BIOMOLECULES
SEM-5, CC-12
PART-3, PPT-23

Part-3: Amino Acids-III, Reactions

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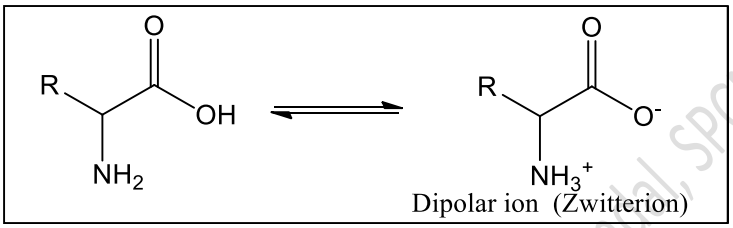
BIOMOLECULES (PART-3, PPT-23)

Part-3: Amino Acids-III, Reactions

Resolution of *Racemic* Amino Acids

Amino acids remain in zwitterionic form because of the presence of both basic (-NH_2) and acidic (-CO_2H) groups in the same molecule and therefore, cannot be resolved like ordinary active acids or bases. Consequently, either the amino or the carboxyl group should be derivatized to achieve resolution.



Amino acids are usually resolved in the form of their *N*-benzoyl, N-formyl, *N*-tosyl and *N*-phthaloyl derivatives which are no longer endowed with zwitterionic properties and may be resolved as typical active acids with optically pure brucine or strychnine. The usual method is to protect the amino group and then convert the product into diastereomeric salts with optically active bases. (±)-Alanine can be resolved as brucine salts of *N*-benzoylalanine as shown in Figure 1.

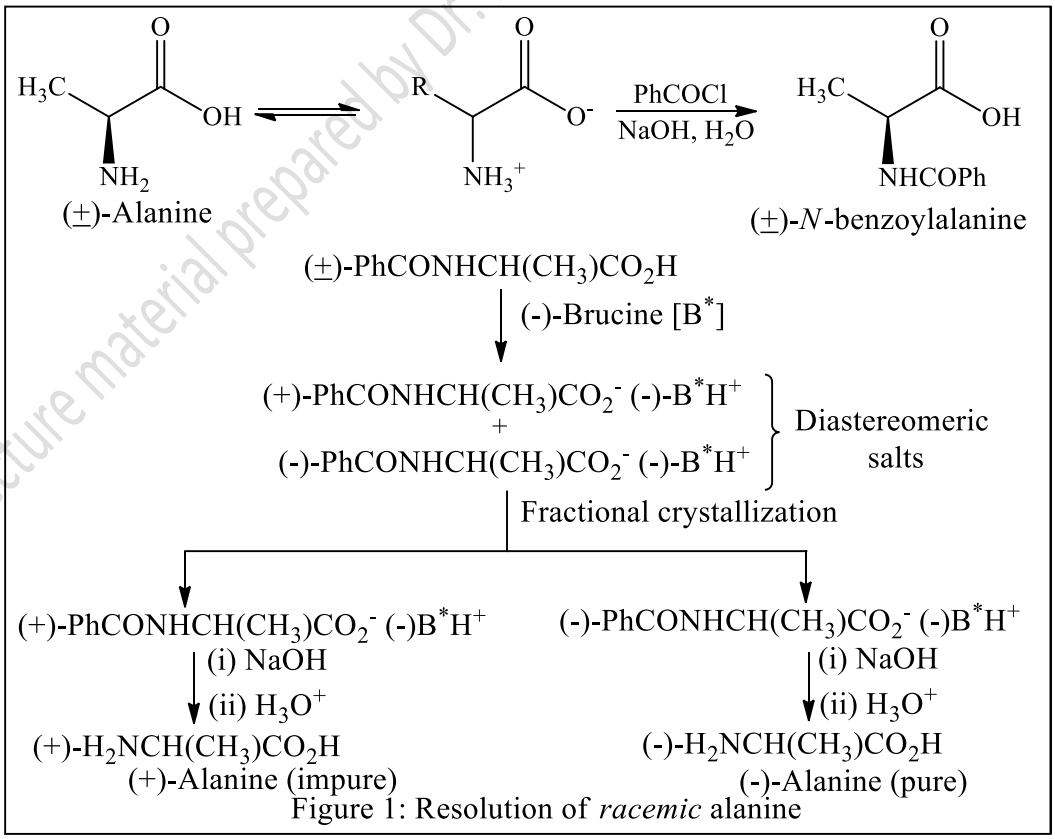


Figure 1: Resolution of *racemic* alanine

The *racemic N*-benzoylalanine is resolved in the usual way using optically pure brucine or strychnine. If brucine is used, the brucine salt of the D(-)-enantiomer of *N*-benzoylalanine is less soluble. If strychnine is used, the strychnine salt of the L(+)-enantiomer of *N*-benzoylalanine crystallizes. Acidification of the salt yields the D(-)- and L(+)-enantiomers of *N*-benzoylalanine. Basic hydrolysis gives the pure enantiomeric alanine.

The enantiomer that forms the less soluble salt is usually obtained in an optically pure condition. Since the more soluble enantiomer is usually obtained by evaporation of the solution, it will be eventually optically impure by the presence of less soluble salt in solution. The impure *N*-benzoyl-L(+)-alanine may then be treated with strychnine to give the insoluble strychnine salt. Usual work-up gives the (+)-alanine in pure form. Following this procedure both enantiomers may be isolated in an optically pure form.

In the removal of benzoyl group from the resolved derivative by acid hydrolysis, some racemization may occur due to the presence of acidic hydrogen atom on the *chiral* centre. This difficulty can be avoided by subjecting the *N*-formyl derivative of alanine to resolution. Formyl group from *N*-formylalanine, $OHCNHCH(CH_3)CO_2H$ can be removed under mild conditions and does not lead to racemization of the resolved material.

Some basic amino acids, which contain a free as well as a zwitterionic amine function, have been resolved by means of organic acids. Therefore, (+)-tartaric acid has been used to resolve (±)-histidine, and (±)-lysine may be resolved by means of (+)-glutamic acid. Alternatively, the carboxyl end of the amino acid [H_2N-CH(R)-CO_2H] can be protected by esterification and resolution can be done through salt formation with optically active acids. Many *racemic* α-amino acids have been successfully resolved by preparing isobutyl or benzyl esters and using dibenzoyl tartaric acid as the resolving agent.

A few exceptions are known where specific amino acids can be resolved without derivatization. One known example is the resolution of phenylalanine, $PhCH_2CH(NH_2)CO_2H$. This has been resolved using optically pure camphor-10-sulfonic acid which itself is a very strong acid, pK_a 1.2.

Reaction of Amino Acids with Ninhydrin

The presence of amino acids can be detected by the formation of a purple colour on treatment with ninhydrin. The same compound responsible for the purple colour is formed from all amino acids in which the α-amino group is primary.

Ninhydrin is the hydrate of indane-1,2,3-trione (Figure 2). With the exception of proline and hydroxyproline, all of the α-amino acids found in proteins react with ninhydrin to give the same intensely colored purple anion (λ_{max} 570 nm) (Figure 3) and the formation of which is illustrated mechanistically in Figure 4.

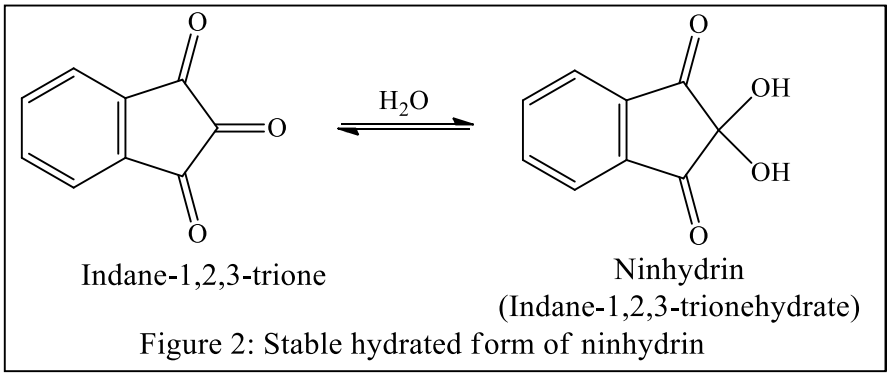


Figure 2: Stable hydrated form of ninhydrin

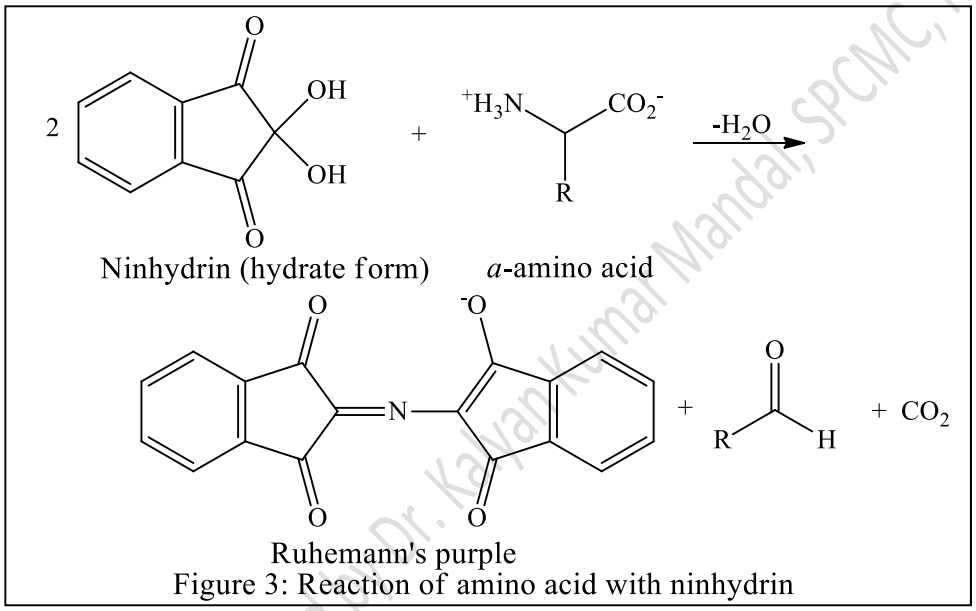


Figure 3: Reaction of amino acid with ninhydrin

It is to be noted that the only portion of the anion that is derived from the α-amino acid is the nitrogen as shown in Figure 3.

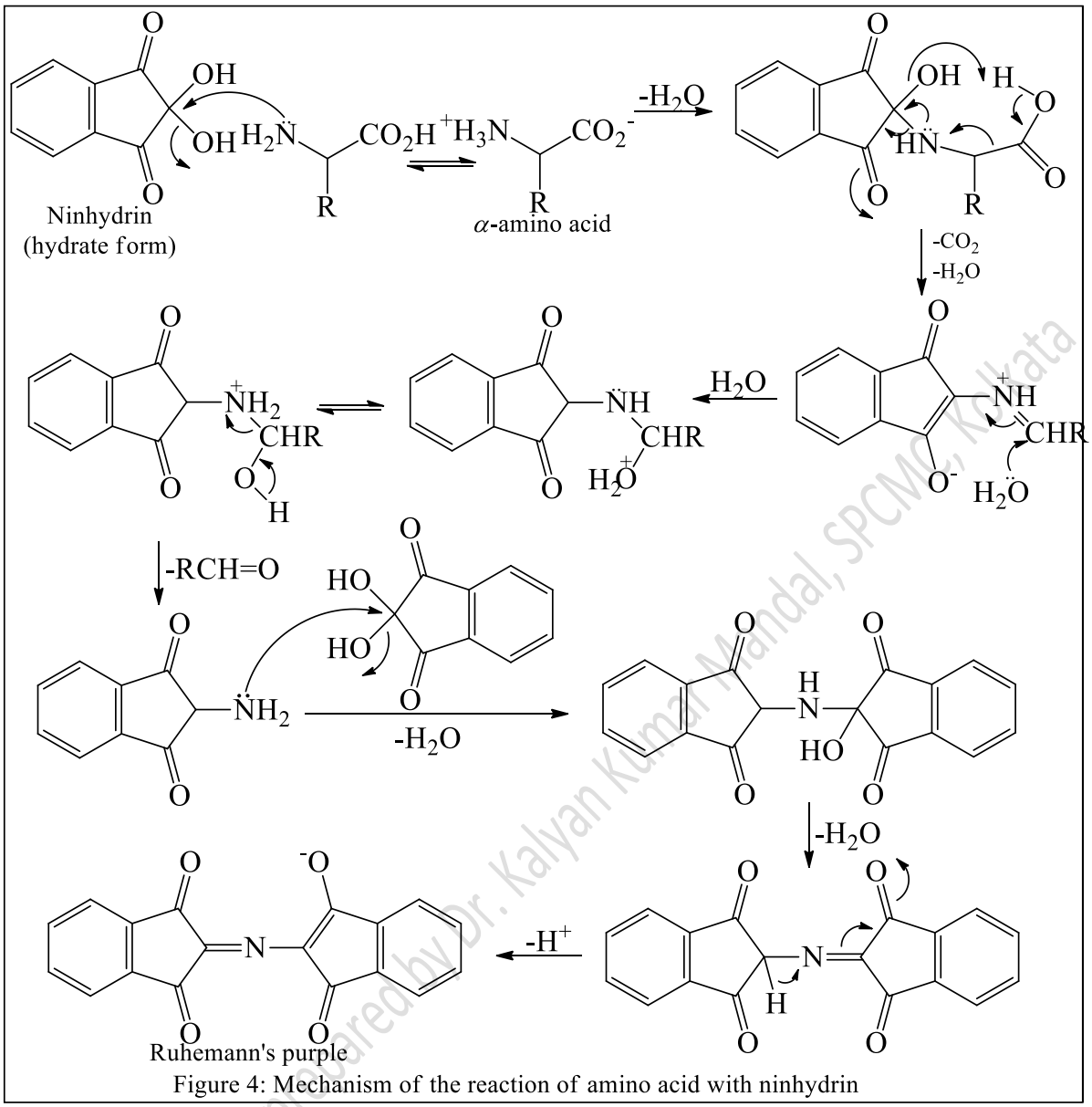


Figure 4: Mechanism of the reaction of amino acid with ninhydrin

Reaction of Imino Acids with Ninhydrin

The ninhydrin is used as a spraying reagent in the identification and quantitative estimation of amino acids. All α-amino acids give the same purple (or blue) product. Proline and hydroxyproline do not react with ninhydrin in the same way because their α-amino groups are secondary amines and part of a five-membered ring. However, they give an orange (or yellow) compound on reaction with ninhydrin.

The reaction of imino acids with ninhydrin proceeds initially in a similar manner to that of α-amino acids. Imino acids, e.g., proline and hydroxyproline, react with ninhydrin to give a orange-yellow color. At higher temperatures (~100 °C), the yellow compound (A) is transformed to the purple-red compound (B). The reaction is shown in Figure 5.

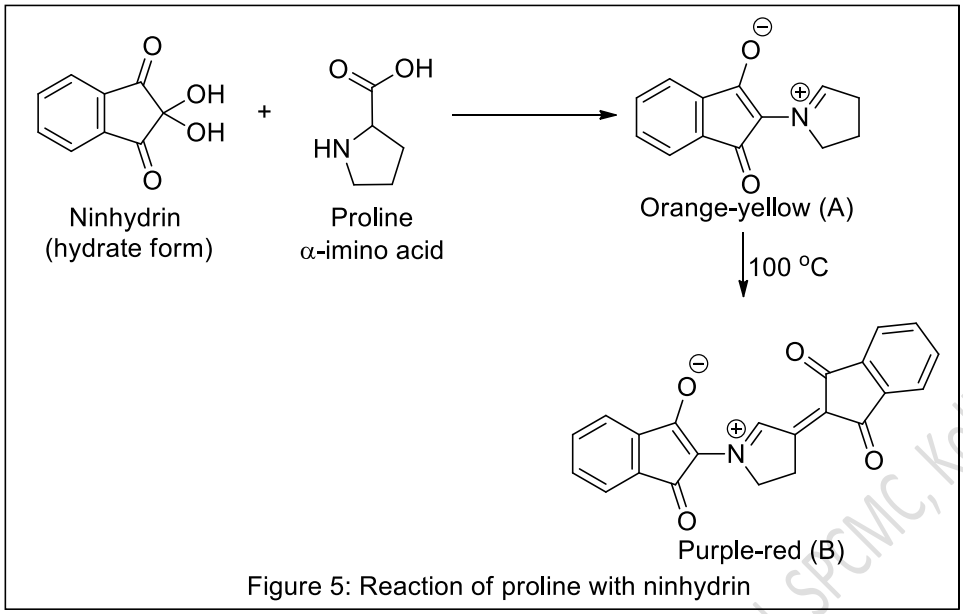


Figure 5: Reaction of proline with ninhydrin

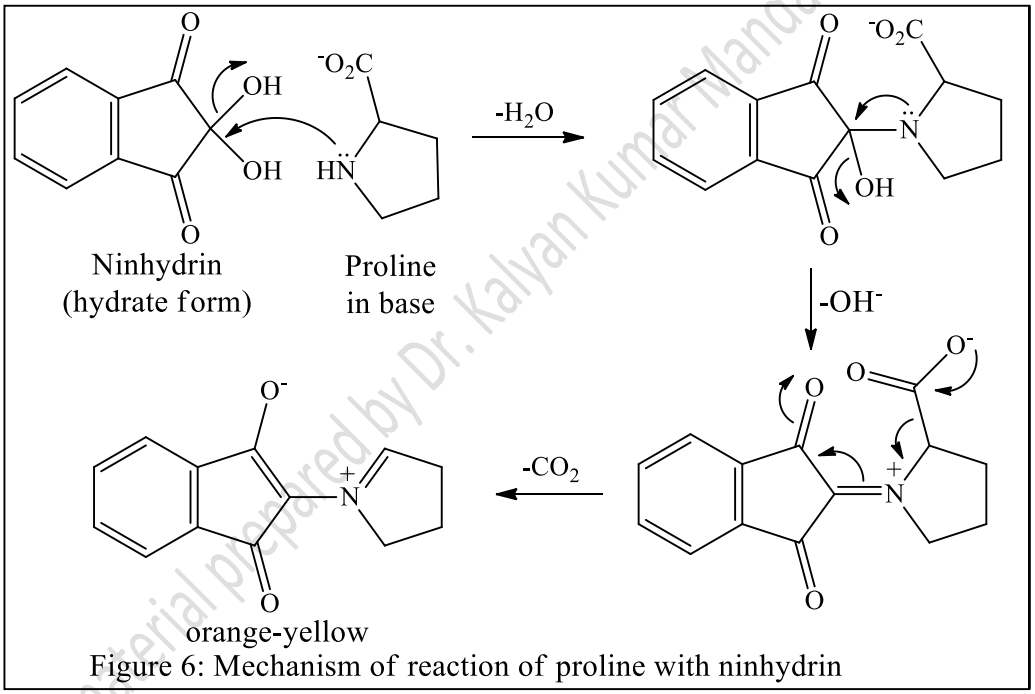


Figure 6: Mechanism of reaction of proline with ninhydrin

Other reagents are also used, e.g., sodium 2,4,6-trinitrobenzene-1-sulfonate (TNBS). Also, specific reagents may be used to detect particular amino acids, e.g., diazotized sulfanilic acid couples with tyrosine and histidine in alkaline solution to give a red colour.

Dakin-West Reaction

When heated with acetic anhydride (Ac_2O) in pyridine solution, α-amino acids are converted into methyl α-acetamido ketones. This reaction is referred to as Dakin-West reaction.

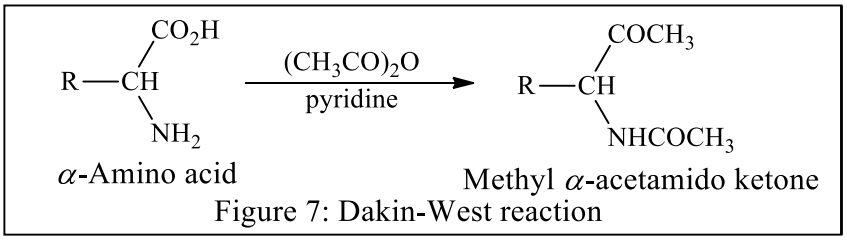


Figure 7: Dakin-West reaction

The reaction begins with acylation of the amino acid. An intramolecular reaction then follows to give azlactone intermediate. A series of addition and elimination steps and the release of carbon dioxide gas provide the acylamino ketone product. With pyridine as a base and solvent, refluxing conditions are required. However, with the addition of 4-dimethylaminopyridine (DMAP) as a catalyst, the reaction can take place at room temperature.

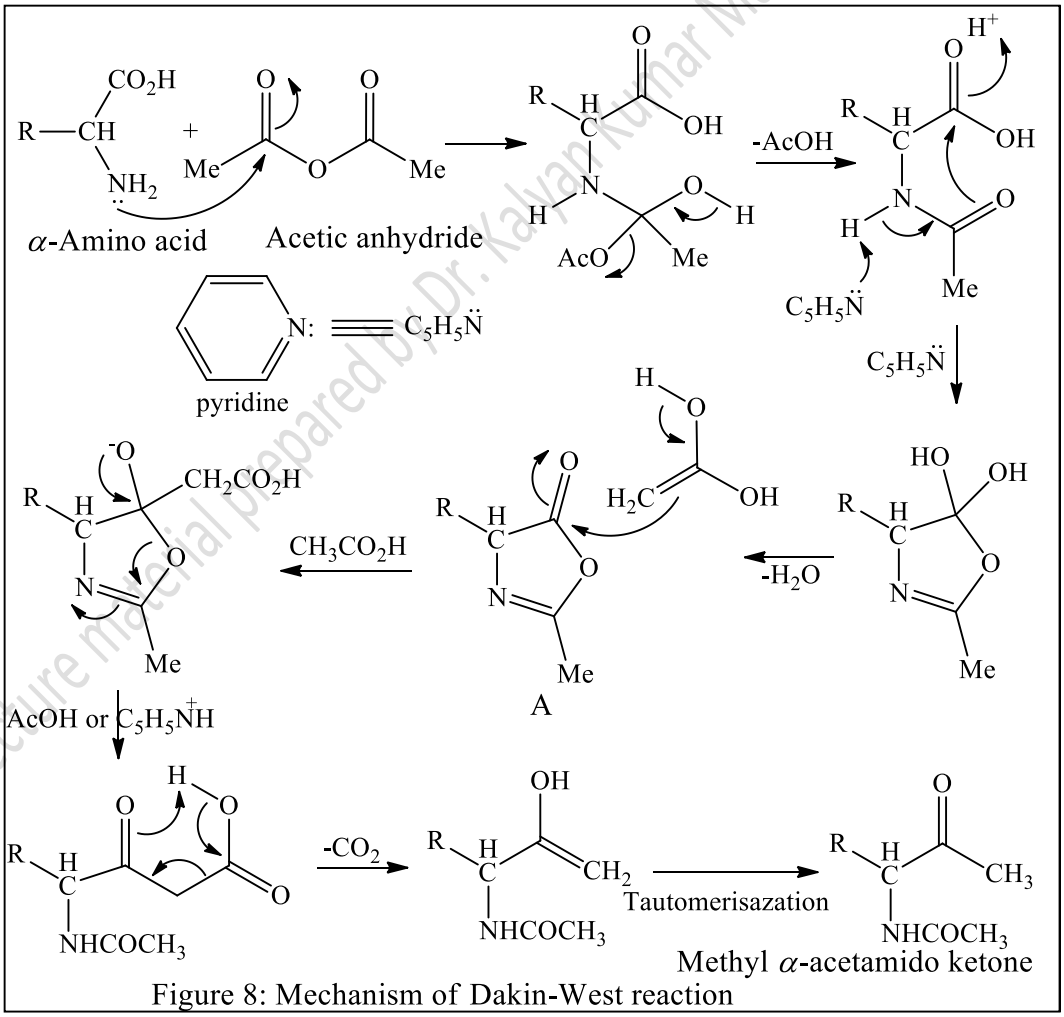


Figure 8: Mechanism of Dakin-West reaction

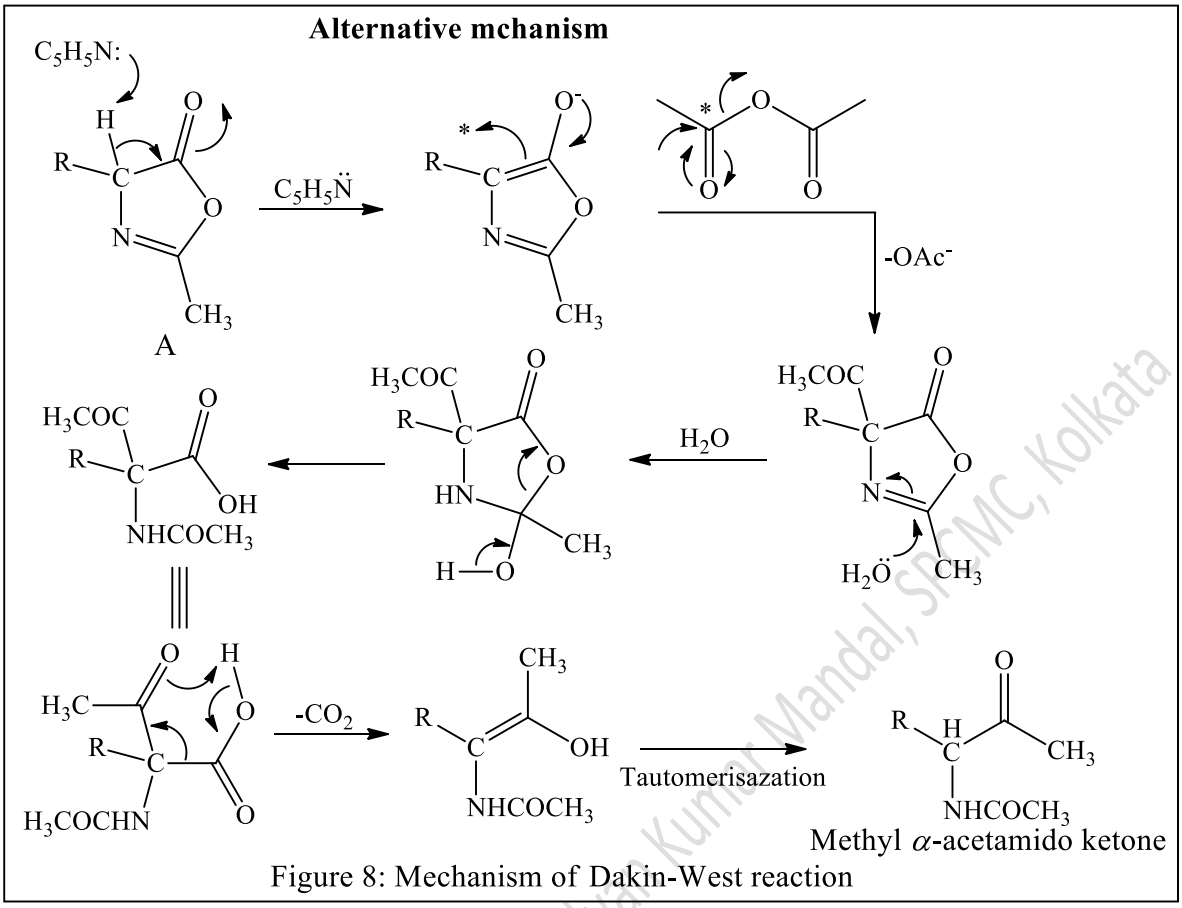
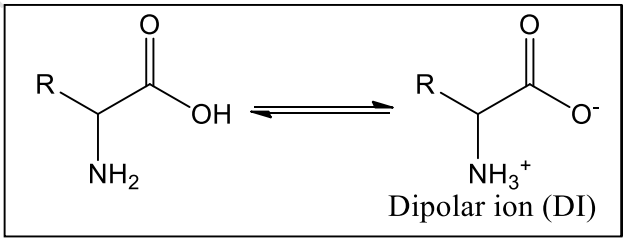


Figure 8: Mechanism of Dakin-West reaction

Estimation of Amino Acids by Sörensen Formol Titration (SFT)

The Sörensen formol titration (SFT) (1907) is a titration of an amino acid with potassium hydroxide in the presence of formaldehyde. It is used in the determination of protein content in samples. In aqueous solution of an amino acid, $H_2NCH(R)CO_2H$, the following equilibrium exists:



The dipolar ion (also known as zwitterion) structure accounts for the absence of any acidic and basic property of aqueous solution of the amino acid. For this reason, amino acids cannot be titrated directly with an alkali. However, in the presence of neutralized formaldehyde solution, amino acids behave as strong monoprotic acids and can be quantitatively titrated with standard alkali. Formalin stabilizes the amino ($-NH_2$) group by forming the Schiff base linkage (-N=CH-), as a result, zwitterion cannot be formed and the carboxylate group of the

amino acids is free. Consequently, this can be titrated with a strong base (e.g., NaOH) using phenolphthalein as indicator.

$$H_2C{=}O + H_2N\text{-}CH(R)\text{-}CO_2H \rightarrow H_2C{=}N\text{-}CH(R)\text{-}CO_2H + H_2O$$

$$H_2C{=}N\text{-}CH(R)\text{-}CO_2H + NaOH \rightarrow H_2C{=}N\text{-}CH(R)\text{-}CO_2^-Na^+ + H_2O$$

The reaction between glycine and formaldehyde may be different than that shown above. In this case, the reaction may lead to the formation of dimethylol glycine, which behaves as a strong monoprotic acid.

$$2H_2C{=}O + H_2N\text{-}CH_2\text{-}CO_2H \rightarrow (HOH_2C)_2N\text{-}CH_2\text{-}CO_2H$$

$$(HOH_2C)_2N\text{-}CH_2CO_2H + NaOH \rightarrow (HOH_2C)_2N\text{-}CH_2CO_2^-Na^+ + H_2O$$

In either way,

1000 mL of (N) NaOH ≡ 1 g equivalent of glycine

≡ 75 g of glycine

≡ 1 F. W. of amino acid

In the formol titration method of Sörensen, neutralized formaldehyde is added to the solution of the amino-acid and standard alkali run in until the production of a red colour with phenolphthalein. An explanation of this method is that the original amino acid is neutral because the basic $-NH_2$ neutralizes the acidic $-CO_2H$, and that with the addition of formaldehyde the basic character of the $-NH_2$ is destroyed, with the result that the acidic $-CO_2H$ is free to be titrated.

It can also be viewed in the way that a neutral amino-acid is so because the $-NH_2$ and the $-CO_2H$ are neither appreciably ionized, *i.e.,* functioning neither as base nor acid in neutral solution. The cause of the neutrality of a monoamino-monocarboxylie acid, like glycine, is that a neutral solution of a monoamino-monocarboxylic acid consists of neutral undissociated molecules of the ampholyte.

Accuracy in Formol Titration

There have been some inaccuracies of the SFT caused by the differences in the basicity of the nitrogen in different amino acids. For instances, proline (an imino acid, p*I* 6.30), histidine (p*I* 7.59), and lysine (p*I* 9.74) yields too low values compared to the theory. Unlike alpha, monobasic (containing one amino group per molecule) amino acids, these amino (or imino) acids nitrogens have inconstant (frequently changing) basicity, which results in partial reaction with formaldehyde.

On the contrary, in case of tyrosine (p*I* 5.66), the actual results are too high due to the electron withdrawing hydroxyl group (-OH), which acts as a base. This explanation is supported by the fact that phenylalanine (p*I* 5.48) can be accurately titrated.

Reaction of *α*-Amino acids with Nitrous acid van Slyke Method

Nitrous acid liberates nitrogen from amino acids. The nitrogen is evolved quantitatively, and this forms the basis of the van Slyke method (1911) for analyzing mixtures of amino acids (Figure 9).

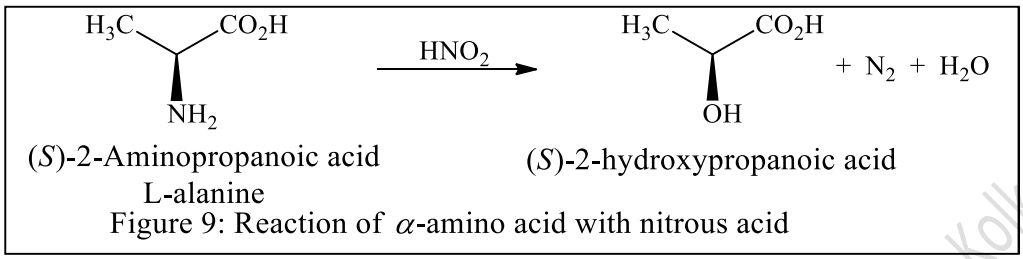


Figure 9: Reaction of *α*-amino acid with nitrous acid

The van Slyke determination is a chemical test for the determination of amino acids containing a primary amine group. To quantify aliphatic amino acids, the sample is diluted in glycerol and then treated with a solution of sodium nitrite, water and acetic acid. The resulting diazotization reaction produces nitrogen gas which can be observed qualitatively or measured quantitatively.

(*S*)-2-aminopropanoic acid (A) upon treatment with nitrous acid undergoes a substitution reaction leading to the formation of (*S*)-2-hydroxypropanoic acid (B). This reaction is seen to occur with *retention* of configuration at a stereogenic centre as shown in Figure 9. The explanation of this stereochemical outcome is that not one but two substitution reactions are occurring, each of which inverts the configuration of the stereocentre.

After formation of the diazonium salt (I), the carboxylate ion participates in an intramolecular substitution reaction producing *α*-lactone (II) with *inversion* of configuration at the stereocentre. The reaction is then completed by an S_N2 reaction in which water reacts with *α*-lactone (II), producing hydroxy acid (B) again with *inversion* of configuration at the stereocentre. The stereochemical outcome of this reaction is illustrated mechanistically in Figure 10.

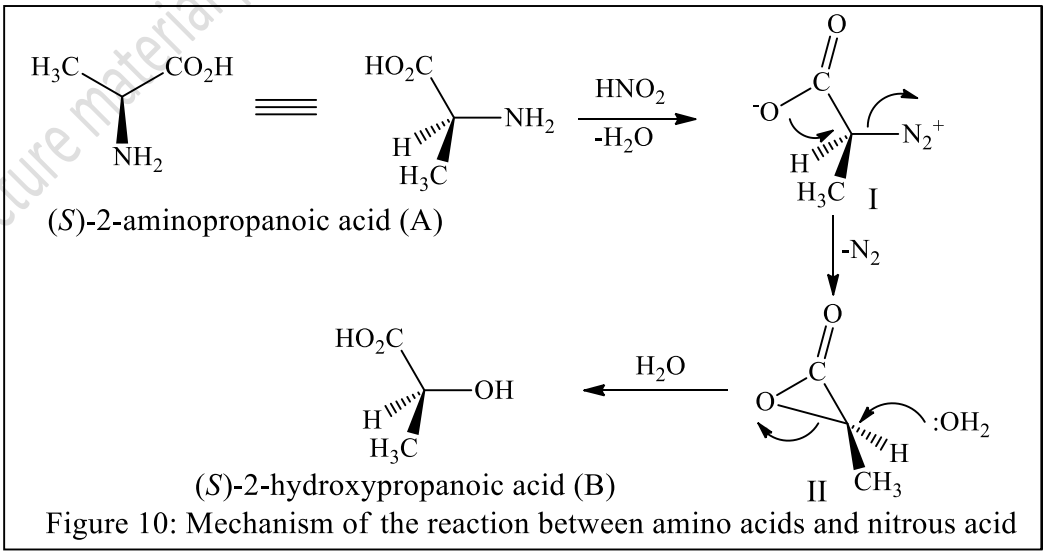


Figure 10: Mechanism of the reaction between amino acids and nitrous acid

The carboxylate ion in I is a neighbouring group which participates in the reaction and alters the stereochemical outcome. Neighbouring group participation in this substitution reactions is expected as the internal nucleophile is suitably placed in the molecule to form a three-membered ring intermediate, an α-lactone (II).

Formation of Hydantoins

Amino acids react with phenyl isocyanate to form phenylhydantoic acid (A), and these on treatment with hydrochloric acid, readily form hydantoins (B; Figure 11). If phenyl isothiocyanate (C) is used instead of the phenyl isocyanate, then thiohydantoins (D) are produced.

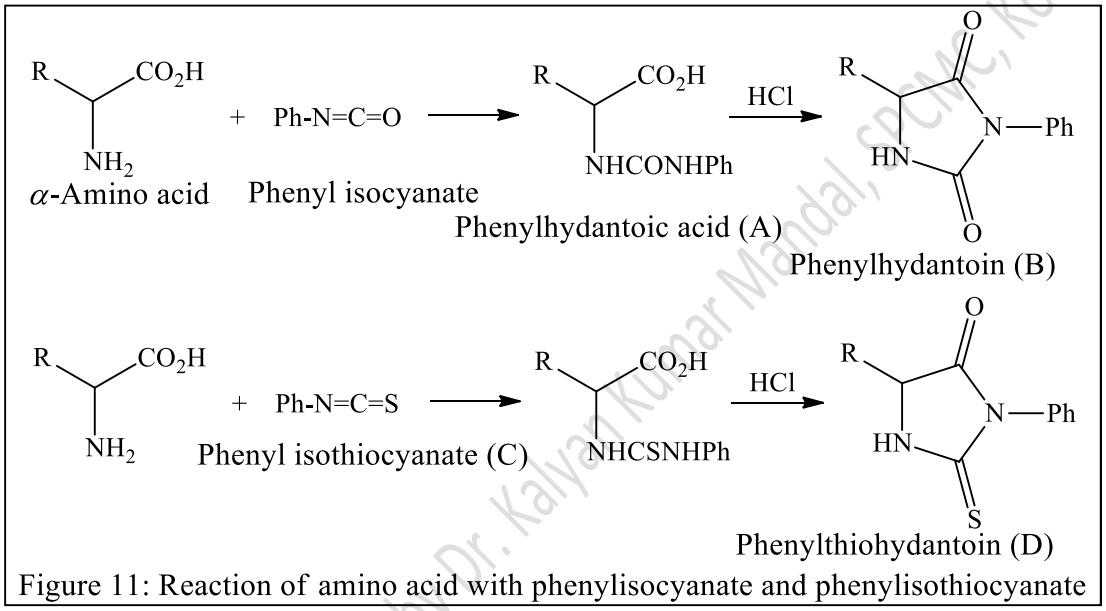


Figure 11: Reaction of amino acid with phenylisocyanate and phenylisothiocyanate

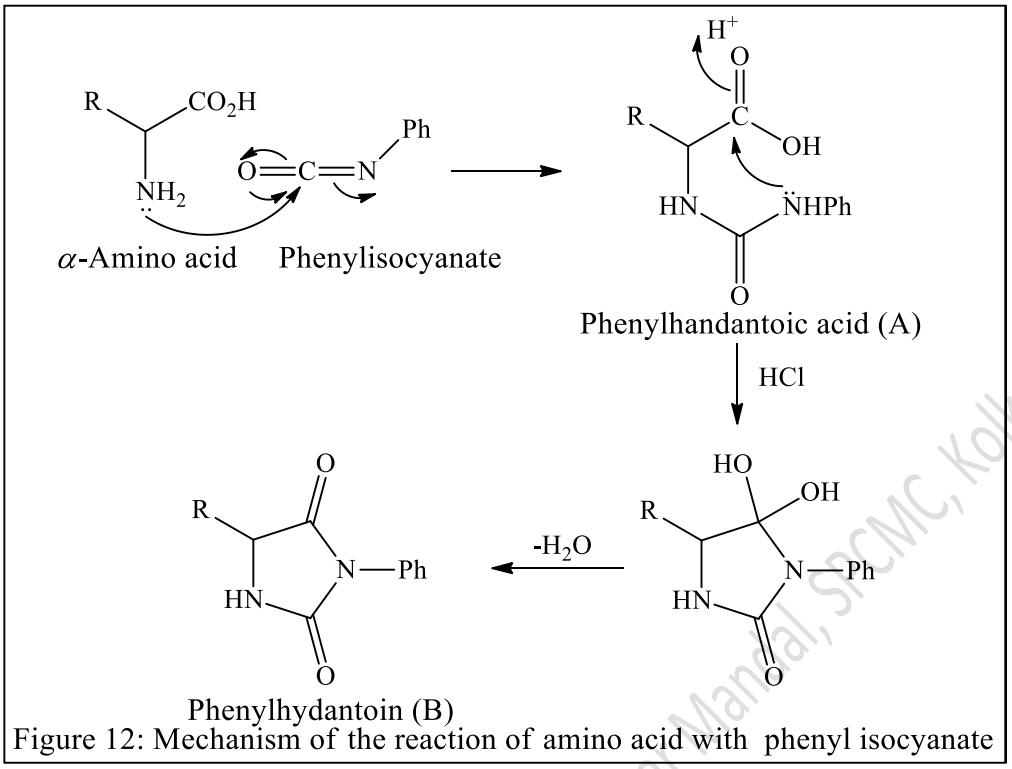


Figure 12: Mechanism of the reaction of amino acid with phenyl isocyanate

Reaction of *N*-Substituted Amino Acids with Nitrous Acid and Acetic Anhydride

N-alkyl or arylamino acids form *N*-nitroso derivatives with nitrous acid, and these may be dehydrated to sydnones by means of acetic anhydride.

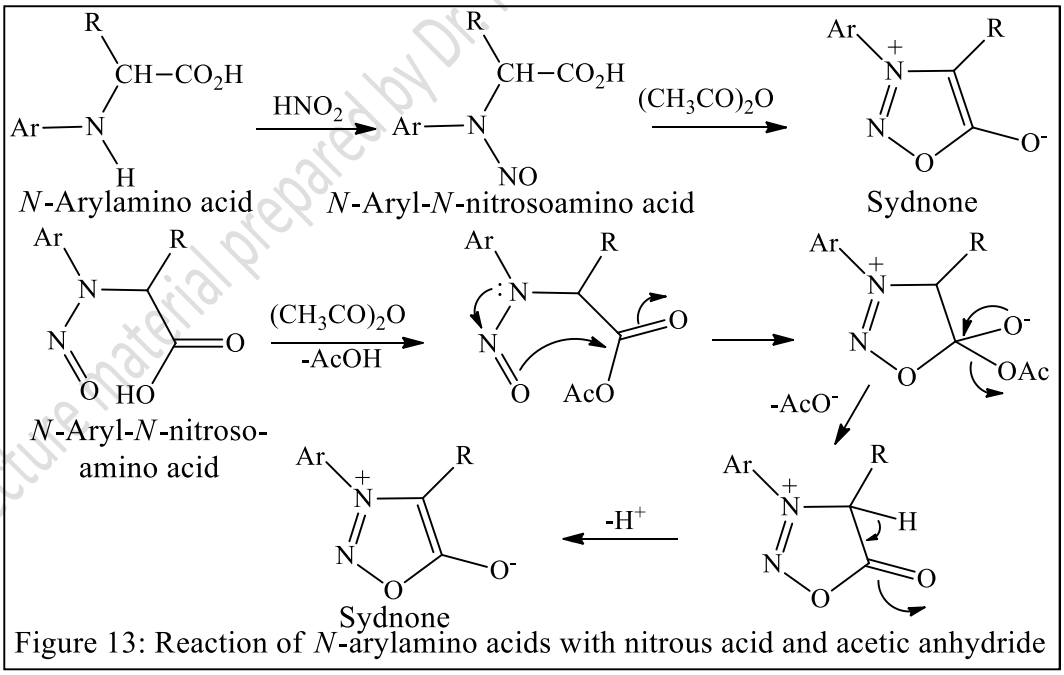


Figure 13: Reaction of *N*-arylamino acids with nitrous acid and acetic anhydride

Dimerization of Amino Acids

When heated, α-amino acids dimerize to form 2,5-diketopiperizines. The corresponding esters give better yields, e.g., glycine ester gives 2,5-diketopiperazine.

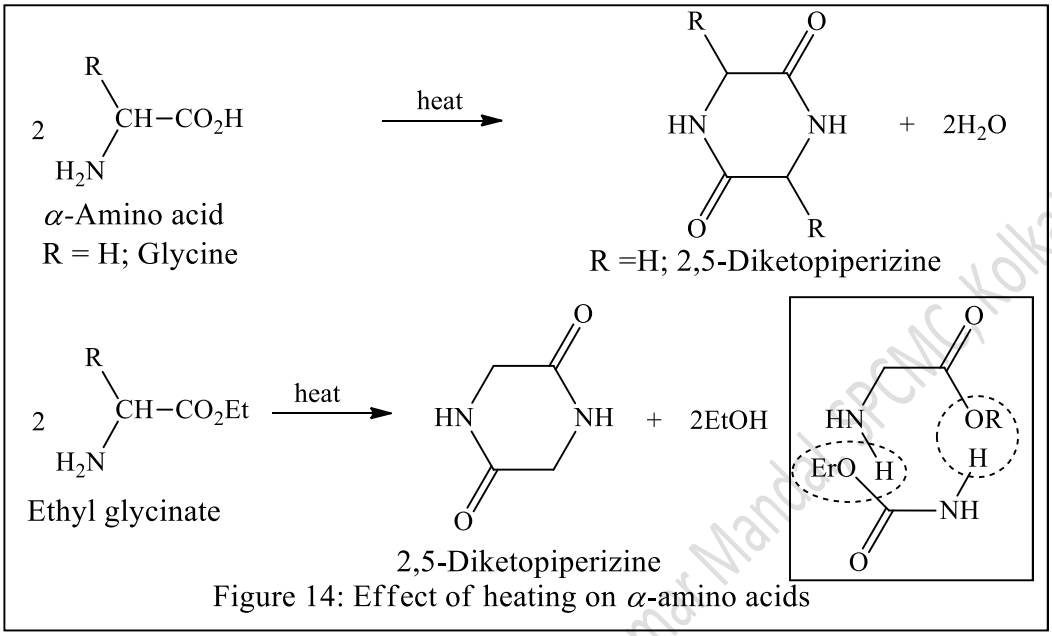


Figure 14: Effect of heating on α-amino acids