Experiment 31
Amino Acids and Proteins

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Name:

Date:

Key Objectives

1. Identify major macromolecules - carbohydrates, lipids and proteins.
2. Diagnostic tests for proteins and amino acids
3. Perform a separation and filtration

Discussion

Amino Acids

Amino Acids (AA) are the fundamental building block for the larger molecules that are polypeptides and proteins, similar to what was seen with carbohydrates and glucose being the fundemental building block of disaccharides and polysaccharides (amylose, amylopectin, glycogen and cellulose). As shown in Figure 31.1 amino acids all share a common structure, with only the identity of the R-group being changed. Note that the R-group or side chains are responsible for the properties of the amino acids. They can be grouped according to the general classifications of hydrophobic, hydrophilic, acid, and base. A complete list of 20 amino acids used by humans can be found in your textbook or on the cheat sheet for Chapter 29.

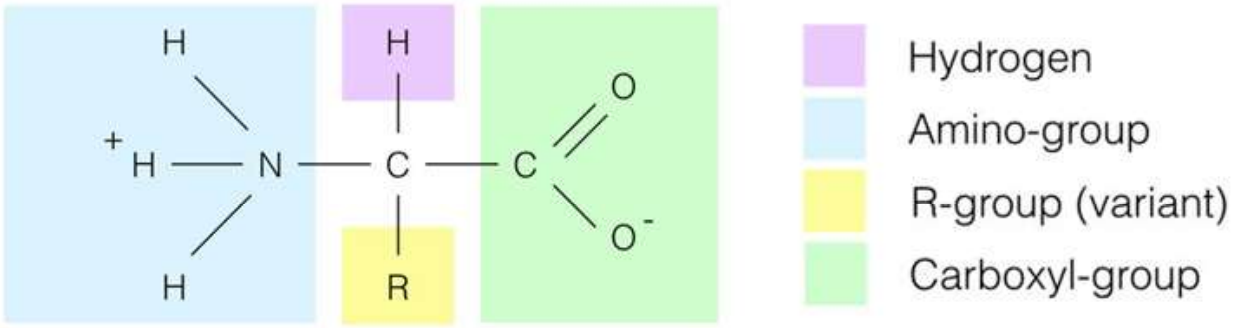

Figure 31.1: Generic representation of an Amino Acid. credit: unknown

Polypeptides and Proteins

Polypeptides and Proteins are formed when amino acids are bonded together to form a polymer. The primary structural difference is the polypeptides generally contain less than 50 amino acids bonded together while proteins can contain 100-1000's of amino acids bonded together.

Polypeptides are formed by a dehydration reaction between the amine group of one amino acid and the carboxylic acid group of the next amino acid to form an amide bond. Biologist often refer to this as a peptide bond or linkage. Polypeptides are generally drawn from the N-terminal end to the C-terminal end. The order the amino acids are bonded in matters, thus two amino acids (A and B) can form two polypeptides (AB and BA). The number of possible combinations is given by N! (N factorial).

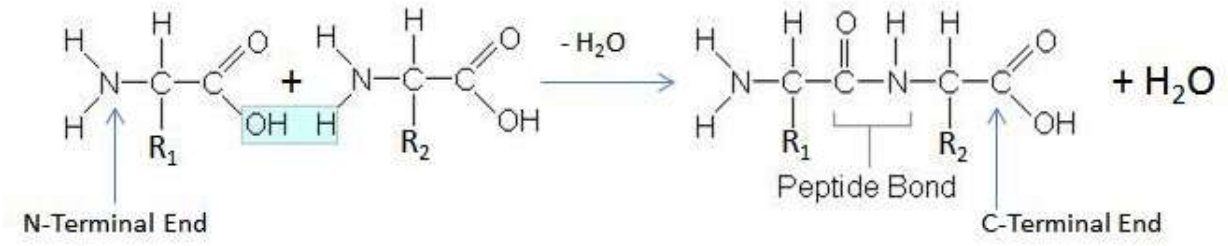

Figure 31.2: Formation of a amide (peptide) bond by dehydration. credit: unknown

A. Chemical Tests

There are many chemical tests available to test for and distinguish between amino acids, polypeptides and proteins. We will examine a couple of the more standard tests.

Biuret Test

The biuret test is utilized to identify the presence of 2 or more peptide bonds in a molecule by reaction with Cu^{+2} ions to form a brightly colored complex. It will test negative for individual amino acids (they lack peptide bonds) but will test positive for any polypeptide of 3 or more amino acids. The test is very sensitive and the color change can be correlated to the amount of protein in a sample or the size of the protein by using a spectrometer, similar to the Beer's Law experiment performed last semester.

The test is named for biuret which is formed when urea is heated, and produces a pink/purple color when reacted with Cu^{+2} ions and will act as a positive control for the experiment. In the reaction 4 amide groups form a complex with 1 copper ion. The resulting solution turns from the typical light blue color of Cu^{+2} to a purple/violet/dark blue color.

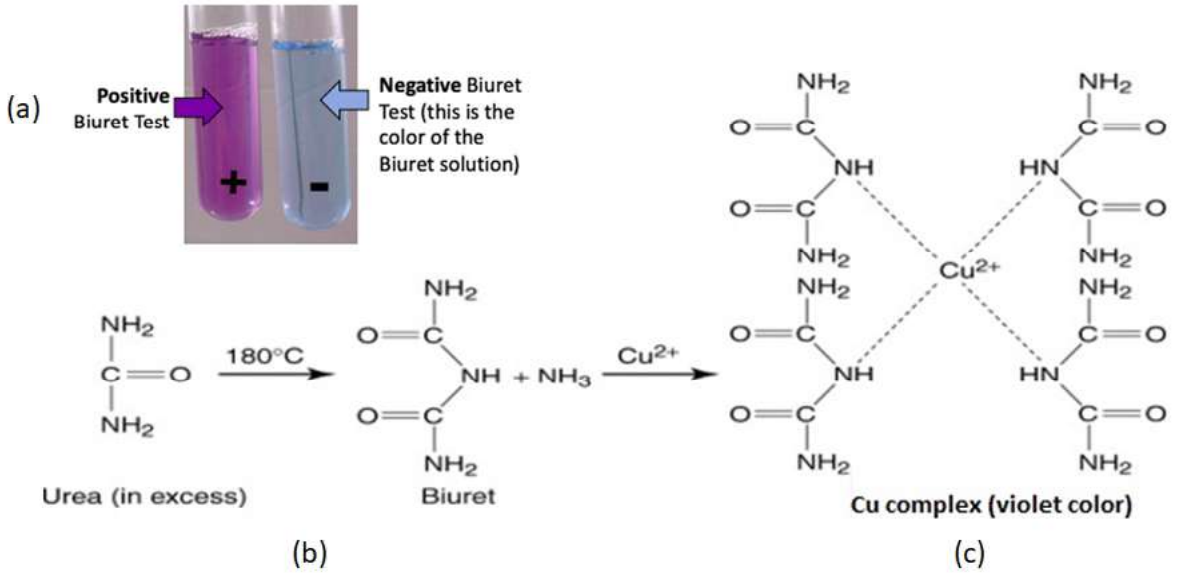

Figure 31.3: (a) Positive and negative results for the biuret test. (b) Reaction of urea to form biuret. (c) The biuret complex formed after reaction with Cu^{+2}. credit: unknown

Ninhydrin Test

The Ninhydrin test is used because it is extremely sensitive to small quantities of proteins (10^{-6} g) and is commonly used in fingerprinting because of this. The reaction occurs between Ninhydrin and the free amine group of amino acids to form a colored complex through a series of reactions that are too complicated to go into detail about. The reaction generally results in a blue-purple colored solution, however, Proline and Hydroxyproline give a bright yellow color change.

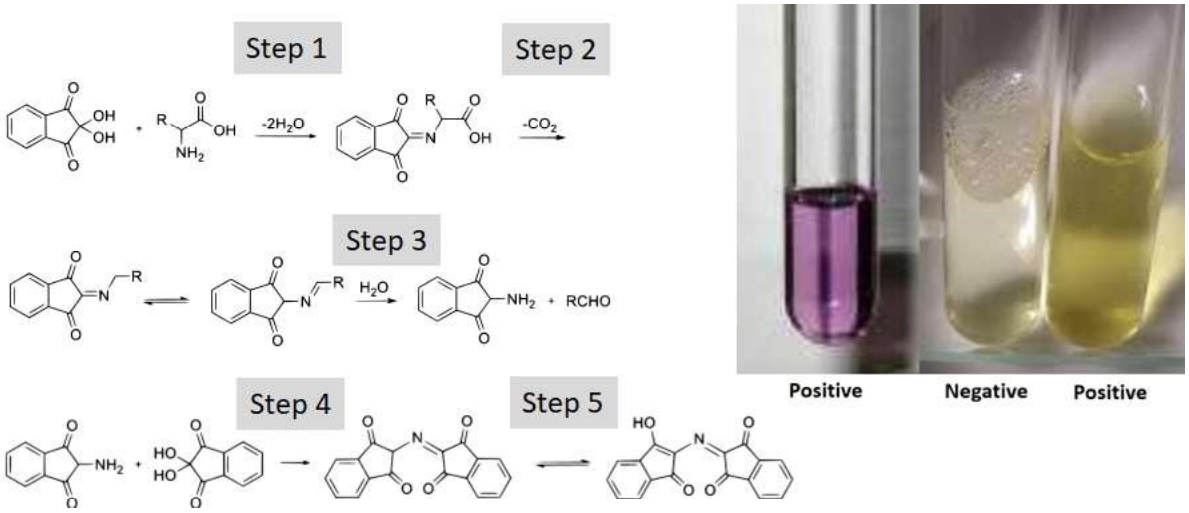

Figure 31.4: The ninhydrin test is quite complex and requires a number of steps to complete. Do not memorize this, just analyze it. A positive test is either purple or if proline is present yellow. credit: unknown

Tyrosine Test

The tyrosine test is very sensitive to the presence of the amino acid tyrosine (big surprise!) either by itself or in a protein. The reaction is so complicated I couldn't actually find it using Google! The test is positive for tyrosine if the final solution is red colored, often with a precipitated observed.

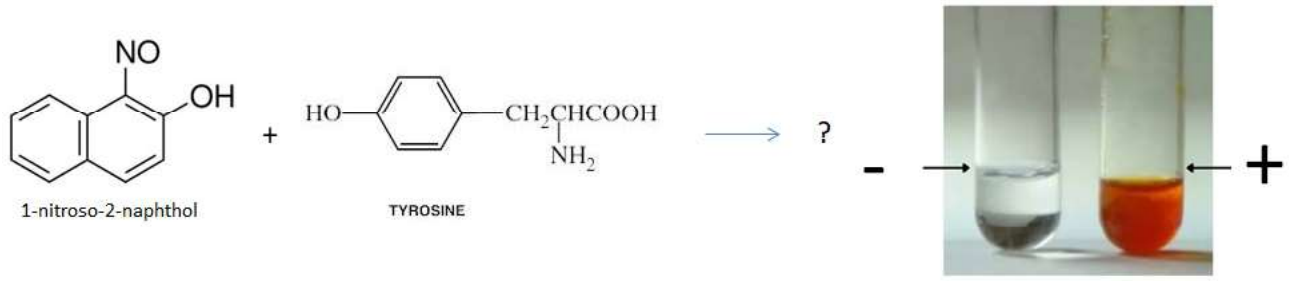

Figure 31.5: Tyrosine test, a positive test results in the formation of a yellow/orange/red color if a protein contains Tyrosine or Tryptophan. credit: author modified from `https://microbenotes.com/wp-content/uploads/2020/11/Xanthoproteic-Test.jpeg`

Xanthoproteic Test

This is used Tests for the presence of a aromatic (phenyl) group in (Phe, Tyr, and Try) which reacts with nitric acid and is heated to form colored compounds, generally yellow/orange in color. Oddly enough Phenylalanine (Phe) by itself is too stable and does not react with the nitric acid under the conditions in this test giving a negative result. Phenylalanine (PHE) can give a positive if the heating period is extended.

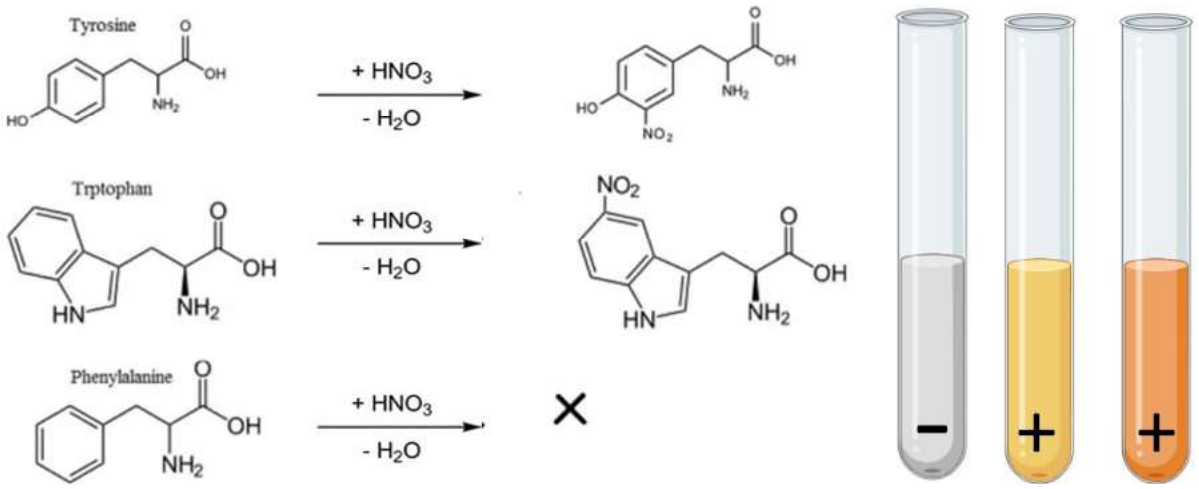

Figure 31.6: Xanthoproteic test results. credit: unknown

Cystine/Sulfur Test

When amino acids or proteins containing cystine (Cys) is heated in a basic solution, the sulfur atoms are converted to sulfide ions (S^{-2}) which are then reacted with lead (II) acetate to form a black precipitate of lead (II) sufide.

Isolation of Proteins from Milk

Milk contains many nutrients including proteins, fats, carbohydrates and several important vitamins and minerals. Casein typically makes up 80% of the protein in cows milk, and is generally found bonded to calcium. Casein can be separated from the calcium ion and precipitated from the milk, by reacting with dilute acetic acid, though care must be taken to not add excess acetic acid as it can be redissolved.

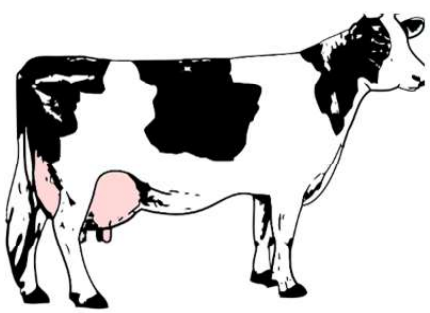

Figure 31.7: Milk comes from cows! Label for skim milk. credit: `https://freesvg.org/vector-image-of-cow-full-of-milk` and `https://commons.wikimedia.org/wiki/File:Oat_milk_glass_and_bottles.jpg`

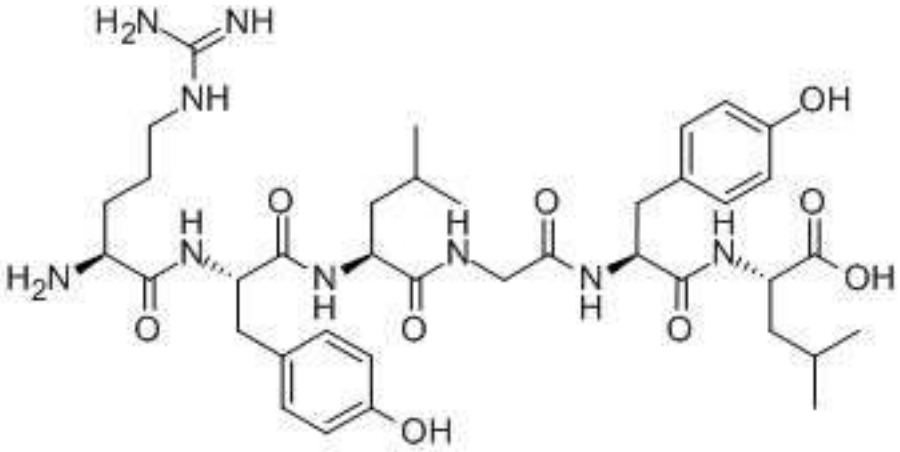

Figure 31.8: Structure of Casein isolated from cow milk. credit: unknown

Procedure

Begin by performing parts A and B first. After that the tests may be done in any order.

A. Separation of Casein from Cows Milk - Day 1

1. Mix 50 mL of nonfat milk with 50 mL of water in a 250 m: Erlenmeyer flask.
2. Slowly add 10% acetic acid to the solution with vigorous stirring until a flocculent precipitate forms (the Casein). Generally 2-3 mL of acetic acid is required.
3. Avoid adding excess acetic acid and redissolving your casein.
4. Allow the solution to settle until you observe the precipitate has settled to the bottom of the flask (5-10 minutes).
5. Set up a funnel with filter paper to separate the precipitate and solution.
6. Pour the solution through the filter paper. It may take a long time to finish filter, so proceed to the rest of the tests and perform the tests on the casein on day 2.
7. The solid is the casein, yo umay discard the liquid in the sink.
8. Once the casein is filtered, carefully remove the excess moisture by pressing the precipitate between paper towels.
9. Once dry, store your casein in the provided container for use on day 2.
10. Be sure to label the container with your name.
11. For each test (C-G) use a pea sized piece of Caesin dissolved in the appropriate amount of water.
12. Dispose of the liquid portion of the filtration down the sink.

B. Preparing a Biuret Solution

1. Prepare a solution of biuret by placing 1.5 grams of Urea in a large (50-100 mL)test tube.
2. Heat the test tube over a bunsen burner until the urea has melted and continue to gently heat for 30 seconds. Do not overheat the solution
3. When done heating carefully note the odor of the resulting solution.
4. Allow the test tube to cool (you may hold it under running water).
5. Once cool, add 20 mL of water to the test tube and mix until you have dissolved the residue.
6. The Biuret solution is now ready to be used.
7. Save your Biuret solution in an sealed Erlenmeyer flask for use on day 2 if required.

C. Biuret Test

1. In a clean test tube add 4.0 mL of the solution to be tested, 1.0 mL of 10% NaOH, and 4 drops of 1.0% $CuSO_4$ solution.
2. Note any color changes that occur.
3. Discard the contents of the test tubes in the waste container labeled "AA - Waste".

D. Tyrosine Test

1. In a clean test tube add 1.0 mL of the solution to be tested.
2. Add 2 drops of 0.1% 1-nitroso-2-naphthol (in acetone) and mix, the resulting solution should have a very light color at this point.
3. Add 3 drops of 6.0 M nitric acid to the solution. Drop them such that they **DO NOT** splash, or touch the sides of the test tube. They should form a small bead at the bottom of the solution. **DO NOT** mix the solution.
4. Place the test tube in boiling water for 1 minute.
5. Note any color changes observed.
6. Discard the contents of the test tubes in the waste container labeled "AA - Waste".

E. Ninhydrin Test

1. In a clean test tube add 5 mL of each solution to be tested.
2. Add 10 drop of Ninhydrin Solution.
3. Mix the test tube and place in a boiling water bath for 2 minutes.
4. Note any color changes that occur.
5. Discard the contents of the test tubes in the waste container labeled "AA - Waste".

F. Xanthoproteic Test

1. In a clean test tube add 3 mL of each solution to be tested.
2. Add 10 drop of concentrated Nitric Acid.
3. Mix and note any color changes.
4. Place the test tube in boiling water for 4 minutes and note any color changes.
5. Cool the solutions (use running water, or simple leave them sit for 15-20 minutes.

6. Add 10% sodium hydroxide to the solution in 1 mL increments until the solution is basic when tested with litmus paper.
7. Note any color changes.
8. Discard the contents of the test tubes in the waste container labeled "AA - Waste".

G. Cysteine/Sulfur Test

1. In a clean test tube add 3 mL of each solution to be tested.
2. Add 2 ml of 10% sodium hydroxide to the solution and mix.
3. Heat the solution in boiling water for 2 minutes.
4. Remove the test tube and add 4 drops of lead (II) acetate to the solution.
5. Record any changes observed.
6. Add 6 M hydrochloric acid to the solution until any precipitates are dissolved (2-3 mL).
7. After any precipitates have redissolved carefully smell the solution and record your observations.
8. Discard the contents of the test tubes in the waste container labeled "AA - Waste".

Name: _____ Date: _____ Score: _____/80

Results

Substance	C. Biuret Test		D. Tyrosine Test		E. Ninhydrin Test	
	Observations	+/-	Observations	+/-	Observations	+/-
Water						
Biuret						
Tyrosine						
1% Glycine						
1% Phenol						
2% Gelatin						
Cholesterol						
1% Glucose						
Casein						
Albumin						

Substance	F. Xanthoproteic Test				G. Cysteine/Sulfur Test	
	Initial	After Heating	After NaOH	+/-	Observations	+/-
Water						
Biuret						
Tyrosine						
1% Glycine						
1% Phenol						
2% Gelatin						
Cholesterol						
1% Glucose						
Casein						
Casein						
Albumin						

Post Lab Questions

General Questions

1. What is meant by the term negative control?

2. What is meant by the term positive control?

Biuret Test

3. What was the odor emitted by the Biuret Solution? Based on the odor, what is the most likely identity of the gas?

4. What is another test one could have performed on the Biuret Solution to give additional evidence as to the identity of the gas? Explain.

5. What structural feature (functional group) is required for the Biuret test to give a positive result? Draw an example of it.

6. For the Biuret test did any compounds give unexpected results, list any compounds giving a false positive or false negative result. Explain.

   False Negative:

   False Positive:

Tyrosine Test

7. What structural feature (functional group) is required for the Tyrosine test to give a positive result? Draw an example of it.

8. For the Tyrosine test did any compounds give unexpected results, list any compounds giving a false positive or false negative result. Explain.

   False Negative:

   False Positive:

9. Draw a tri-peptide that would give a positive result for the Tyrosine test.

Ninhydrin Test

10. What structural feature (functional group) is required for the Ninhydrin test to give a positive result? Draw an example of it.

11. For what Amino Acid does the Ninhydrin give a non-standard positive test for? Draw the structure of the Amino Acid. Why does this particular Amino Acid give a different result?

12. For the Ninhydrin test did any compounds give unexpected results, list any compounds giving a false positive or false negative result. Explain.

    False Negative:

    False Positive:

Xanthoproteic Test

13. What structural feature (functional group) is required for the Xanthoproteic test to give a positive result? Draw an example of it.

14. For the Xanthoproteic test did any compounds give unexpected results, list any compounds giving a false positive or false negative result. Explain.

    False Negative:

    False Positive:

15. Did your fingers turn yellow while performing this test? Why would this occur?

Cystine/Sulfur Test

16. What structural feature (functional group) is required for the Cystine/Sulfur test to give a positive result? Draw an example of it.

17. For the Cystine/Sulfur test did any compounds give unexpected results, list any compounds giving a false positive or false negative result. Explain.

    False Negative:

    False Positive:

18. What evidence is there that sulfur is present (2 reasons)?

19. What is the identity of the product formed during the reaction when lead (II) acetate is added to the test solution (S^{-2})? Write the complete chemical reaction for its formation. What type of reaction occurred?

20. After adding hydrochloric acid to the precipitate a gas was formed. What is the identity of the gas? Write the complete chemical reaction for its formation. What type of reaction occurred?

Caesin

21. What is the sequence of AA that make up Casein? Use the three letter abbreviations in your answer.

22. Based on the structure in the previous question, did Casein give any unexpected results when tested? Explain.

Name: _____ Class: _____ Date: _____

Prelab Questions

1. What color of litmus paper should you use to test if a solution is:

   basic? acidic?

2. For each of the following tests, what is the visual evidence for a positive result?

   (a) Biuret:

   (b) Tyrosine:

   (c) Ninhydrin:

   (d) Xanthoproteic:

   (e) Cystine/Sulfur:

3. Complete the table below by writing the chemical formula: Sodium hydroxide, Hydrochloric acid, Sulfuric acid, Nitric acid, Copper (II) sulfate, and Lead (II)acetate.

Name	**Structure**	**Name**	**Structure**
Sodium Hydroxide		Hydrochloric Acid	
Sulfuric Acid		Nitric Acid	
Copper (II) Sulfate		Lead (II) Acetate	

4. Complete the table below by drawing the line drawing or lewis structure of - Acetic Acid, Urea, Biuret, Tyrosine, Glycine, Phenol, Cholesterol, and Glucose.

Name	**Structure**	**Name**	**Structure**
Acetic Acid		Urea	
Biuret		Tyrosine	
Glycine		Phenol	
Cholesterol		Glucose	