AVIT AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY | VINAYAKA MISSION'S RESEARCH FOUNDATION (Deemed to be University under section 3 of the UGC Act 1956) | Accredited by NAAC | Approved by AICTE

DEPARTMENT OF CHEMISTRY

BIOORGANIC CHEMISTRY LAB - 17CHBS81

LIST OF EXPERIMENTS

1. Synthesis of Aspirin
2. Hydrolysis of Sucrose
3. Preparation of Pyruvic acid from Tartaric acid.
4. Preparation of Oleic acid
5. Preparation of alpha D- glucopyranose pentaacetate
6. Preparation of Lycopene from Tomato paste
7. Preparation of L-Proline.
8. Preparation of 1,2,5,6 di- O-Cyclohexylidine-alpha-D-glucofuranose.
9. Preparation of s-ethyl hydroxybutonate from ethyl acetoacetate using Yeast.
10. Preparation of s-ethyl hydroxybutonate using 3,5 dinitrobenzoate.

HOD – H & S

STANDARD OPERATING PROCEDURES (SOP) FOR LABORATORY SAFETY

One should remember that, in the laboratory there is no such thing that can be regarded as a harmless substance. Following are the guidelines that are commonly practiced as SOP in the laboratory.

(i) Lab coats or ankle length aprons must be worn while handling toxic, corrosive and flammable materials.

(ii) Long hair, neckties, or loose clothing should be tied or otherwise secured.

(iii) Gloves should be worn, while handling corrosive and highly toxic chemicals.

(iv) Open shoes are not worn in the laboratory.

(v) Bare legs are not acceptable, while handling hot, cold, toxic, corrosive or sharp materials.

(vi) Appropriate eye protection should be worn at all times in laboratories.

(vii) Always wash hands with soap after working with chemicals, even though gloves have been used.

(viii) Do not mouth pipette or siphon toxic chemical reagents, corrosive liquids, organic solvents, strong acids and alkalies

(ix) Do not directly smell, sniff or taste any chemical. Avoid inhalation.

(x) Containers should be closed when not in use.

(xi) When working with flammable chemicals, make sure that there are no sources of ignition near by, in order to avoid fire or explosion.

(xii) Handle toxic, corrosive chemicals and flammable solvents in a chemical safety hood or a fume hood.

(xiii) No smoking in any area of a laboratory.

(xiv) No eating, drinking of beverage or application of cosmetics in the laboratory, except in designated areas in which no chemicals are used or stored.

(xv) Avoid working alone in the laboratory.

(xvi) Perform only those experiments or procedures that are authorised by the instructor.

(xvii) Report all injuries, fires and accidents to the lab supervisor or the instructor, as soon as possible.

EXPT. NO. :

DATE :

PREPARATION OF ASPIRIN

AIM

To prepare Aspirin from salicylic acid in an acidic medium.

THEORY

Aspirin, also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication.

Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecules together to create a patch over damage of the walls within blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots. It has also been established that low doses of aspirin may be given immediately after a heart attack to reduce the risk of another heart attack or of the death of cardiac tissue.

The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents, aspirin is no longer used to control flu - like symptoms or the symptoms of chickenpox or other viral illnesses, because of the risk of Reye's syndrome.

Aspirin was the first discovered member of the class of drugs known as Non - Steroidal anti - Inflammatory Drugs (NSAIDs), not all of which are salicylates, although they all have similar effects and most have inhibition of the enzyme cyclooxygenase as their mechanism of action.

PRINCIPLE

Phenol readily undergoes acetylation with acetic anhydride in the presence of an acid catalyst such as concentrated sulphuric acid to acetyl salicylic acid, which is commonly known as aspirin. Salicylic acid reacts with acetic anhydride in acidic medium to give aspirin with a byproduct of acetic acid.

MATERIALS REQUIRED

(i) Salicylic acid
(ii) Acetic anhydride
(iii) Concentrated Sulphuric acid
(iv) Acetic acid
(v) Distilled water
(vi) Water bath
(vii) Thermometer
(viii) Vacuum pump
(ix) Filter paper
(x) Test tubes
(xi) Conical flask

PROCEDURE

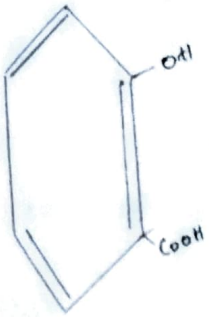
(i) Take 5g of salicylic acid in a conical flask or in a boiling test tube.
(ii) Add 7mL of acetic anhydride to it.
(iii) Add 5 drops of Concentrated Sulphuric acid to the solution to create an acidic medium.
(iv) Now, stir the flask for proper mixing.
(v) Warm the contents in the water bath at 50-60 °C for 15 minutes.
(vi) Cool the contents to the room temperature.
(vii) Stir the cooled contents continuously and mix it with 75mL of Distilled water.
(viii) Then, filter it using a vacuum pump.
(ix) Recrytallisation of aspirin was done by adding the crude in acetic acid and water in the ratio of 1:1 and then it is filtered.
(x) Moisture content is removed from aspirin by heating it in an oven.

OBSERVATION

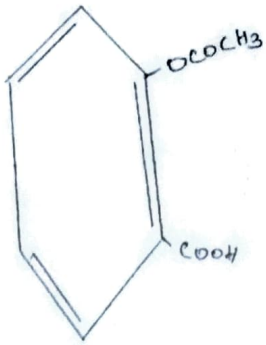
RESULT

Thus aspirin of _____ g is obtained from 5g of salicylic acid.

PREPARATION OF ASPIRIN



+ $(CH_3CO)_2O$ $\xrightarrow{\text{Conc. } H_2SO_4}$



Salicylic acid

Acetic anhydride

Aspirin + CH_3COOH

Acetic acid

EXPT. NO. :

DATE :

HYDROLYSIS OF SUCROSE BY ACID

AIM

To hydrolyse sucrose by acid.

THEORY

Sucrose is the organic compound commonly known as table sugar and sometimes called saccharose. This white, odourless, crystalline powder has a pleasing, sweet taste. It is best known for its role in human nutrition. The molecule is a disaccharide derived from glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$. When sucrose is hydrolyzed it forms a 1:1 mixture of glucose and fructose. This mixture is the main ingredient in honey. It is called invert sugar because the angle of the specific rotation of the plain polarized light changes from a positive to a negative value due to the presence of the optical isomers of the mixture of glucose and fructose sugars.

PRINICPLE

Sucrose is a disaccharide, which on hydrolysis with an acid yields the constituent monosaccharides namely glucose and fructose. In the hydrolysis of any di - or poly saccharide, a water molecule helps to break the acetal bond as shown in red. The acetal bond is broken, the H from the water is added to the oxygen on the glucose. The -OH is then added to the carbon on the fructose. By the end of hydrolysis process, the presence of glucose or fructose can be checked by performing the respective confirmatory test as a proof for hydrolysis.

MATERIALS REQUIRED

(i) 1 % sucrose solution

(ii) 3M HCl

(iii) Test tubes

(iv) Pipettes

(v) Test tube stand

PROCEDURE

(i) Label the two test tubes as A1 and A2.

(ii) Take 1mL of 1% sucrose solution in A1 and A2.

(iii) Add 2mL of Benedict's solution to A1.

(iv) Place the test tube A1 in a boiling water bath for 5 – 6 min.

(v) Observe the colour change and record.

(vi) Add 2 – 3 drops of 3M HCl in A2 and keep it in the boiling water bath for 5 – 6 min.

(vii) Add 2mL of Benedict's solution to A2.

(viii) Place the test tubes in the boiling water bath for 5 to 6 min.

(ix) Note the colour change.

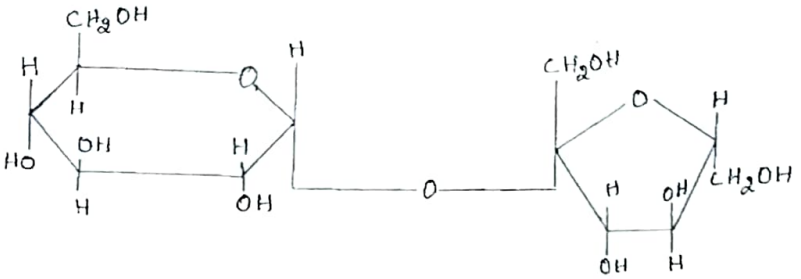
(x) The solution without the addition of HCl serves as control.

OBSERVATION

RESULT

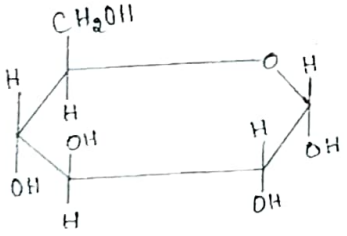
Sucrose on hydrolysis yields Glucose and Fructose, which was confirmed by the presence of brick red colour.

HYDROLYSIS OF SUCROSE BY ACID



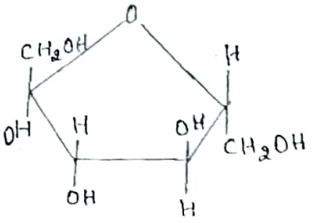
SUCROSE

Concentrated HCl



α-D GLUCOSE

+

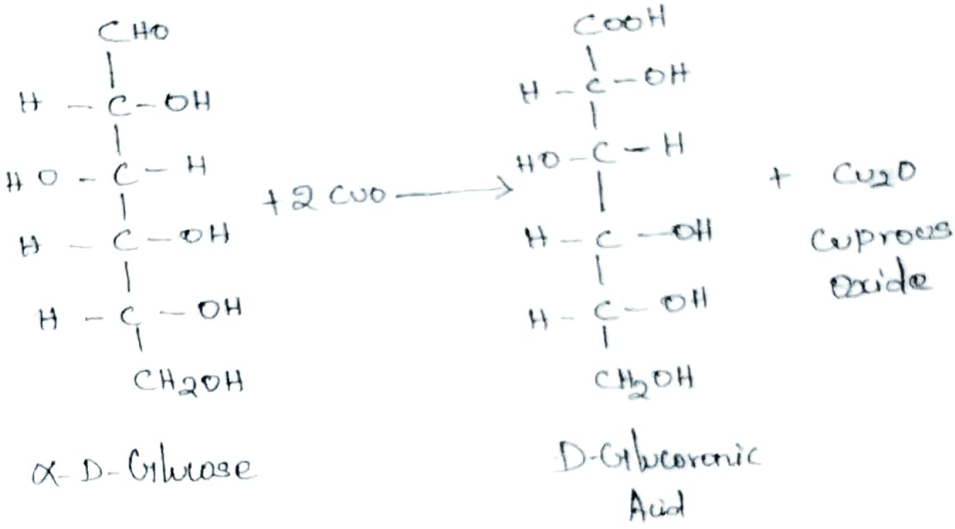


β-D FRUCTOSE

$$Na_2CO_3 + H_2O \longrightarrow 2NaOH + H_2CO_3$$

$$2NaOH + CuSO_4 \longrightarrow Cu(OH)_2 + Na_2SO_4$$

$$Cu(OH)_2 \longrightarrow CuO + H_2O$$



α-D-Glucose

D-Gluconic Acid

EXPT. NO. :

DATE :

HYDROLYSIS OF SUCROSE BY YEAST

AIM

To hydrolyse sucrose using the enzyme invertase.

THEORY

Sucrose is the organic compound commonly known as table sugar and sometimes called saccharose. This white, odourless, crystalline powder has a pleasing, sweet taste. It is best known for its role in human nutrition. The molecule is a disaccharide derived from glucose and fructose with the molecular formula $C_{12}H_{22}O_{11}$. When sucrose is hydrolyzed it forms a 1:1 mixture of glucose and fructose. This mixture is the main ingredient in honey. It is called invert sugar because the angle of the specific rotation of the plain polarized light changes from a positive to a negative value due to the presence of the optical isomers of the mixture of glucose and fructose sugars.

PRINCIPLE

The enzyme invertase catalyzes the hydrolysis of disaccharide sucrose into invert sugar. Invert sugar is a mixture of Glucose and Fructose which are monosaccharides. For yeast to utilize sucrose as an energy source it must be first converted into the fermentable monosaccharide Glucose and Fructose. By the end of hydrolysis process, the presence of glucose or fructose can be checked by performing the respective confirmatory test as a proof for hydrolysis.

MATERIALS REQUIRED

(i) Yeast
(ii) Sucrose
(iii) Glucose
(iv) Funnel
(v) Filter paper
(vi) Beaker
(vii) Test tubes
(viii) Test tube rack
(ix) Hot plate

REAGENTS REQUIRED

(i) **Preparation of Yeast Filtrate Solution**

Mix 7g of dry yeast with 80mL of distilled water. Allow it to stand for 20 minutes and stir it occasionally. Filter the resulting suspension and save the filtrate solution. Refrigerate the extract if held overnight.

(ii) **Preparation of 5% Sucrose Solution**

Dissolve 5g of sucrose in 100mL of distilled water.

(iii) **Preparation of 5 % Glucose solution**

Dissolve 5 g of glucose in 100mL of distilled water.

PROCEDURE

(i) Three test tubes are labeled as A1, A2, A3.
(ii) Take 2.5mL of 5% sucrose solution in tube A1.
(iii) Take 2.5mL of 5% sucrose solution and 1mL of invertase extract in tube A2.
(iv) Take 2.5mL of 5% distilled water and add 1mL of invertase extract in tube A3.
(v) Mix the tubes well.

(vi) Incubate the tubes for 30 minutes in warm water.

(vii) Take 4 test tubes and label it as B1, B2, B3 and B4.

(viii) Take 5mL of Benedict's solution in all the four test tubes.

(ix) Transfer the content of A1 to B1.

(x) Transfer the content of A2 to B2.

(xi) Transfer the content of A3 to B3.

(xii) Add 2.5mL of Glucose solution to B4.

(xiii) Mix the content of the tubes.

(xiv) Place the test tubes B1, B2, B3 and B4 into a boiling water bath.

(xv) After 3 – 4 min., remove the tubes from the water bath.

(xvi) Note the colour change

OBSERVATION

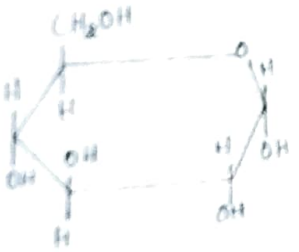
RESULT

Sucrose on hydrolysis yields Glucose and Fructose, which was confirmed by the appearance of brick red colour.

HYDROLYSIS OF SUCROSE BY YEAST

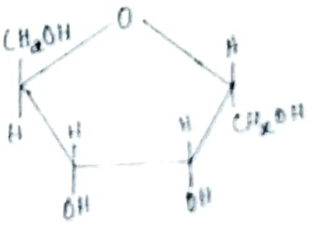
SUCROSE

Invertase



α D Glucose

+



β D Fructose

EXPT. NO. :

DATE :

PREPARATION OF PYRUVIC ACID FROM TARTARIC ACID

AIM

To prepare pyruvic acid from tartaric acid.

THEORY

Pyruvic acid is miscible with water, soluble in ethanol, diethyl ether, boils at 165°C, may be solidified and when pure melts at 13.6°C. A derivative of pyruvic acid is known as phenyl pyruvic acid, occurs in the urine of people who suffer from ketonuria. This is a disorder in which the aminoacid phenyl alanine is not metabolised normally.

The carbohydrate anion of pyruvic acid is known as pyruvate. Pyruvate is an important intermediate in metabolism, being produced during glycolysis and converted to acetyl co-enzyme A required for citric acid cycle (Kreb's Cycle). Under anaerobic conditions, pyruvate is converted to lactate or ethanol. Pyruvate is a key intersection in the network of metabolic pathways. The pyruvic acid derivative bromopyruvic acid is being studied for potential cancer treatment applications by researchers.

Other possible benefits of pyruvate may include as an antioxidant to help slow aging, to help lower blood pressure and cholesterol, increased glycogen storage, retention of lean muscle mass, increased anabolism or body protein uptake and increased fat utilization and resting metabolic rate

PRINCIPLE

Tartaric acid when fused with potassium hydrogen sulphate and heated to about 210 – 220°C gives pyruvic acid.

MATERIALS REQUIRED

(i) Tartaric acid

(ii) Potassium hydrogen sulphate

(iii) Oil bath distillation setup

(iv) Round bottomed flask

(v) Condenser

(vi) Heating mantle

(vii) Acetic acid

(viii) Mortar and pestle

(ix) Sodium nitroprusside.

PROCEDURE

(i) Take 50g of tartaric acid and 75g of potassium hydrogen sulphate in a mortar and pestle. Ground well to ensure proper homogenization

(ii) Transfer the ground mixture to round bottom flask and subject it to distillation of 210 – 220°C using an oil bath setup.

(iii) Allow it to distil until the liquid no longer distils.

(iv) Collect the distillate and redistill it to collect the fractionate under reduced pressure at 75 – 80°C.

(v) The fractionate is pyruvic acid, which is confirmed by performing the confirmatory test.

CONFIRMATORY TEST FOR PYRUVIC ACID

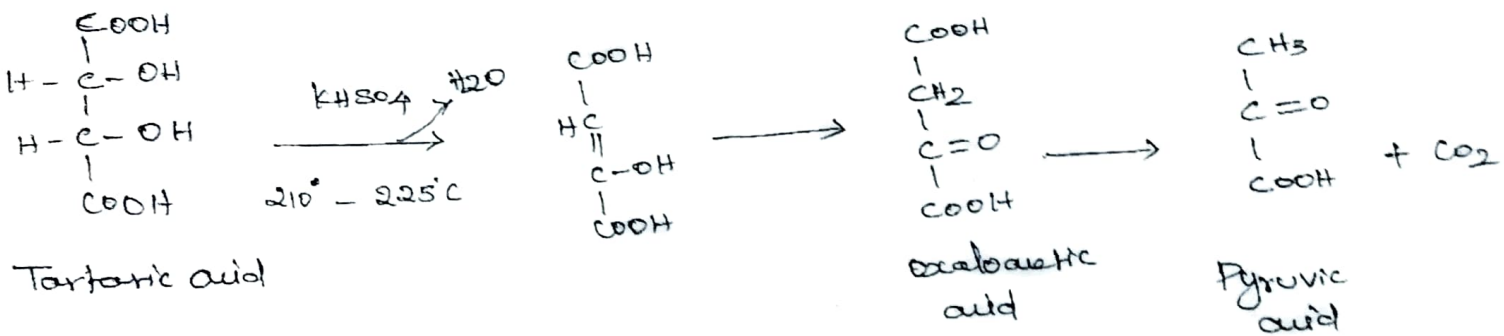
Take 2mL of distillate and add 2mL of sodium nitroprusside until the occurrence of wine red colour. The sodium hydroxide is added dropwise until the appearance of violet colour. On addition of acetic acid, the solution turns yellow.

OBSERVATION

RESULT

The prepared pyruvic acid is confirmed by the confirmatory test.

PREPARATION OF PYRUVIC ACID FROM TARTARIC ACID



Tartaric acid

oxaloacetic acid

Pyruvic acid

EXPT. NO. :

DATE :

PREPARATION OF OLEIC ACID

AIM

To prepare Oleic acid from olive oil by saponification.

THEORY

The term Oleic means related to, or derived from, oil or olive. Oleic acid is a mono unsaturated omega - 9 fatty acid found in various animal and vegetable sources. It has the formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$. The trans isomer of oleic acid is called elaidic acid.

Triglyceride esters of oleic acid comprise the majority of olive oil, though there may be less than 2.0% as actual free acid in the virgin olive oil, while higher concentrations make the olive oil inedible. It also makes up 36 – 67% of peanut oil, 15 - 20% of grape seed oil, sea buckthorn oil , sesame oil and 14% of poppyseed oil.

Oleic acid is the most abundant fatty acid in human adipose tissue. As an excipient in pharmaceuticals, oleic acid is used as an emulsifying or solubilizing agent in aerosol products. Oleic acid may hinder the progression of ALD, or Adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands. Oleic acid may help boost memory. Oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil

PRINCIPLE

Saponification of triglyceride such as olive oil will give sodium salts of the constituent fatty acids and glycerol backbone. In the case of olive oil, the three fatty acids are oleic acid, stearic acid and palmitic acid. On saponification, the sodium salts

of fatty acids are obtained. On treatment of these fatty acids with lead acetate, they form their corresponding lead salts as precipitate. From this mixture of lead salts of fatty acids, the lead salts of oleate alone dissolve in ether. This characteristic feature is used in the separation of oleic acid from olive oil.

MATERIALS REQUIRED

(i) Olive oil
(ii) 3N Sodium hydroxide
(iii) 3N Lead acetate
(iv) Ether
(v) 1N HCl
(vi) Bromine solution in CCl_4
(vii) Conical flask
(viii) Beaker
(ix) Funnel
(x) Filter paper

PROCEDURE

(i) Dissolve 10mL of olive oil in 10mL of 3N NaOH and stir in a magnetic stirrer.

(ii) To this mixture, add 10mL of lead acetate and mix well.

(iii) Filter the precipitate obtained and dry it.

(iv) To dry lead salt of fatty acids, add 10mL of ether, mix well and filter it.

(v) Evaporate the filtrate and to this residue, add 1N HCl, to liberate oleic acid as an oily layer.

CONFIMATORY TEST FOR OLEIC ACID

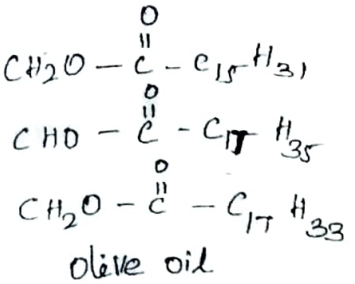
Oleic acid when taken in water, will not turn blue litmus to red. However, it will turn blue litmus to red when taken in alcohol.

RESULT

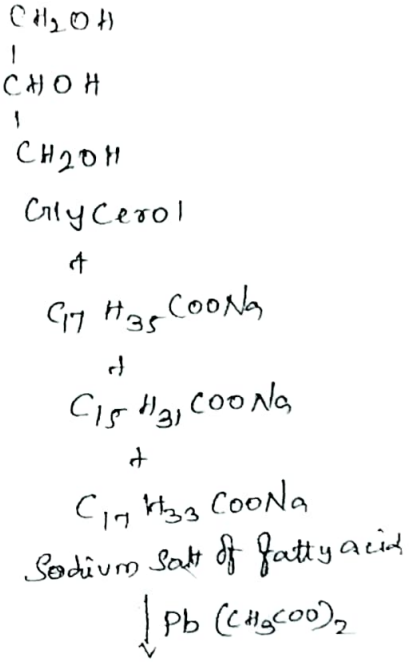
The prepared Oleic acid was confirmed by the confirmatory test.

PREPARATION OF OLEIC ACID

$CH_3(CH_2)_7 CH=CH(CH_2)_7 COO- \quad -COO- \quad$ cis 9-octadecanoic acid (Oleic acid)



olive oil



Glycerol + $C_{17}H_{35}COONa$ + $C_{15}H_{31}COONa$ + $C_{17}H_{33}COONa$

Sodium salt of fatty acid

$\downarrow Pb(CH_3COO)_2$

$C_{17}H_{33}(COO)_2Pb$ + $C_{15}H_{31}(COO)_2Pb$ + $C_{17}H_{35}(COO)_2Pb$

lead salt of fatty acid

$\downarrow$ ether

$C_{17}H_{33}(COO)_2Pb$ — lead acetate

$\xleftarrow{HCl}$

$C_{17}H_{33}COOH + PbCl_2$

oleic acid — lead chloride

EXPT. NO. :

DATE :

PREPARATION OF α - D - GLUCOPYRANOSE PENTAACETATE

AIM

To prepare α – D - glucopyranose pentaacetate from Glucose.

PRINICIPLE

α - D - glucopyranose undergoes acetylation when treated with excess of acetic anhydride in the presence of zinc chloride and gives α - D- glucopyranose pentaacetate. It is an intermediate in organic synthesis used for the production of industrial chemicals and cosmetics.

MATERIALS REQUIRED

(i) Acetic anhydride
(ii) Anhydrous zinc chloride
(iii) Ice
(iv) Rectified spirit
(v) α - D- glucose
(vi) conical flask
(vii) Pipette
(viii) Round bottomed flask
(ix) Boiling water bath
(x) Funnel
(xi) Reflux condenser

PROCEDURE

(i) Take 1g of anhydrous zinc chloride in a 100mL round bottomed flask.

(ii) Add 25mL of acetic anhydride to it.

(iii) Fix the reflux condenser onto the round bottomed flask and keep it in boiling water bath for 5 min.

(iv) Ensure that the mixture undergoes dissolution by gently shaking the flask.

(v) Add 5g of powdered glucose to the mixture while shaking.

(vi) Replace the setup in hot water bath and allow it to condense for one hour.

(vii) Pour the contents in 100g of ice crystals, immediately after refluxing.

(viii) Stir the mixture and leave it for 30min so that the oily layer was separated and allowed to solidify for crystallization.

(ix) Filter the precipitate.

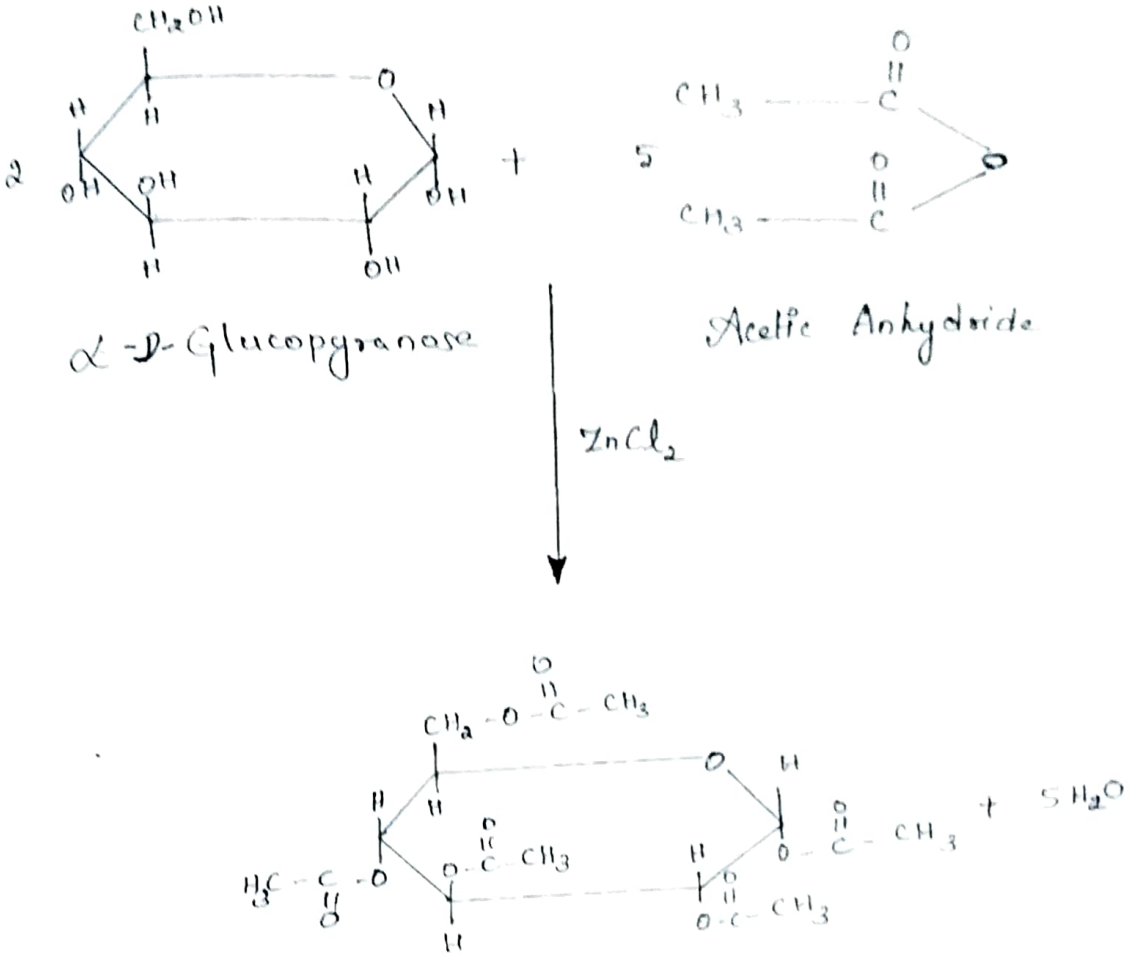
(x) Wash the crystals obtained using rectified spirit and calculate the yield obtained.

OBSERVATION

RESULT

White powdery crystals of α - D- glucopyranose pentaacetate is found to be g.

PREPARATION OF α - D - GLUCOPYRANOSE PENTAACETATE



α - D - Glucopyranose + Acetic Anhydride

α - D - Glucopyranose Penta acetate

EXPT. NO. :

DATE :

ISOLATION OF LYCOPENE FROM TOMATO

AIM

To isolate Lycopene from tomato.

THEORY

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes, other red fruits and vegetables, such as red carrots, watermelons and papayas (but not strawberries or cherries). Although lycopene is chemically a carotene, it has no vitamin A activity.

In plants, algae, and other photosynthetic organisms, lycopene is an important intermediate in the biosynthesis of many carotenoids, including beta carotene, responsible for yellow, orange or red pigmentation, photosynthesis, and photo-protection. Structurally, it is a tetraterpene assembled from eight isoprene units, composed entirely of carbon and hydrogen, and is insoluble in water. Lycopene's eleven conjugated double bonds give it its deep red colour and are responsible for its antioxidant activity. Due to its strong colour and non-toxicity, lycopene is a useful food colouring.

Lycopene has been considered as a potential agent for prevention of some types of cancers, particularly prostate cancer. Lycopene may be the most powerful carotenoid quencher of singlet oxygen, being 100 times more efficient in test tube studies of singlet – oxygen quenching action than vitamin E, which in turn has 125 times the quenching action of glutathione (water soluble). Singlet oxygen produced during exposure to ultraviolet light is a primary cause of skin aging.

Given its antioxidant properties, substantial scientific and clinical research has been devoted to a possible correlation between lycopene consumption and general health. Early research suggested some amelioration of cardiovascular disease, cancer, diabetes, osteoporosis, and even male infertility.

Processing of tomatoes increases the concentration of bioavailable lycopene. Lycopene in tomato paste is four times more bioavailable than in fresh tomatoes. For this reason, tomato sauce is a preferable source as opposed to raw tomatoes.

MATERIALS REQUIRED

(i) Ethanol
(ii) Dichloromethane
(iii) Sodium sulphate
(iv) Alumina
(v) Ligroin
(vi) Concentrated sulphuric acid
(vii) Reflux condenser
(viii) Funnel
(ix) Round-bottomed flask
(x) Spatula
(xi) Separating funnel
(xii) Fume hood
(xiii) Pasteur pipette
(xiv) Microscope
(xv) UV spectrophotometer.

PROCEDURE

(i) Take 10g of tomato paste in 100mL round bottomed flask.

(ii) Add 10mL of ethanol and heat under reflux for 5min.

(iii) Filter the hot mixture using a funnel.

(iv) Squeeze the liquid out of the solid residue with a spatula and return it to the flask.

(v) Add 10mL of dichloromethane to the refluxed product.

(vi) Again filter it. Combine the yellow extract.

(vii) Repeat the extraction for 2 to 3 times with 10mL of dichloromethane.

(viii) Combine all the extract and take it in a separating funnel.

(ix) Add water and sodium chloride solution to the acid layer and shake it well.

(x) Run the coloured lower layer through a cone of sodium sulphate into a dry flask and evaporate it to dryness in a fume hood.

(xi) Then a 12cm column of alumina using ligroin (40 – 60°c) was prepared as solvent.

(xii) The excess solvent was run out.

(xiii) The crude product was dissolved in 2-3mL of toluene and transferred into the column using a Pasteur pipette. The column was eluted with ligroin and the elute was collected in a flask. Care should be taken to discard the initial colourless elute.

(xiv) The main fraction collected is coloured due to the presence of carotene in tomato.

(xv) The solution was evaporated at room temperature to obtain pure lycopene.

(xvi) The crystals of lycopene can be examined under microscope.

(xvii) The product was treated with concentrated sulphuric acid to give a characteristic colour.

(xviii) UV spectrum of the product can be recorded between 200 and 600nm.

OBSERVATION

RESULT

Thus lycopene was isolated from tomato.

EXPT. NO. :

DATE :

PREPARATION OF L-PROLINE

AIM

To prepare L - Proline from gelatin.

THEORY

Pure proline is a white, non – deliquescent solid. It crystallizes quite easily from strong aqueous solutions in the form of long needles. It is not very soluble in cold absolute alcohol, but dissolves readily in the hot solvent, crystallising out on cooling in needle - shaped crystals. It may also be recrystallised from iso-propyl alcohol. It gives absolutely no amino - nitrogen in the Van Slyke apparatus.

Proline and its derivatives are often used as asymmetric catalysts in organic reactions. The CBS reduction and proline catalysed aldol condensation are prominent examples. L - Proline is an osmoprotectant and therefore is used in many pharmaceutical and biotechnological applications.

Proline is one of the two amino acids that do not follow along with the typical Ramachandran plot, along with glycine. Due to the ring formation connected to the Beta-carbon, the ψ and φ angles about the peptide bond have less allowable degrees of rotation.

Proline is the only amino acid that does not form a blue / purple colour when developed by spraying with ninhydrin for uses in chromatography. Proline, instead, produces an orange / yellow colour.

Gelatin is a translucent, colourless, odourless, brittle, nearly tasteless solid substance, derived from the collagen inside animals' skin and bones. It is commonly used as a gelling agent in food, pharmaceuticals, photography, and cosmetic manufacturing.Gelatin is unusually high in the non - essential amino acids glycine and proline, while lacking in certain essential amino acids. It contains no tryptophan and is deficient in isoleucine, threonine and methionine. The approximate amino acid

composition of gelatin is glycine 21%, proline 12%, hydroxyproline 12%, glutamic acid 10%, alanine 9%, arginine 8%, aspartic acid 6%, lysine 4%, serine 4%, leucine 3%, valine 2%, phenylalanine 2%, threonine 2%, isoleucine 1%, hydroxylysine 1%, methionine and histidine <1% and tyrosine <0.5%. These values vary, especially the minor constituents, depending on the source of the raw material and processing technique.

MATERIALS REQUIRED

(i) Sheet gelatin

(ii) Concentrated HCl

(iii) Reflux condenser

(iv) Decolouring charcoal

(v) Magnetic stirrer bottle shaker

(vi) Rotary vacuum evaporator.

PROCEDURE

(i) Place 150g of good quality sheet of gelatin (cut into conveniently sliced pieces) in a 1L flask.

(ii) Boil the mixture gently for 8h (fume cupboard) or boil under reflux for about 3h and leave it on a steam bath for over night. Biuret negative reaction indicates complete hydrolysis.

(iii) Concentrate the hydrolysate into syrup by distillation under pressure (Rotary evaporator).

(iv) Remove the excess HCl by dissolving the syrup in 500mL water.

(v) Boil the solution with 3g of decolouring charcoal.

(vi) Filter the mixture, cool it and dilute with water to 1200mL.

(vii) Add this solution to the filtered solution of 125g of ammonium rhodanilatein in 250mL of methanol by continuous stirring.

(viii) Keep it at 0°C for 2h to allow complete separation of proline rhodanilate.

(ix) Filter and wash with water and evaporate it to dryness.

(x) Dissolve this damp crude extract in 400mL of ethanol.

(xi) Add 800mL of 0.5M HCl by continuous stirring.

(xii) Cool the mixture again for 2h at 0°C.

(xiii) Filter the purified product from rhodanilate and wash it with 250mL of cold water

(xiv) Dry the product at 50°C for preliminary softening blankening.

(xv) Suspend the purified salt in 850mL of water in a stoppered bottle.

(xvi) Add 25mL of pure pyridine and shake it for about 4 – 5h.

(xvii) Remove the insoluble pyridine rhodonilate by filtration and wash with 100mL of cold water.

(xviii) Add glacial acetic acid drop wise to the pale pink filtrate until a small pink precipitate forms.

(xix) Filter it and evaporate the colourless filtrate to dryness (using rotary evaporator) and suspend the residue in absolute ethanol and evaporate twice

(xx) Dry the resulting faintly pink crude proline in a vaccum desicator over silica gel.

(xxi) Using minimum volume of ethanol, recrystallize the product.

RESULT

Thus L - proline was prepared from gelatin sheets.

EXPT. NO. :

DATE :

PREPARATION OF 5, 10, 15, 20 TETRA PHENYL PORPHYRIN

AIM

To prepare 5, 10, 15, 20 Tetra phenyl porphyrin.

THEORY

Porphyrin is a class of cyclic tetrapyrrolic ring structure compounds joined by four methane bridges (=C-) through alpha-carbon atoms of four pyrroles. Porphyrins are water-soluble nitrogenous biological pigments occurring widely in animal and plant tissues to take part in important biological functions such as metal-binding cofactors in hemoglobin in the blood of animals, chlorophyll in plants for photosynthesis, and certain enzymes for cell respiration, the cytochromes, enzymes that occur in minute quantities in most cells and are involved in oxidative processes; and catalase, also a widely distributed enzyme that accelerates the breakdown of hydrogen peroxide.

Numerous types of porphyrins can be obtained through the modification of a natural porphyrins and total synthesis. Not only their chemical transformations through nucleophilic and electrophilic substitution, substituent modification, reduction, oxidation but also their metalation and demetalation properties with iron, zinc, copper, nickel, and cobalt give valuable biological applications in molecular biology, fluoroimmunoassay, catalysts and new pharmaceutical developments.

Tetraphenylporphyrin, abbreviated H_2TPP, is a synthetic heterocyclic compound that resembles naturally occurring porphyrins. The study of naturally occurring porphyrins is complicated by their low symmetry and the presence of polar substituents. Tetraphenylporphyrin is hydrophilic, symmetrically substituted, and easily synthesized. The compound is a purple crystalline powder, with absorption 413 - 417 nm, Melting Point 450°C and dissolves in nonpolar organic solvents such as

chloroform and benzene. H_2TPP is a photosensitizer for the production of singlet oxygen

PRINCIPLE

The condensation of pyrrole and benzaldehyde gives macrocyclic porphyrin called 5, 10, 15, 20 tetra phenyl porphyrin.

MATERIALS REQUIRED

(i) Propionic acid

(ii) Reflux condenser

(iii) Pyrrole

(iv) Benzaldehyde

(v) Round bottom flask

PROCEDURE

(i) Take 40mL of propionic acid in 250mL round bottom flask.

(ii) Fix a reflux condenser with the setup and keep it in a boiling water bath for 10 minutes.

(iii) Add 1.6mL of benzaldehyde and 1mL of pyrrole by pouring the solution

(iv) down the reflux condenser while propionic acid begins to boil.

(v) Reflux the mixture for one hour. Filtration is done using vacuum pump.

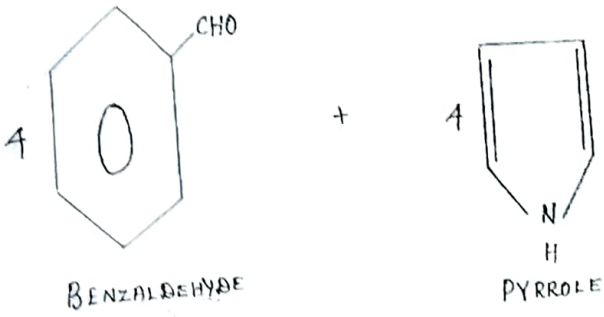
(vi) Filtered crystals are washed using 5mL of methanol and air dried.

OBSERVATION

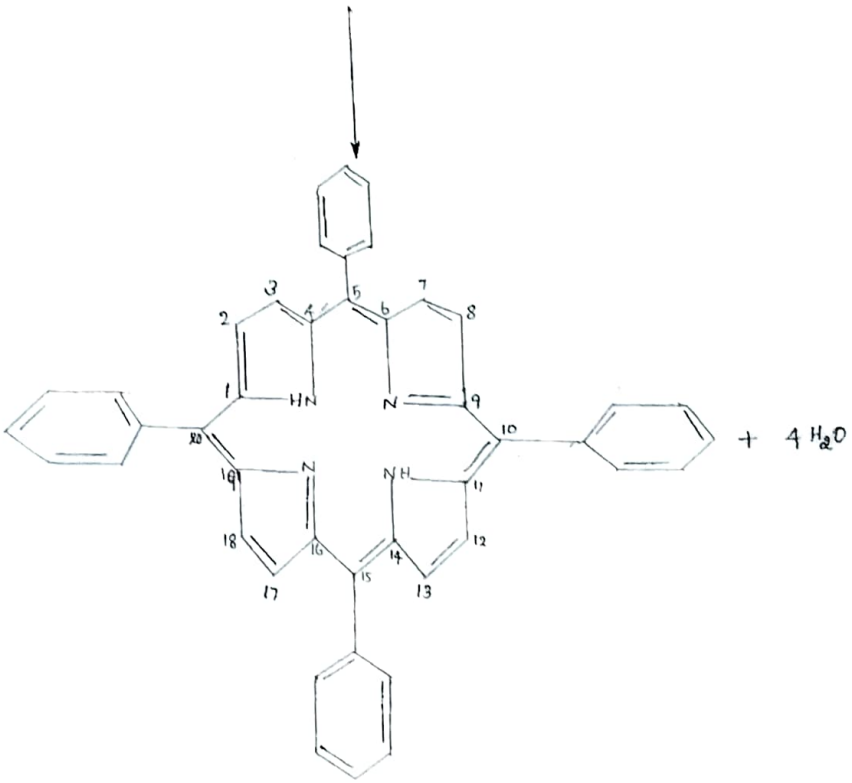
RESULT

_____ g of 5,10,15,20 Tetra phenyl porphyrin is obtained.

PREPARTION OF 5, 10, 15, 20 TETRA PHENYL PORPHYRIN



BENZALDEHYDE PYRROLE



5, 10, 15, 20 Tetra Phenyl Porphyrin

EXPT. NO.:
DATE:

PREPARATION OF1, 2, 5, 6 DI- O-CYCLOHEXYLIDINE-ALPHA-D-GLUCOFURANOSE

AIM

To prepare crystals of 1,2,5,6 di-O-cyclo hexylidine α-D-Glucopyranose

CHEMICALS REQUIRED

i. Cyclo hexanose
ii. Conc. H_2SO4
iii. Dry α-D-Glucose

APPARATUS REQUIRED

i. Round bottomed flask
ii. Cooling bath with an intimate mixture of ice and salt
iii. Shaker

PROCEDURE

i. Take a round bottomed flask. Immerse it in a large plastic or metal container filled with freezing mixture.
ii. Add 10.6 ml of cyclo hexanose to the flask and cool it at 0.C.
iii. Add 1 ml of Conc.H2SO4 using separating funnel into the vigorously stirred cyclo hexanone. Final solution should be light straw color.
iv. Add slowly with vigorously stirring 4.5 gm of finely powdered dry -D-Glucose.
v. Remove the cooling bath and allow the reaction mixture to reach ambient temperature continuously stirring it.
vi. Put the flask in the shaker for 8 hours so that there is a continuous mechanical stirring.

vii. The reaction mixture becomes progressively more viscous and finally sets into the solid between crystalline mass on cooling.

viii. Now keep it in room temperature for some time.

ix. Keep the conical flask in a slant position so that the solvent separates from the solid mass. Remove the solvent; take the solid mass in a filter paper and dry it well to get crystals of 1,2,5,6 di-o-cyclo hexylidine α-D-Glucopyranose.

RESULT

EXPT. NO.:
DATE:

PREPARATION OF S-ETHYL HYDROXYBUTONATE FROM ETHYL ACETOACETATE USING YEAST

AIM

To Prepare S-Ethyl Hydroxy butonate from Ethyl Acetoacetate using Yeast.

CHEMICALS REQUIRED

i. Commercially available sugar (sucrose) from a grocery store is used
ii. Commercially available baker's yeast can be used
iii. Ethyl acetoacetate is freshly distilled before use (bp 65°C/12 mm)

APPARATUS REQUIRED

i. using temperature controller
ii. running a reaction for a long time
iii. extraction and using simple drying agents
iv. gravity and vacuum filtration
v. bulb-to-bulb distillation
vi. using Kugelrohr
vii. determination of specific rotation

PROCEDURE

i. A 4-L, three-necked, round-bottomed flask equipped with mechanical stirrer, bubble counter, and a stopper is charged with 1.6 L of tap water, 300 g of sucrose, and 200 g of baker's yeast, which are added with stirring in this order.

ii. The mixture is stirred for 1 hr at about 30°C, 20.0 g (0.154 mol) of ethyl acetoacetate is added, and the fermenting suspension is stirred for another 24 hr at room temperature.

iii. A warm (ca. 40°C) solution of 200 g of sucrose in 1 L of tap water is then added, followed 1 hr later by an additional 20.0 g (0.154 mol) of ethyl acetoacetate

iv. Stirring is continued for 50–60 hr at room temperature.

v. When the reaction is complete by gas chromatographic analysis, the mixture is worked up by first adding 80 g of Celite and filtering through a sintered-glass funnel (porosity 4, 17-cm diam).

vi. After the filtrate is washed with 200 mL of water, it is saturated with sodium chloride and extracted with five 500-mL portions of ethyl ether

vii. The combined ether extracts are dried over magnesium sulfate, filtered, and concentrated with a rotary evaporator at 35°C bath temperature to a volume of 50–80 mL.

viii. This residue is fractionally distilled at a pressure of 12 mm through a 10-cm Vigreux column, and the fraction boiling at 71–73°C (12 mm) is collected to give 24–31 g (59–76%) of (*S*)-(+)-ethyl 3-hydroxybutanoate

ix. the specific rotation $[\alpha]^{25}D$ + 37.2° (chloroform, *c* 1.3) corresponds to an enantiomeric excess of 85%

x. The enantiomeric excess may be enhanced by several crystallizations of the 3,5-dinitrobenzoate derivative or else by using "starved" yeast

RESULT