8336 C

Interconversion of Ketose and Aldose Sugars in Dilute Aqueous Solution.

HELEN R. GARBUTT AND ROGER S. HUBBARD.

From the Buffalo General Hospital and the Medical School of the University of Buffalo.

The present article contains a study of certain changes produced when dilute aqueous solutions of various sugars are boiled. Interest in the subject was aroused because it was observed that in a solution of glucose so treated fructose was apparently produced.[1] Although the interconversion of glucose, fructose, and mannose in alkaline solution has been repeatedly studied since Lobry de Bruyn and Van Eckenstein first observed the phenomenon,[2] results of quantitative studies similar to those reported do not seem to be available.

The technical procedure was as follows: Pure glucose from the Bureau of Standards was dried over calcium chloride and dissolved in distilled water. Seventy-five cc. of a solution so prepared were boiled on an electric hot plate under a reflux condenser. At intervals the flask containing the solution was rapidly cooled in ice water and a sample removed for analysis. The solution was then reheated rapidly and the boiling continued. The changes in the concentration of reducing substances were determined by the copper reduction method of Benedict[3] and variations in the intensity of the resorcinol reaction by the technique described by Roe[4] for the determination of fructose in blood. The purest glucose and fructose obtainable were used as standards. The reaction values were estimated colorimetrically at room temperature. Pure commercial mannose and galactose prepared for the bacteriological laboratory were used in some experiments.

Water solutions of glucose were studied first. It soon became evident that several conditions affected the composition of the boiled mixture. One of these was the concentration of glucose initially present. When the solution was very dilute the maximum concentration of "fructose" was generally reached after boiling for 4 hours, but in stronger ones such a value was not reached in very much

---

[1] Hubbard, R. S., and Garbutt, H. R., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 986.

[2] Armstrong, E. F., The Simple Carbohydrates and the Glucosides, second edition, London, 1912, p. 40.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, **64**, 207.

[4] Roe, J. H., *J. Biol. Chem.*, 1934, **107**, 15.

longer periods. Results of analyses made after 6 hours are given in Table I. It can be seen that the concentration of "fructose" present at this time varied with the concentration of glucose initially present, but was not parallel to it. Since there was very little difference between the percentage composition of the products obtained by boiling 0.02 and 0.1% glucose in a neutral buffer mixture (Table) it seems possible that variations in acidity may have been one of the causes of this lack of parallelism.

TABLE I.*

Formation of "Fructose" on Boiling Aqueous Solutions of Glucose for Six Hours.

Initial concentration of pure glucose mg./100 cc.	"Glucose" after boiling mg./100 cc.	"Fructose" formed by boiling[1] mg./100 cc.	Reaction pH	Proportion of "total sugar" as "fructose" %
5	4.05	1.36	—	36
10	8.70	2.38	6.7	27
20	18.6	5.30	6.8	27
40[2]	40	6.72	—	17
100	98	10.9	—	11
1000	1000	49.6	6.5	5
2000[3]	2000	62.0	5.9	3
20[4]	18.4	5.60	7.0	30
100[4]	101.4	29.8	7.0	29
20[5]	19.5	4.18	6.9	21

*Under "glucose" and "total sugar" are given the reducing power in terms of glucose. Under "fructose" is given a quantitative measure of the resorcinol reaction as fructose. Reaction values were determined at room temperature.

[1]Corrected for the resorcinol reaction given by the unboiled glucose solution.

[2]Results after boiling for 4 hours.

[3]Nitrogen gas led through the solution continuously during the experiment.

[4]Phosphate solution in M/15 concentration giving a pH of 7.0 present in these experiments. Boiled only 4 hours.

[5]One per cent sodium fluoride present in this experiment.

A decrease in reducing power was found in the dilute but not in the stronger solutions studied. Since the reducing power of the carbohydrates probably present in such a mixture varies (fructose gave results approximately 6% higher, and mannose results 12% lower than glucose by the method used), this diminution in reducing power might have been due to the same type of molecular rearrangement as leads to the production of a ketose. The authors believe, however, that some destruction of one of the sugars in the mixture must have taken place or some significant change in the reducing power of the one and 2% solutions would have been found.

It will be noted that the formation of ketose occurred in these experiments at a reaction decidedly more acid than pH 12.3—the value which corresponds to the acidic dissociation exponent of glucose.[5] Although the reaction at 100° is not correctly given by measurements at room temperature, it seems to the authors that even those values must have been distinctly less alkaline than the one indicated by the constant given above. It seems possible, however, that some acidic dissociation of the sugar was produced by heat.

The results of an experiment in which sodium fluoride was added to the glucose are included in Table I. Because the reaction was somewhat more acid during the course of this experiment than during that of the others recorded, it does not seem proper to emphasize the significance of the relatively slight destruction of sugar noted in the presence of this salt.

Sugars in 0.02% solution seemed suitable for study by the methods selected, and glucose, fructose and mannose were treated in this concentration in various ways. Results with mannose were similar to those shown in Table I, but the changes were less marked and less consistent. Only 10% of the total reducing substance was in the form of a ketose after 6 hours' boiling. Determinations of the reducing power gave very irregular results, probably because destruction and conversion into a substance with greater reducing power than mannose (glucose?) occurred simultaneously.

TABLE II.*

Dilute Aqueous Sugar Solutions Boiled in the Presence of Oxygen and Nitrogen.

Time of boiling hr.	0.02 % glucose studied: "Glucose" mg./100 cc.	"Fructose" mg./100 cc.	Reaction pH	Time of boiling hr.	0.02 % fructose studied: "Glucose" mg./100 cc.	"Fructose" mg./100 cc.	Reaction pH
Oxygen gas led continuously through the solutions.							
0	20	0.25	5.8	0	21.3	20	5.9
2	19.9	3.63	6.8	2	19.1	17.6	5.9
4	19.1	5.20	6.8	4	18.5	15.7	6.1
6	17.9	6.15	6.9	6	17.4	13.7	6.7
Nitrogen gas led continuously through the solutions.							
0	20	0.2	6.0	0	21.3	20	6.0
2	20	4.0	7.2	2	18.6	17.0	5.6
4	19.1	6.1	7.2	4	18.0	15.0	5.8
6	18.3	6.5	7.0	6	17.5	13.3	6.4

In Table II are given details of representative experiments with glucose and fructose. In these experiments oxygen and nitrogen were bubbled at a slow rate through the boiling solution throughout the 6-hour period. There was probably some loss of water vapor with the escaping gas, but the results were not significantly different from those of other experiments in which gas was not used.

The results of the experiments with the 2 gases are seen to be

[5] Clark, W. M., The Determination of Hydrogen Ions, third edition, Baltimore, 1928, p. 678.

almost exactly alike; such differences as exist between the sets of quantitative analyses are certainly not greater than can be attributed to the differences in reaction found. The results upon each of the sugars will be separately discussed.

Glucose when boiled gave rise to a substance giving the resorcinol reaction. The rate at which this substance was produced was most marked during the first part of the experiment. After 4 hours equilibrium had been approximately reached in the experiment with nitrogen. There was no measurable decrease in reducing power during the first 2 hours of boiling. Thereafter a significant change could be demonstrated. All of the substances in the boiled mixture were fermentable when treated by massive inoculation with yeast cells for short periods. This observation was confirmed on other similar preparations. The non-fermentable sugar "glutose"[6] could not be demonstrated.

Fructose when boiled showed a loss both in the reducing power and in the intensity of the resorcinol reaction. These losses were regularly greater in the latter factor. The differences between the changes can properly be interpreted as resulting from a conversion of fructose into a sugar, or mixture of sugars, which do not give the ketose test. The loss in reducing power, which was shown during the first period of the experiment, the authors interpret as a change of part of the sugar into some non-reducing substance. The boiled mixture after fermentation with yeast did not give either a reducing or resorcinol reaction.

When the results with the 2 sugars are compared it is seen that the proportion between ketose and total sugar was lower when glucose was used than when fructose was studied. This suggests that a ketose was more readily formed from glucose than was an aldose from fructose. Since the reaction values in the experiments upon the 2 sugars were quite different, great emphasis should not be placed upon this contrast.

A conversion of glucose, fructose and mannose into mixtures of fermentable compounds which show reducing and resorcinol reactions can be demonstrated in dilute aqueous solutions. It seems probable that these changes closely resemble those observed in alkaline solutions, although the reaction did not approach the pH value of the acid dissociation constant of glucose (12.3). Some loss in reducing power was also found. The changes were not measurably influenced by oxygen.

[6] Benedict, E. M., Dakin, H. D., and West, R., *J. Biol. Chem.*, 1926, **68**, 1.