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ON THE CONDITIONS FOR SYNTHESIS

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On the Conditions for Synthesis

Historical

One of the first experiments in synthesizing a complex carbohydrate from a simple one was preformed by Musculus (1) in 1872. By the action of 30 garms of concentrated sulfuric acid on 30 grams of glucose in 800 cc of absolute alcohol, he obtained a dextrin of the formula $C_{16}\ H_{10}\ O_5$, which did not reduce copper solution, was not acted upon by yeast extracts, and was hydrolyzed into glucose by dilute sulfuric acid. Schützenberger and Naudin (2) accomplished the synthesis of a disaccharide by condensing glucose with acetic anhydride, which product the authors called an isomer of sucrose because it had the formula $C_{12}\ H_{22}\ O_{11}$. Gautier (3) 1874, also prepared a condensation product using glucose in absolute alcohol and passing dry HCl gas through the mixture. This product also had the formula $C_{12}\ H_{22}\ O_{11}$. Several years later in 1886, Grimaux and Lefevre (4) did a similar experiment by dissolving pure glucose in eight times its weight of hydrochloric acid, sp. gr. 1.026, evaporating in vacuum, and recovering after precipitation with alcohol, a dextrin in the precipitate and a mixture of glucose and maltose in the filtrate. Emil Fischer (5) was unable to confirm the presence of maltose in repeating the above experiment. Fischer's (5) experiment on the synthesis of the disaccharide, isomaltose, was published in 1890. He dissolved by heating, 100 grams of pure glucose in 400 grams of concentrated hydrochloric acid and allowed the mixture to stand at 10-15°C for 15 hours. He was able to isolate from the mixture 2.5 grams of the pure isomaltosazone.

In 1898, J.H. van't Hoff (6) suggested the possibility of the reversibility of enzyme action. In this same year and in 1903, A. Croft Hill (7), published his well known experiments on the synthesis of the disaccharide, maltose, from glucose. Working with an approximately 40% glucose/water mixture, Hill observed after two and one half months, an increase in optical rotation, and a decrease in reducing power of the solution, amounting to a change of 15% of the glucose into maltose. On further investigation, he found two separate sugars, one which he isolated and called revertose, and the other maltose. Wroblewski (8) 1901 did the first experiment on the synthesis of sucrose by the action of invertase on a dilute water solution of invert sugar. He observed a shift to the right of the optical rotation of 7.2° and a corresponding decrease in the reducing power. O. Emmerling (9) 1901 repeated the experiments of Hill on 40% glucose solutions and obtained similar results, except that he found the end product to be isomaltose instead of maltose. Emil Fischer and E.F. Armstrong (10) 1902 synthesized isolactose in 15 days from 200 cc of kephirlactase containing 100 grams of galactose and 100 grams of glucose. Armstrong (11) repeated the experiments of Hill and those of Fischer, finding isomaltose by Hill's method and both isomaltose and maltose by Fischer's. Bourquelot, Herrisey, and Coirre (12) 1913 isolated a crystalline disaccharide, gentiobiose from a 50% glucose solution and emulsin of almonds kept at 15-20° for one month. By the action of emulsin on saturated aqueous solutions of galactose Bourquelot and Aubry (13) 1916 obtained in five months, two crystalline galactobioses. Bourquelot and Bridel (14) 1920 reported the production of sucrose by the action of gentiobiase on gentianose. One molecule of glucose was removed as β methyl glucoside and sucrose was isolated from the remaining material. Again in 1920 Bourquelot (15) using Hill's procedure, and substituting galactose for glucose, observed a change of optical rotation but could not isolate any crystals. According to Marc Bridel (16) 1923, the prolonged attempts of Bourquelot and Bridel to synthesize sucrose had been unsuccessful. Bridel found that invertase displayed no reversible reaction on mixtures of concentrated aqueous solutions of glucose and levulose in various proportions. H. Pringsheim and S. Leibowitz (17) 1924, working with 20 grams of glucose in 30 cc of maltase and buffer, obtained maltose and revertose.

In 1927 Pictet and Vogel (18) synthesized maltose and lactose. The former was produced by heating together equimolar mixtures of α and β glucose in vacuum at 160° and isolating the condensation product, maltose. Lactose was synthesized by heating together equal weights of β galactose and β glucose with a small amount of $ZnCl_2$ under pressure at 175°. In 1928 the same workers combined tetraacetylglucose and g-tetraacetylfructose in chloroform solution under the influence of P_2O_5, and obtained octaacetylsucrose. In the same year Irvine, Oldham, and Skinner (19) , using the general methods of Pictet and Vogel obtained from the condensation of tetraacetylchlrorglucose with tetraacetylfructose only a very small yield of disaccharide; from the condensation of g-tetraacetylglucose with tetraacetylchlorofructose, mainly pentaacetylglucose together with a disaccharide isomeric with sucrose; from the condensation of tetraacetylglucose and g-tetraacetylfructose, reducing sugars and the disaccharide isomeric with sucrose. Zemplen and Gerces (20) repeating similar experiments observed a disaccharide formation to the extent of 10-15%, but were unable to isolate octaacetylsucrose.

A. Blagowestschenski (21) 1930 accomplished a synthesis of a trisaccharide, raffinose, by the action of emulsin on a mixture of 17.9712 grams of galactose and 34.1765 grams of sucrose in 1000cc of 80% acetone. S. Mishimura (22) 1930 observed the effect of yeast autolysate on the formation of polysaccharides of the type of dextrin and starch. In 1931 Oparin and Kurssanov (23) again synthesized sucrose by the use of invertase. They introduced phosphoric acid into the sucrose molecule to slow down the formation of β fructose from g-fructose and thus accelerate the resynthesis. They found that 20% of the monosaccharide was converted into sucrose.

It will be noted in reviewing these various attempts at biological synthesis, that the more successful ones have been accomplished in a very concentrated medium. Very often the authors will mention the fact that they obtained better results when their materials were concentrated or when the system in which thay worked was non-aqueous. Musculus (1) was able to synthesize a carbohydrate with concentrated sulfuric acid and to hydrolyze it again with dilute sulfuric acid. Gautier emphasized the necessity of having all materials completely dry, in his synthesis. Emil Fischer's successful disaccharide synthesis was carried out in a concentrated hydrochloric acid medium. A.C. Hill observed that the more concentrated the substrate, the greater the synthesis, and says in his paper, "in the present case the sugar solutions must be of high concentration." Fischer and Armstrong in synthesizing isolactose, worked in a very concentrated aqueous medium. Bourquelot, Herrisey, and Coirre synthesized gentiobiose in a 50% glucose solution. Bourquelot and Aubry worked in a concentrated aqueous medium in synthesizing the galactobioses. After obtaining negative results in aqueous dilute solutions, Blagowestschenski carried out his experiment in 80% acetone. In his own words, "These negative results in aqueous solution obliged me to seek the solution of the problem in the use of a non-aqueous medium."

These workers have observed in practically every case an increased synthesis with an increase in the concentration of materials and a decrease in the water content. Even though positive syntheses were accomplished in some instances the period fpr their production was long while the yield of materials synthesized was very low, not going above 20% in any of the experiments just reviewed. This percentage does not even approximate those found in nature. In explanation, Martin H. Fischer (24) says,"Living matter is normally a practically anhydrous medium; the chemical characteristics of the normal life of the cell occur in an anhydrous medium and their course and products must, in consequence, be entirely different from the course and products of these same reactions occurring in and familiar to us from the study of the ordinary aqueous solution."....."Nature always makes her analyses in an aqueous solution and her syntheses in an anhydrous one."

The following are used by Fischer to illustrate the syntheses of materials in anhydrous media and their hydrolyses or analyses in aqueous media. 100% soap can be synthesized from suitable quantities of fatty acid and alkali if mixed with less water than can be taken up as hydration water by the soap. As soon as more water than this amount is present, the fraction of soap synthesis falls or such as is present suffers hydrolysis into fatty acid and alkali.

In a water solution iron and iodine combine to form a small quantity of iron iodide, with a large excess of iron hydroxide and hydriodic acid. As soon as sugar is added, (which combines with the water), the reaction goes completely to iron iodide.

Ethyl butyrate in a water solution hydrolyzes into ethyl alcohol and butyric acid. In reverse, absolute alcohol and butyric acid combine slowly to form ethyl butryate. If any third substance, any drier like H_2SO_4, P_2O_5, $CaCl_2$, a lyophilic colloid, or any enzyme, is added, a complete synthesis takes place in minutes to days.

This explains why Musculus was able to synthesize a carbohydrate in an anhydrous concentrated sulfuric acid medium, and was able to hydrolyze the product again with a mere dilution. Likewise many of the enzymatic syntheses are reversed by the addition merely of excess water.

The present work was undertaken in an effort to produce the synthesis of a disaccharide from a solution of monosaccharides from which the free water had been removed by a hydrophilic colloid. Fibrin was chosen because it has been shown by Fischer (25) that fibrin is capable of taking up extra large quantities of water in the presence of acids, and acids are the material commonly employed to hydrolyze polysaccharides. Fibrin, moreover, while anhydrous is still sufficiently "liquid" to permit the easy removal of samples from time to time.

Experimental

The general method consisted of hydrolyzing 50-67% sucrose solution by heating it with N/20 hydrochloric acid until complete inversion had taken place. This was ascertained by carrying out reducing sugar determinations, according to the method of Benedict, every fifteen minutes until three consecutive readings gave a constant value which accounted for all the sugar as monosaccharide. After cooling the solution about 1 gram of fibrin for every 5 cc of solution was added in addition to two drops of toluene as a preservative. This mixture was allowed to remain at room temperature from three to five weeks and at intervals during this period, samples were removed from the mixture. The fibrin was filtered off from the sample by suction through asbestos, and a clear yellow syrup remained. 2 cc of the syrup were diluted to 250 cc. Two 50 cc aliquots were used for the determinations. One sample was made up to 100 cc and a reducing sugar determination was made immediately. The other 50 cc portion was hydrolyzed by heating it with 5 cc of conc. HCl for 15 minutes, neutralized with sodium carbonate and made up to 100cc. A quantitative test for reducing sugar was also made for this portion. The total reducing sugar in the sample at the beginning of the experiment was taken as 100% and the other percentages determined on this basis.

Experiment A

100 grams of sucrose were dissolved in 100 cc of N/20 hydrochloric acid and the mixture warmed for two hours, and allowed to stand over night. 30 grams of fibrin (Difco 40-80 mesh) and two drops of toluene were added. The samples were removed at weekly intervals and percentages of reducing and non-reducing sugars determined. The results are recorded in Table I.

Insert Table I

Experiment B

100 grams of sucrose were dissolved in 50 cc of N/20 hydrochloric acid and the mixture warmed until complete inversion had taken place (2 hrs). 2 drops of toluene and 25 grams of fibrin (Difco 40-80 mesh) were added. The percentages were recorded weekly for two weeks but the increases noted were so great that readings were taken every four days. The results are recorded in table II

Insert Table II

Experiment C

300 grams of sucrose were dissolved in 150 cc of N/20 hydrochloric acid and heated until completely inverted. 2 drops of toluene and 75 grams of powdered fibrin (Armour) were added. Percentages were determined every week for four weeks and then every four days. The results are recorded in Table III.

Table I

Date	Reducing Sugar	Non-reducing sugar
1/22/36	100%	0
1/29/36	89.41%	10.59%
2/5/36	62.22%	37.78%
2/12/36	54.03%	46.97%
2/19/36	38.20%	61.80%

Table II

Date	Reducing sugar	Non-reducing sugar
2/19/36	100%	0
2/26/36	87.21%	12.79%
3/5/36	56.89%	43.11%
3/9/36	44.25%	55.75%
3/13/36	36.48%	63.56%
3/17/36	16.89%	83.11%

Table III

Date	Reducing sugar	Non-reducing sugar
4/5/36	100%	0
4/12/36	74.70%	25.30%
4/19/36	57.79%	42.21%
4/26/36	36.44%	63.56%
4/30/36	24.39%	71.61%
5/4/36	11.54%	88.46%

Control Experiment

In order to prove that the sugar (disaccharide) did not have its origin in the fibrin, 25 grams of fibrin was allowed to react with 50 cc of N/20 hydrochloric acid for a period of five weeks. After this interval the fibrin was filtered off, the filtrate neutralized with sodium carbonate and the reducing sugar determined. The entire filtrate from the 25 grams of fibrin was found to contain only 35 milligrams of sugar.

Experiment D

Polariscopic determinations were made on samples before and after inversion. The inversions were accomplished by hydrochloric acid and by invertase.

Solution I contained 3.33% total sugar, 2.02% disaccharide and 1.31% reducing sugar. The solution was inverted with a commercial invertase, "convertit". Polariscopic readings were taken before and after inversion, using a sodium light and a 5 cm tube.

Before inversion the solution gave a reading of	+5.51
After inversion the solution gave a reading of	-1.52

Assuming that the disaccharide present is sucrose and that the remaining reducing sugar is invert sugar, we can calculate the degrees of rotation that such a solution would give, and compare it with the reading actually obtained in the experimental solution. Sucrose $\alpha = 66.5^\circ$, glucose $\alpha = 53.2^\circ$, and levulose $\alpha = -92.0$. These rotations are calculated on the basis of 10% solutions in 10 cm tubes, so that substituting in the following equations, the rotations for sucrose, glucose and levulose can be calculated for their percentages, and for the 5 cm tube.

2.02% sucrose solution gives $\alpha = \frac{66.5^{\circ} \times 2.02}{10 \times 2} = +6.716$

0.655% levulose solution $\alpha = \frac{-92.0 \times 0.655}{10 \times 2} = -2.846$

0.655% glucose solution $\alpha = \frac{53.2 \times 0.655}{10 \times 2} = +1.742$

Total optical rotation for such a solution + 5.612

Actual optical rotation observed on unknown + 5.51

Solution II contained 1.02% total sugar, 0.67% disaccharide, and 0.35% reducing sugar. It gave the following readings before and after inversion with hydrochloric acid.

Polariscope reading before inversion = + 1.81

Polariscope reading after inversion = - 0.38

Assuming the disaccharide to be sucrose and calculating the rotation as in the previous experiment, we have the following result.

0.67% sucrose solution gives $\alpha = \frac{66.5 \times 0.67}{10 \times 2} = +2.227$

0.175% levulose solution $\alpha = \frac{-92.0 \times 0.175}{10 \times 2} = -0.805$

0.175% glucose solution $\alpha = \frac{53.2 \times 0.175}{10 \times 2} = +0.463$

Total optical rotation for the solution	+ 1.885
Actual optical rotation observed in the test solu.	+ 1.81

All attempts to isolate the disaccharide met with negative results. No cahracteristic osazone was obtainable. The sugar was not precipitated as the strontium bisaccharate, although the procedure was followed closely and according to several different authorities. Precipitation with absolute alcohol gave only a partly crystalline, partly sticky mass, from which the crystals could not be separated.

Discussion

The reaction taking place in the system may be represented by the following equation; using sucrose as the disaccharide:

sucrose + water = glucose + levulose

The two sides of the equation are in equilibrium, so that the products of the components on each side is equal to a constant. In other words, no one component can be increased or decreased without increasing or decreasing another. For instance, if the water in the system is increased, the sucrose must be decreased and the glucose and levulose increased, or an increase in water makes for an increase in hydrolysis. But if, in the case of this experiment, the water is decreased, the sucrose is increased and the glucose and levulose decreased, or synthesis is increased.

Fibrin was used as a drier to remove water from the system because, with the hydrochloric acid used in the hydrolysis of sucrose, it formed a compound with a high hydration capacity. Because of this ability of combining with the acid, fibrin also acted as a buffer, keeping the system close to the neutral point throughout the experiment.

Summary

In a concentrated invert sugar solution made anhydrous by the use of fibrin, a decrease in reducing sugars of 88.46% was accomplished in 29 days.

The product was hydrolyzed by hydrochloric acid and also by a commercial invertase.

The optical rotation of a solution in which the disaccharide was calculated as sucrose checked closely with the actual value obtained with the experimental solution.

No characteristic osazone was obtainable.

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