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Lab. Biochemistry

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Xanthoproteic Test

Objective:

To differentiate between aromatic amino acids which give positive results [yellow color] and other amino acids.

Principle:

Concentrated nitric acid react with aromatic nucleus present in the amino acid side chain [nitration reaction] giving the solution yellow color.

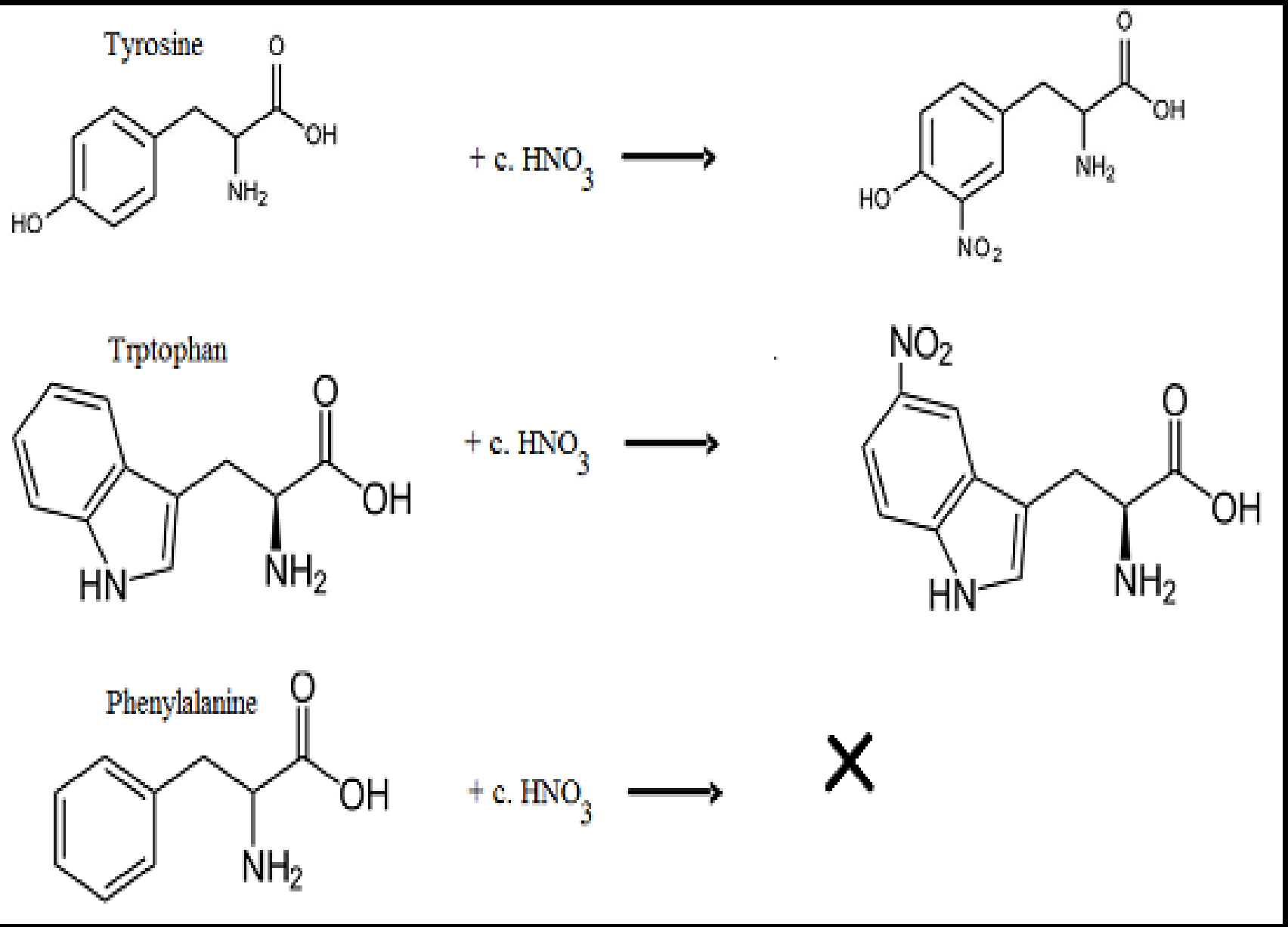
Note:

Amino acids tyrosine and tryptophan contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.

The aromatic ring of **phenylalanine** does not react readily with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react

Phenylalanine still reacts with nitric acid, just not as readily as tyrosine or tryptophan because tyrosine and tryptophan have electron donors (hydroxyl and the nitrogen heteroatom), which make the ring a lot more attractive for electrophilic nitration.



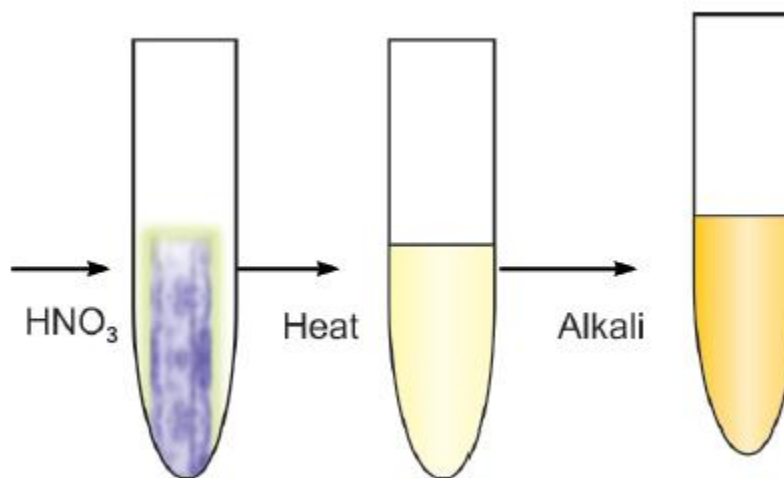


Procedure

To 2 mL amino acid solution in a boiling test tube, add equal volume of concentrated HNO_3 .

Heat over a flame for 2 min and observe the color.

Addition of alkali increases the ionization of compounds hence the color deepens to get final orange color



Xanthoproteic test

One tube is kept as control and the other as test, so as to understand the development of even faint color. To one tube add 40% NaOH or liquor ammonia (ammonium hydroxide) in excess

Observation:

A white precipitate forms on adding nitric acid, which on heating turns **yellow** and then dissolves to impart yellow color to the solution. Upon adding alkali the color deepens to attain **orange** color. Addition of nitric acid causes denaturation of proteins to get white precipitate. Yellow color due to nitration of benzene ring of amino acids – **tryptophan and tyrosine**.

