Lab #5-Benzylidene Acetals As A Protecting Groups For Sugars
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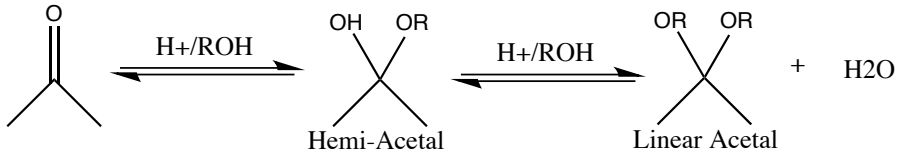
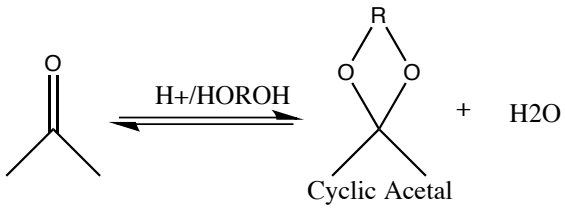
Introduction

Carbohydrates play a diverse role in living systems, they are the primary source of energy and they also play key roles in cell structure, shape and recognition. Carbohydrates usually contained many hydroxy groups and are often offered to as sugars or starches. Oligosaccharides are complex carbohydrates which re present on the surface of cells and act as glycans playing an important part in signal recognition. The amount and types of these carbohydrates on cell surfaces vary greatly; in recent years this diversity has been found useful in designing cell-specific antibodies and vaccines! Due to the complex structure of carbohydrates, nature and chemists require efficient mechanisms which certain functional groups to react and not others. This can be achieved by the use of protecting groups such as acetals.

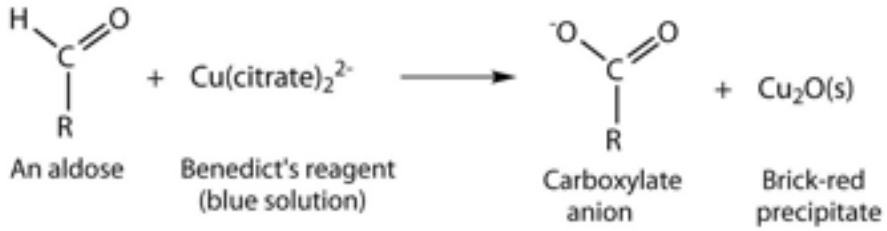
The term acetal is used to describe a carbon atom that is boned to two alkoxy groups ("OR"). For example, under acidic conditions with one equivalent of alcohol, aldehydes and ketones react to form a hemi-acetal (carbon bonded to and OH and OR group). This is a stable intermediate in the overall mechanism to form an acetal. If another equivalent of alcohol is introduced, the hemi-acetal will react to completion and form the acetal (carbon with two OR groups). Instead of adding two equivalents of alcohol, chemists can also add on equivalent of a diol to generate an acetal in the form of a protecting group. Once the protecting group has "covered" the aldehyde or ketone, the reactant can be subjected to stronger reducing agents without affecting the protecting group because it is stable under these conditions. Once the desired reaction has been conducted, chemists can remove the acetal and regenerate the original functional group of the molecule. This can be done simply by the addition on excess water and acid. So overall, reacting a carbonyl with one alcohol gives a hemi-acetal, reassign it with two alcohols gives a linear acetal and reacting it with a diol gives a cyclic acetals known as a protecting group. In Part A of this experiment, methyl-alpha-D-glucopyranoside will be reacted with benzaldehyde dimethyl acetal to form a protecting group at carbons 4 and 6 of the carbohydrate. Techniques such as extraction, filtration and TLC will be used to analyze the reaction and products.





A reducing sugar is any sugar that has the ability to act as a reducing agent due to the presence of free aldehydes, ketones or alpha-hydroxy-ketones. Additionally, if a carbohydrate

contains a hemi-acetal it will likely be a reducing sugar as well. All monosaccharides are reducing sugars along with certain disaccharides, olgiosaccharides and polysaccharides. One way of testing for the presence of reducing sugars in a sample is by using Benedict's Reagent. If a sample contains reducing sugars it will be capable of reducing Cu2+ into Cu+, this will be indicated with a red colour. If there are no reducing sugars present int eh sample the solution will remain blue. Depending on the amount of reducing sugars present, the solution will change colours from blue to green, yellow, organ and lastly red. In Part B, sample of milk, sucrose, glucose and juice will be tested with Benedict's Reagent to test for the presence of reducing sugars.



Mechanism

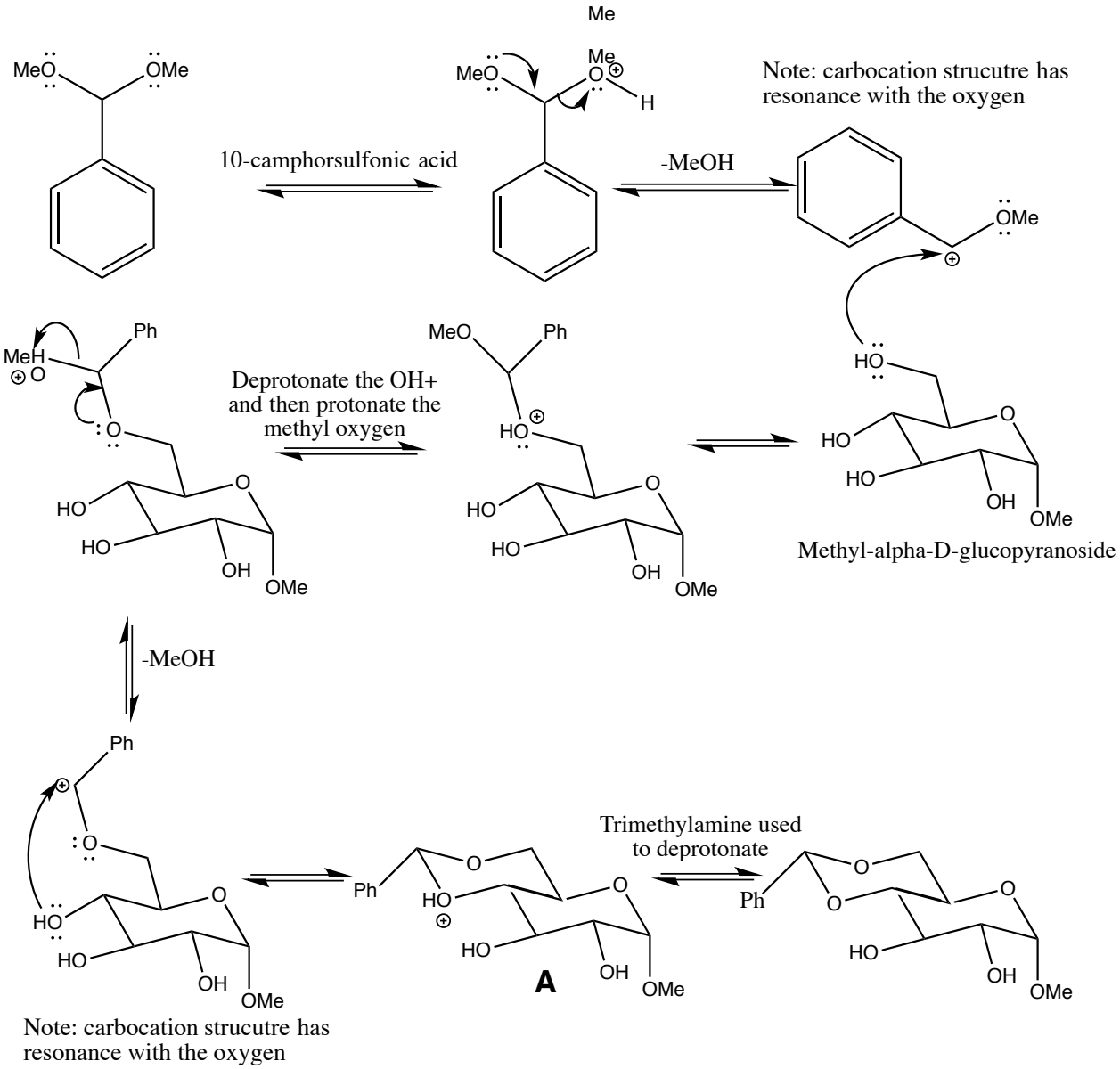


Table of Reagents

Compound	Molar Mass (g/mol)	Moles (n)	Quantity	Density (g/cm3)	Equivalent
Alpha-D- Glucopyranoside	194.183	0.00257	0.5g	1.46	-
Tetrahydrofuran	72.11	0.123	10ml	0.889g/ml	-
Benzaldehyde Dimethyl Acetal	152.19	0.00465	0.7ml	1.01	-
10-Camphorsulfonic Acid	232.30	0.000517	0.12g	-	-
10% Sulfuric Acid in Methanol (Solvent)	-	-	-	-	-
Triethylamine	101.19	-	Few Drops	0.73g/ml	-
Ethyl Acetate	88.11	0.511	50ml	0.902g/ml	-
Distilled Water	-	-	45ml	-	-
Sodium Sulfate	142.04	-	-	2.66	-
Dichloromethane	84.93	0.204	13ml	1.33g/ml	
Hexanes	86.18	0.566s	75ml	0.65g/ml	
3:7 Hexanes:Ethyl Acetate	-	-	-	-	-
Sucrose	342.3		0.1g	1.59	
Glucose	180.16		0.1g	1.54	
Milk	-	-	1ml	-	-
Coke	-	-	1ml	-	-
Benedict's Solution	-	-	12ml	-	-
Concentrated HCL	36.46	-	4 drops	1.19	-

Experimental Procedure

Please refer to "CHM2123 Laboratory of Organic Chemistry II" pg.63-70. Modification for Part A was that the mixture was placed in an ice bath after evaporation because no precipitate had formed. Also for Part A the manual says to use trimethylamine but triethylamine was used instead. Modification for Part B was that there was no juice present to test thus coke was used instead.

Table of Observations

Key Step	Observation
Part A: Protection Of A Monosaccharide	
Steps 1-2	-obtained the following substances: 10 ml of tetrahydrofuran-clear liquid 0.5 g of methyl-alpha-D-glucopyranoside-white powder 0.12 g of Camphorsulfonic acid 0.7 ml of benzaldehyde dimethyl acetal(given)-clear liquid -upon mixing became slightly cloudy and strong odour (bad smell)
Step 4	-after 45min the TLC was taken-meanwhile part B was completed -the TLC was dipped in sulfuric acid- (had to dip each “end” of the plate separately because the whole thing wouldn't fit in at once) -another TLC of the same materials was taken to give better analysis
Step 6 and 7	-added 15 ml of water and 50 ml of ethyl acetate to the reaction mixture and extracted it three times in total (added 15 ml of water each time) -while rinsing the reaction flask with ethyl acetate to ensure maximum transfer, some of the mixture spilled on the counter and was lost -for all three extractions the organic phase was on top and aqueous was on the bottom -organic was cloudy and slightly more vicious than the aqueous
Steps 8	-**a lot** of drying agent was required to form the hydrates -filtration was succesful but there was a lot of crystals present
Step 9	-after evaporation the mixture was not syrup-like and roughly 1-2ml of it was left
Step 10	-13 ml of DCM and 75 ml of Hexanes was added to the evaporated mixture -even after addition there was no ppt. so the beaker was placed in an ice bath -after some time there was very minimal ppt formed-we proceeded to the next steps-0.87g (mass of crystals)
Step 11	-final TLC was developed and it was seen that all three lanes had one spot each and all with very similar Rf values
Part B: Benedict’s Test For Reducing Sugars	
Steps 1-4	-sucrose and glucose were both white-powdery solids -milk was thick and cloudy -we used coke instead of juice
Step 5/6	-roughly 3 ml of Benedict's’ solution was added to all tubes and then placed in a beaker with boiling water -the following colours were observed sucrose-still light blue and kind of clear glucose-brick red milk-orange and thicker/chunky coke-dark red/almost brick red

Key Step	Observation
Step 7	-after the addition of 4 drops of HCl to the sucrose, the mixture separated into two layers -the bottom layer has a brown/dark red colour and the top is still light blue

Calculations-Percent Yield

$$n(product) = (0.87g)/(228.29g/mol)$$
$$= 0.003810942$$

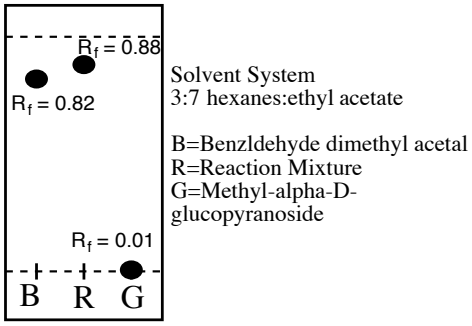
$$\%yield = \frac{0.00381942n}{0.00257n}(100)$$
$$= 148\%$$

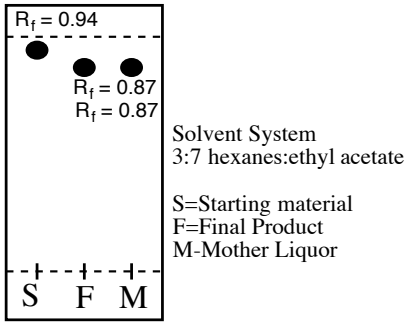
Table of Results

-Refer to TLC plates for part A results and quantitative observations for part B results.

Product	Mass	Molar Mass	Yield
4,6-O-benzylidene-alpha-D-glucopyranoside	0.87g	228.29g/mol	148%

TLC Plates





Discussion

Part A of this experiment explored the reaction between methyl-alpa-D-glucopyranoside, a sugar and benzaldehyde dimethyl acetal, a protecting group. The goal of this lab was to use techniques such as reflux, extraction and TLC to monitor the reaction progress and analyze the products. To begin, the sugar, benzaldehyde dimethyl acetal, tetrahydrofuran(solvent), and 10-camphorsulfonic acid were combined in a round bottom flask and the reaction was allowed to proceed under reflux conditions (see mechanism). Upon addition it was noted that the mixture had a bad odour and was cloudy. The purpose of the acid was to protonate the oxygen on the benzaldehyde dimethyl acetal which “initiates” the rest of the reaction. The tetrahydrofuran is a polar aprotic solvent which is suitable because it eliminates the possibility of any side acid-base reactions with the reactants. After about 45min the first TLC plate was developed which compared the two starting materials with the reaction mixture. Looking at the first TLC plate form above, it can be

proven that the reaction was complete because the mixture no longer contains either of the starting materials. Theoretically, the spot in the reaction lane should represent structure A in the mechanism shown which is charged and thus should be more polar than the benzaldehyde dimethyl acetal. However, this is not the case according to the experimental TLC. According to the TLC the structure A is less polar than the benzaldehyde dimethyl acetal which doesn't seem reasonable; possibly the reaction was still incomplete in which case the reaction spot would have represented an intermediate in the reaction; or there was some kind of contamination with the capillary tubes for TLC spotting.

Next, triethylamine was added to the cooled reaction mixture to deprotonate the structure A yielding the final product which is the protected sugar at carbons 4 and 6 (benzyldiene). The triethylamine was an important step because it "removed" the charge form the product which forced it to be more soluble in the organic phase during the extraction. The aqueous phase during the extraction contained, the pronated triethylamine, water, methanol and the deprotonated 10-camphorsulfonic acid whereas the organic phases contained: the product, ethyl acetate and tetrahydrofuran. Following extraction the organic phase was dried using Na2SO4 and then the hydrate crystals were separated via suction filtration. It was noted that A LOT of drying agent was needed to completely remove water form the mixture. The goal of the drying agent was to remove any excess water remaining in the organic phase however it seemed usual that so much of it was required since the majority of the water should have been removed in the aqueous phases. Additionally, it is likely that not all of the water formed hydrates and this may have lead to the very high percent yield that will be discussed later.

After drying the organic phase what is left is ethyl acetate, tetrahydrofuran and the product. To further isolate the product the flask was heated in a steam bath to evaporate the ethyl acetate and tetrahydrofuran with boiling points of about 77 and 66 degrees respectively. After evaporation there was a very small amount of mixture left, roughly 1 to 2 ml. However, unlike the manual states, the flask did not contain a syrup-like substance, rather it was less viscous than excepted. A possible reason for that is that there was still some water remaining. Since the flask was only heated till about 80 degrees the water present would not have evaporated and thus caused the resulting mixture to be less vicious than expected. Lastly, DCM and hexanes were added to force the precipitation of the product. The DCM was required to solubilize the contents of the flask and then hexanes increased the hydrophobicity of the solvent overall. The non-polar solvent does not solubilize the benzylidene very well and as a result it precipitates out. However, while performing this step the precipitate did not form readily and to improve results the flask was placed in an ice bath. The crystals were isolated via another suction filtration and the mass was measured to be 0.87g.

The second TLC was developed using the product dissolved in acetone, the mother liquor and benzaldehyde dimethyl acetal. It is makes sense that the mother liquor and final product have similar spots because some of the final product may have been soluble in the solvent and as a result it ended up in the mother liquor. Additionally it makes sense that the starting material lane does not match with the final product, this indicates that no significant amount of starting material remained in the product. The percent yield was calculated to be 148%, which is extremely high. One reason for this unusual yield is that some water may still have been present due to the inefficient drying of the organic phase as mentioned earlier. Also the crystals may have incorporated an impurity into its lattice structure and therefore falsely increased the yield. Overall this procedure was effective in protecting the sugar and forming the benzylidene crystals, however better purification methods should be used to ensure that the product is pure. Additionally, spectroscopy could be used to help identify any unaccepted species present in the final crystals.

Part B of this lab focused on testing for reducing sugars with the use of Benedict's reagent. Any sugar that has a free aldehyde group or alpha-hydroxy ketone group will be able to reduce the Cu (II) in the reagent to Cu2O. Referring to the table of observations, it can be proven that the sucrose was not able to reduce the copper at all; the milk was a bit more reducing followed by the coke and than glucose being the most reducing. It makes sense that most of the other substance were reducing to some extent and the sucrose was not reducing because it does not contain any free aldehydes or ketones and nor is it a monosaccharide. When the HCl was added to the sucrose test tube the glycosidic bond was cleaved resulting in two reducing monomers (glucose and fructose). This explains why the colour of the sucrose test tube changed from clear blue to green-yellow. This part of the lab can be taken one step further by applying the Benedict's reagent in certain food samples or even testing certain drugs for reducing agents. There were no significant sources of errors for this part of the lab.

Questions

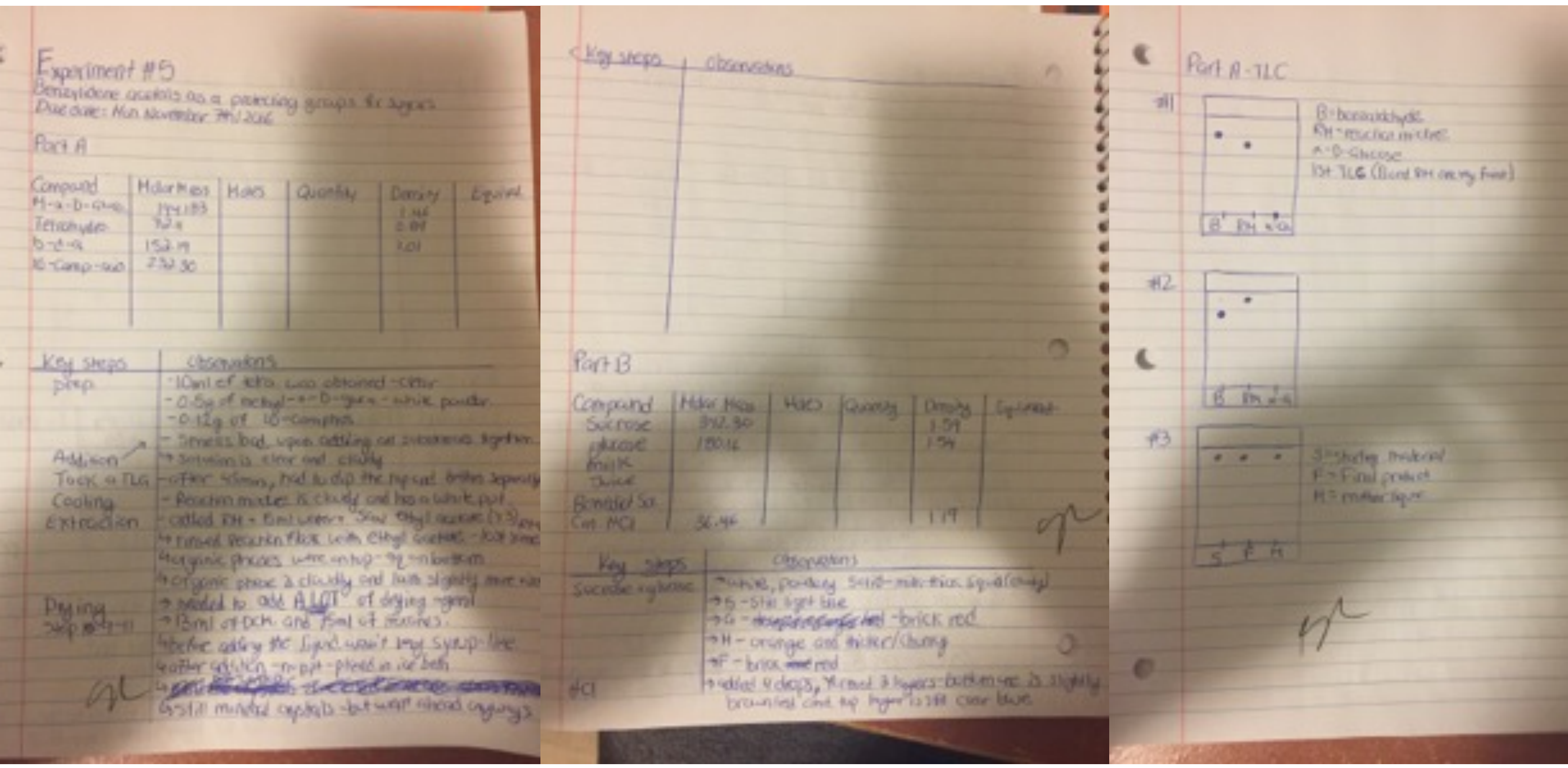
1. Both starch and cellulose are made of the same repeating monomer which is glucose. Starch is formed using alpha glucose units which form a glycosidic bond at carbons 1 and 4. In starch the glucose units are in the same orientation. On the other hand, cellulose if made of beta glucose units and has 1-4 glycosidic linkage. However, to make this linkage possible the glucose units need to alternate orientation. This results in a much strong polymer where cellulose polymers appear to be in a "staircase" pattern and starch is linear. The 3D arrangement of cellulose due to its molecular structure is more tightly packed and stronger. Humans cannot digest cellulose but can digest starch.

2. Methyl-alpha-D-glucopyranoside can be synthesized from alpha-D glucose with an organic base such as 10-camphorsulfonic acid and methanol as the two reactants needed to make the product (plus water). The intermediate in this reaction is an oxonium ion which is planar, this means that the nucleophile can attack form the equatorial or axial positions. The result of this is that not 100% of the product will be Methyl-alpha-D-glucopyranoside some will have the OMe group equatorial and others will have it axial. Note: equatorial positions are more stable so it is likely that the Methyl-alpha-D-glucopyranoside will be slightly minor than the methyl-equatorial

3. The scheme begins with fructose as the starting material and the first reagent is a base such as NaOH. These two reactants undergo a reaction to form an enolate which is the second molecule shown in the scheme. The next step is to add some water (reactant) which can react with the enolate to make one of two possible products, mannose or glucose (molecules 1 and 4 in the scheme). Therefore although fructose is not strictly a reducing sugar it can undergo the reaction explained above to produce two reducing sugars: glucose and mannose giving a positive Benedict's test.

4. Benedict's test is known to give false negatives in food samples because of the presence of certain acids and sugars. For example: in soda the presence of phosphoric acid cleaves the glycosidic bond in sucrose yielding two reducing monomers (glucose and fructose); this gives a false positive test result. Another example is ascorbic acid which has similar affects as the phosphoric acid.

Raw Data


Experiment #5
Part A
Compound
Molar Mass
Density
Key steps
Observations
Addition
Cooling
Extraction
Part B
Compound
Sucrose
glucose
Key steps
Observations
Part A - TLC
#1
#2
#3
F = Final product