EXPERIMENT III

QUANTITATIVE ASCORBIC ACID ANALYSIS

Ascorbic acid is a hexose derivative with endiol structure and lactone ring. It is water soluble, colorless and odorless, crystalline. In dry conditions, it can be stored for a long time in places that are not exposed to light. Many animals and plants can synthesize ascorbic acid, but for some mammals and humans, vitamin C is essential and about 75mg per day is required in the diet. Ascorbic acid, which is abundant in fresh vegetables and fruits, is a strong reducing agent. It acts as an antioxidant in the body. It is thought to play an important role in delaying aging and preventing cancer.

It has been suggested by Linus Pauling that taking very high doses of vitamin C, which is widely used to prevent colds, protects against cancer. In recent studies, it has been suggested that consuming excessive vitamin C triggers cancer. In vitamin C deficiency, scurvy disease occurs in the form of capillary vascular bleeding, gum infections and tooth decay. With the addition of ascorbic acid, which is synthetically prepared, to soft drinks, this disease is rarely encountered today. Excess of vitamin C is excreted in the urine.

Ascorbic acid is stable in acidic solutions. Ascorbic acid, which has a strong reducing effect, loses its effect when heated. The determination of ascorbic acid in foodstuffs, medicines and natural products is based on this reducing property of the compound. Titrimetric techniques are one of the main analytical methods used in determination of ascorbic acid. Titrations with iodine and 2,6-dichlorophenol-indophenol, fluorometric processes, photochemical reactions with methylene blue are among these. When a large number of analyzes are to be made, gas chromatography, electrochemical (polarographic, coulometric, amperometric) methods can also be used for ascorbic acid determination.

METHOD

The solution and chemicals used in the experiment

0.25% starch solution (0.625g starch is weighed and dissolved in 250 ml of hot water and boiled until the solution is clear)
0.7 M sodium thiosulfate (0.1 g Na_2CO_3 and 11.08 g $Na_2S_2O_3$ / 1000 mL water)
0.2% KIO_3 solution (2g KIO_3 / 1000 mL water)
5% KI solution (2.5g / 50 mL water (each group will prepare separately))
0.1% Ascorbic acid standard solution (0.1g ascorbic acid / 100 mL water)
0.3 M H_2SO_4 solution (16.65 mL der H_2SO_4 / 1000 mL)

Experimental

Erlen No	0,3M H_2SO_4 (mL)	Ascorbic acid (mL)	Juice sample (mL)	Water (mL)	KIO_3 (mL)	KI (mL)	Na2S2O3 with Titration (Until it turns light yellow)	Starch (mL)	Na2S2O3 with Titration (Until it is colorless)	Na2S2O3 Consumption
1	50	2,5	0	17,5	15	10		2		
2	50	5	0	15	15	10		2		
3	50	10	0	10	15	10		2		
Sample	50	0	20	0	15	10		2		

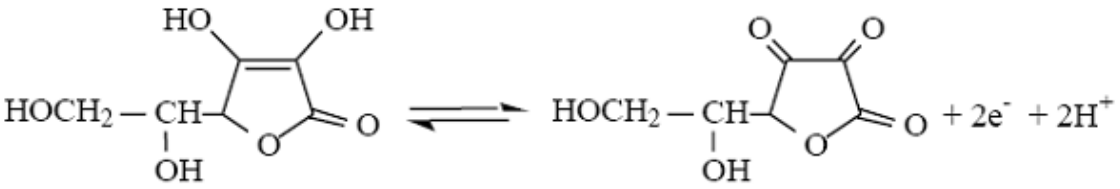
Ascorbic acid (vitamin C) is a moderately strong reducing agent. The reaction of reducing the iodine molecule in water with ascorbic acid can be given as follows;

(1) $KIO_3(aq) + 6\ H^+(aq) + 5\ I^-(aq) \rightarrow 3\ I_2(aq) + 3\ H_2O(l) + K^+(aq)$

(2) $C_6H_8O_6(aq) + I_2(aq) \rightarrow C_6H_6O_6(aq) + 2\ I^-(aq) + 2\ H^+(aq)$

With the 1st reaction, I2 is formed, this I2 formed is oxidized by the 2nd reaction. Both reactions take place in a dilute acidic medium. Also, Reaction 1 requires I^- ions.

Relevant half reactions can be given as follows;
$I_2 + 2e^- \rightarrow 2\ I^-$



The total reaction is as follows;

Ascorbic acid + I_2 (aq) + $H_2O \rightarrow$ Dehydroascorbic + $2I^- + 2H^+$

The equilibrium constant of this reaction is large, and those that enter completely turn into products. However, the solubility of I2 in water is low. Therefore, I_3^- complex is formed by using I^-.

$I_2(aq) + I^- \rightarrow I_3^-$

The I_3^-complex is called triiodide. Triiodide can also be produced using iodate.

$IO_3^- + 8I^- + 6H^+ \rightarrow 3I_3^- + 3H_2O$

Triiodide reacts with ascorbic acid.

Ascorbic acid + I_3^- + H_2O → Dehydroascorbic acid + $3I^-$ + $2H^+$

Ascorbic acid concentration is determined indirectly by determining the remaining I_3- ions. Thiosulfate is used for this purpose.

I_3^- + $2S_2O_3^{2-}$ → $3I^-$ + $S_4O_6^{2-}$

$S_4O_6^{2-}$ is called as thionate ion. Starch is used as an indicator. Triiodide forms a dark blue complex with starch.

Manipulation of Data

The amount of ascorbic acid in the flasks 1, 2 and 3 is calculated in mg and plotted against the thiosulfate consumption. The consumption of the sample is read on the graph and the ascorbic acid concentration is determined and expressed in mg / 100mL.

QUESTIONS

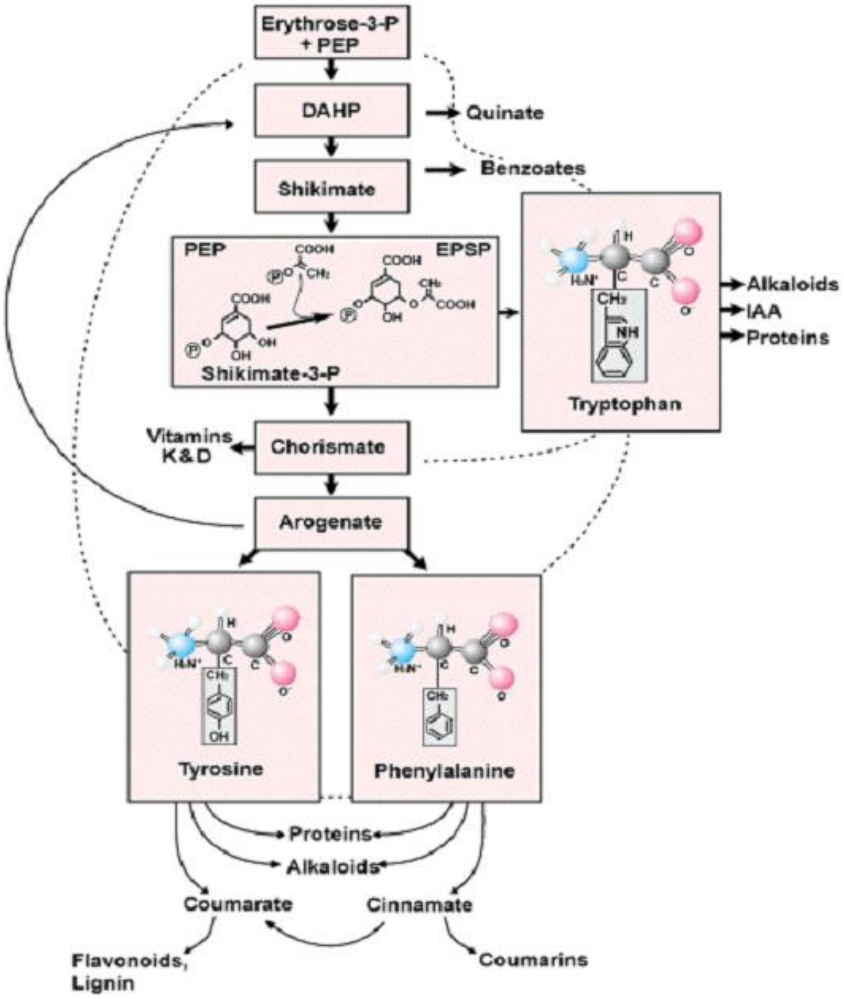
1- By which organisms and by what metabolic pathway is ascorbic acid synthesized?

2- Briefly explain the collagen structure. What are the changes in collagen structure in ascorbic acid deficiency?

EXPERIMENT IV

ISOLATION OF NATURAL COMPOUNDS FROM HERBAL SOURCES

Natural compounds can be defined as organic compounds produced by living organisms. It is known that approximately half of the drugs used today are obtained from natural sources. Plants and bacteria are the most important resources for finding new pharmaceutical agents.
The metabolic products produced by the organism and important for its survival are called primary metabolites. If a living thing cannot produce even one of its primary metabolites, it cannot survive. The products of the main metabolic pathways such as amino acids, nucleic acids, pyruvate etc. are examples of primary metabolites.
Secondary metabolites, on the other hand, can be defined as compounds that are not necessary for the survival of the living organisms, but they increase the survival of the living organisms when produced under certain conditions. Antibiotics produced by bacteria, alkaloids and phenolic compounds produced by plants can be given as examples of secondary metabolites.
Alkaloids are organic bases containing nitrogen and are products of amino acid metabolism. Plants do not excrete nitrogenous products because assimilable N-compounds are often the limiting factor in plant growth. With sufficient nitrogen supply, some amino acids are synthesized in excess and these molecules accumulate by converting into alkaloids, the end product of metabolism.
Formation of secondary metabolites in plants is given in the figure. Common precursors of alkaloids are ornithine, lysine, phenylalanine, tyrosine and nicotinic acid.
Nicotine [1-methyl-2- (3-pyridyl) pyrrolidine] is found mostly in tobacco and small amounts in tomatoes, eggplant and green peppers. N-atoms in pyridine and pyrrolidine rings give nicotine an alkaline character. High doses of nicotine are toxic to humans and used as insecticide and parasiticides in veterinary medicine. Nicotine, which is a colorless oil, boils at 246 °C. It is easily soluble in water and many organic solvents. Nicotine found as complexes with citric and malic acids in dry tobacco and can be extracted with dilute alkaline solutions. Nicotine, which is taken into the ether phase from alkaline solutions, is then purified. Chemical characterization of nicotine is difficult as it is obtained as a liquid in very small amounts. For this reason, the nicotine obtained is isolated by reacting with picric acid and converting into nicotine dipicrate salt.



METHOD

Chemicals and Solutions Used in the Experiment

5% NaOH solution (5g NaOH / 100 mL water), diethyl ether, saturated picric acid solution.

Weigh Approximately 4 grams of tobacco and place in a 100 mL beaker. Add 25 mL of 5% NaOH solution and mix by crushing for 2-3 minutes with the help of a glass rod. The extracts obtained are filtered through a glass cotton fitted funnel and collected in a single flask. The alkaline extract is taken into a separatory funnel and extracted twice with 25 mL of diethyl ether.

Diethyl ether extracts are evaporated to dryness by heating in the fume hood and then dissolved in 5 mL of water and 2-3 mL of ethyl alcohol, and approximately 5 mL of picric acid solution is added. Yellow nicotine dipicrate crystals are formed. The crystals formed are filtered and dried. The melting point of the derivative is 222-224 ^{o}C.

The method described here for obtaining nicotine is a special method. It cannot be used for all natural compounds. Because it is difficult to find a chemical that will specifically precipitate each natural compound. Therefore, when a compound is desired to be isolated from natural sources, various chromatographic methods are used.

Adsorption chromatography, HPLC and GC are the most used methods for this purpose. Organisms do not always synthesize their secondary metabolites, these compounds are usually synthesized during a certain period of the organism's life. What should be applied to obtain a compound from natural sources can be briefly given as follows;

• First of all, it is determined in which period and in which organs or parts (root, leaf, flower, etc.) the living organism synthesized this compound.

• Parts selected within the specified period are collected, generally dried or subjected to other pre-fractionation processes.

• The required solvent to take this substance into solution is determined. For this purpose, by examining the structure of the compound, it is tested in which solvents it can be dissolved and a literature search is made.

• The sample is homogenized with the selected solvent and separated from its insoluble parts. Centrifuge or filtration can be used for this purpose.

• The solution taken is extracted with a volatile organic solvent in which this substance can be dissolved. The appropriate organic solvent is determined by examining the structure and literature research.

• After extraction, the solvent is evaporated and a very small amount of a certain solvent is added again. Thus, the sample is concentrated.

• The desired substance is separated from this mixture using various chromatographic methods. For this, adsorption chromatography on the column, preparative layer chromatography or HPLC can be used.

Then, using mass spectrophotometer and / or NMR, it is determined whether the resulting compound is indeed the desired compound.

Question

1- Investigate how an organic compound is obtained from natural source. Examine the article and briefly write down the method used. You can do this research at www.sciencedirect.com. You can download the article you want by accessing this site from the computers in our school.