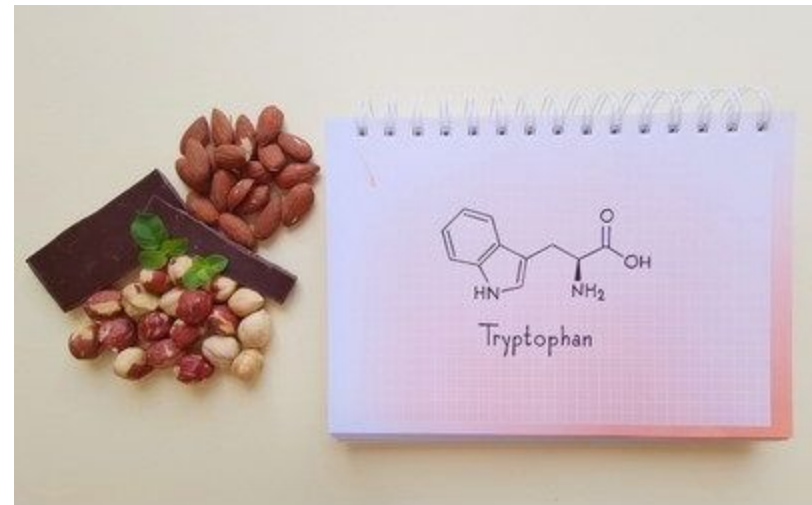
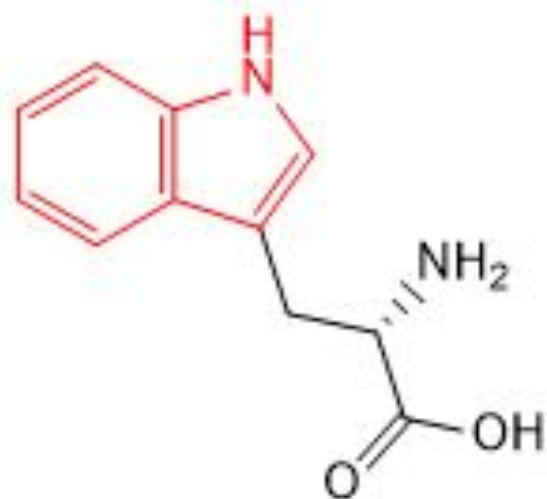


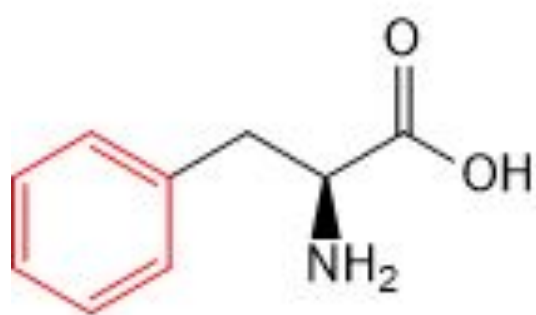
Xanthoproteic Test

- Xanthoproteic test is a test for the detection of amino acids containing aromatic ring like phenylalanine, tyrosine (phenolic ring), and tryptophan (indole ring). The test is named Xanthoproteic test due to the formation of a yellow precipitate of xanthoproteic acid.

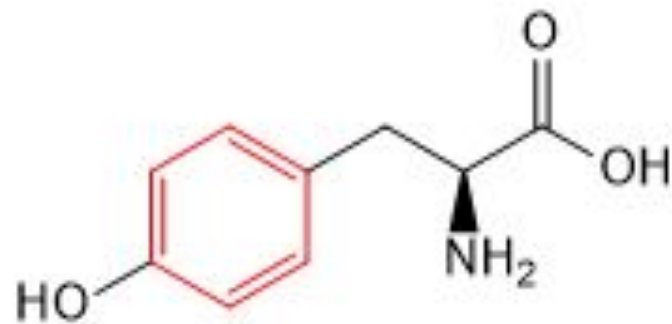




tryptophan



phenylalanine

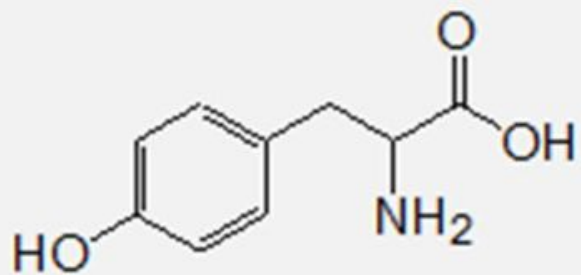


tyrosine

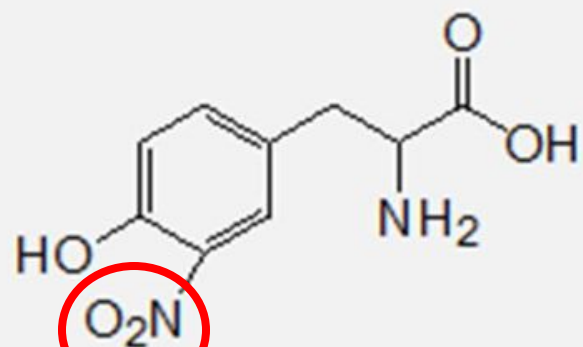
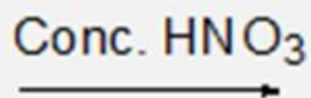
- **Principle:**

- Treating aromatic amino acids with the concentrated nitric acid leads to the nitration of the aromatic ring and formation of **yellow** nitro derivatives product. When the strong basic solution lead to formation of salts, the color change from yellow to **orange**.
- The base is added to neutralize the acid, because some of the colors don't show up if the mixture is still too acidic. So, whether you get yellow or orange doesn't matter. Both are positive test.

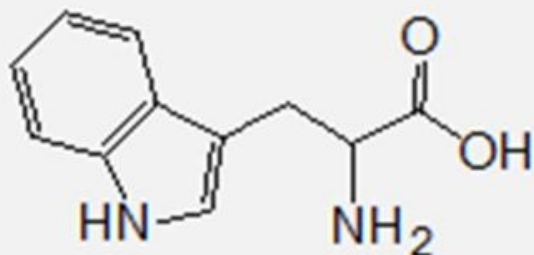
- Amino acids **tyrosine** and **tryptophan** □ contain activated benzene rings [aromatic nucleus] which are easily nitrated to yellow colored compounds.
- The aromatic ring of **phenyl alanine** dose not react readily with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not reacted.



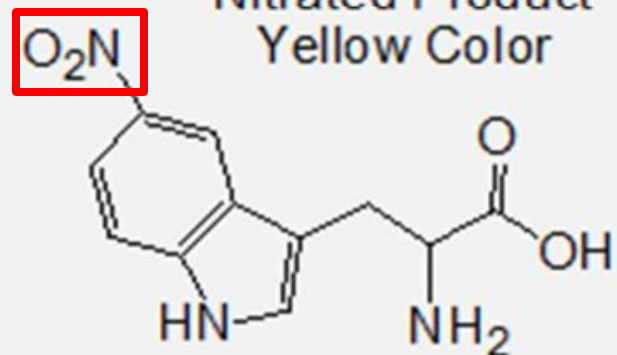
Tyrosine



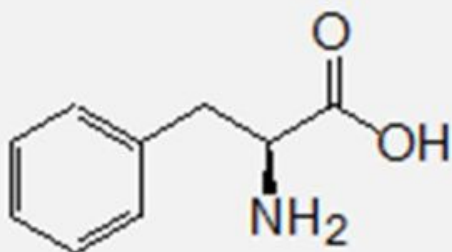
Nitrated Product
Yellow Color



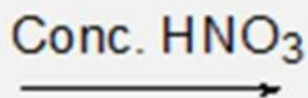
Tryptophan



Nitrated Product
Yellow Color



Phenylalanine



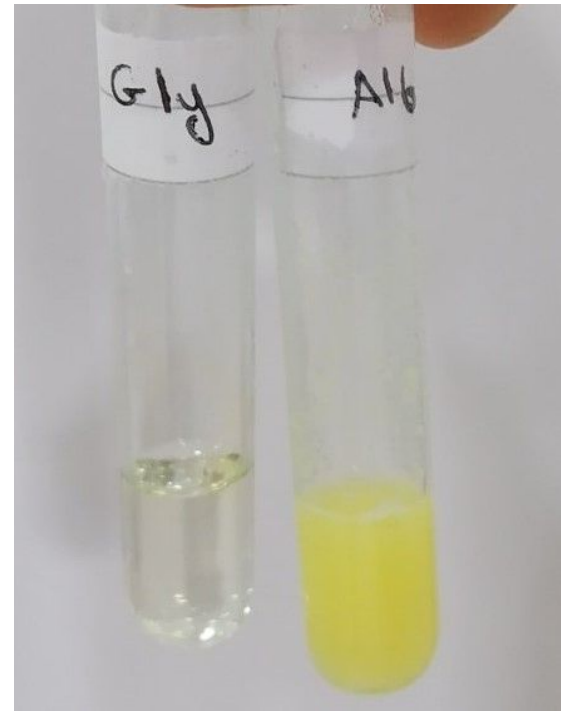
No Reaction

Procedure

1. add 1 ml of sample solution into test tube.
2. add 1ml of concentrated HNO_3
3. Mix and heat until turns yellow on heating.
4. Cool under tap water
5. Add 2 ml of 40% NaOH to make the solution alkaline
6. Observe whether the mixture turns orange in color.

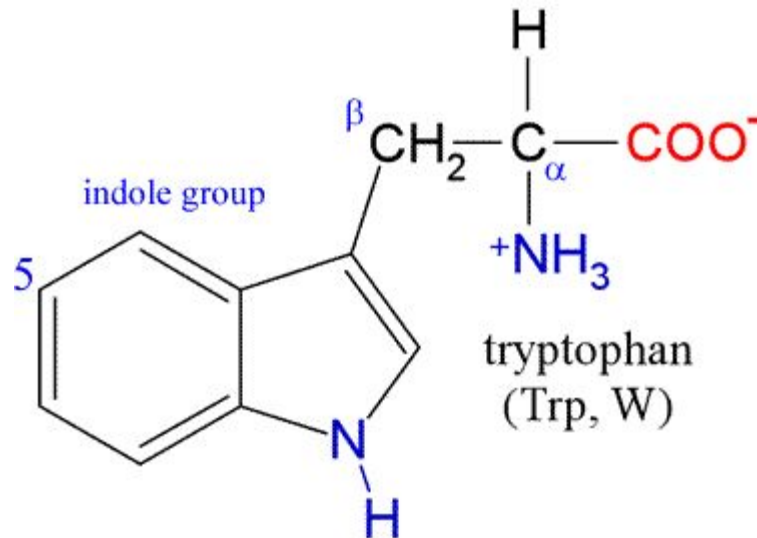
Result

- **Positive test:** yellow or orange indicating the presence of aromatic amino acids.
- **Negative test:** Color does not change indicating absence of aromatic amino acids.



Hopkin's cole Test

- This test is specific test used to detect the presence of tryptophan in proteins. It is also called the **aldehyde test** as the test is based on the presence of aldehyde molecules in the reagent (formaldehyde).

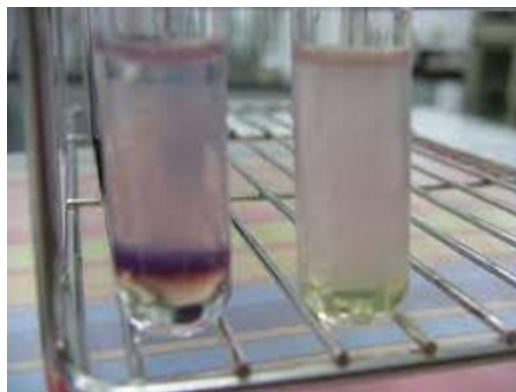


• Principle:

- The test is used to detect tryptophan in a protein solution.
- Hopkin' cole reagent composed of (Mercuric sulfate in sulfuric acid) and formaldehyde. Mercuric sulfate in sulfuric acid which act as an oxidizing agent and it oxidizes the indole ring of tryptophan. Then formaldehyde condensed with the oxidized indole ring to form reddish to violet colored complex.

Procedure

1. Add 2ml of sample solution in a test tube.
2. Add 3 drop of 1/500 formaldehyde.
3. Add 2 drop of 10% mercuric sulfate in sulfuric acid.
4. Add drop by drop of concentrated sulfuric acid through the sides of the test tube.
5. The formation of the reddish to violet ring at the junction of two layers is observed.



Thank
you!!!
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