops with phosphomolybdotungstic acid in alkaline solution due to the presence of phenol group. The test is specific for *tyrosine*.

6. Sakaguchi test. Red colour develops with a-naphthol and sodium hypochlorite. The test is applied for the detection of *arginine*.

7. Pauly test. Red colour develops with diazotized sulfanilic acid in alkaline solution. The reaction is specific for *tyrosine* and *histidine*.

8. Ehrlich test. With *p*-dimethylaminobenzaldehyde in 12 N HCl, *tryptophan* develops a blue colour.

F. REACTIONS INVOLVING SH GROUP

1. Nitroprusside test. Red colour develops with sodium nitroprusside in dilute $NH_4.OH$. The test is specific for *cysteine*.

2. Sullivan test. *Cysteine* develops red colour in the presence of sodium 1, 2-naphthoquinone-4-sulfonate and sodium hydrosulfite.

Thank you