ON THE OXIDATION OF GLUCOSE IN ALKALINE SOLUTIONS OF IODINE.

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In 1918 Willstätter and Schudel (1) described a method for the estimation of sugars which embodied the principle first set forth by Romijn (2), that hexo-aldoses may be quantitatively oxidized to the corresponding hexonic acid in the presence of iodine in alkaline solution. The reaction may be expressed by the equation $RCHO + I_2 + 3NaOH = RCOONa + 2NaI + 2H_2O$. Thus 1 molecule of aldose reacts with 2 molecules of sodium hypoiodite, and when a measured excess of the latter is used the amount of iodine liberated on acidification, after the reaction between aldose and hypoiodite has reached completion, serves as a measure of the quantity of sugar present.

In the original description of their method Willstätter and Schudel state that sodium hydroxide must be slowly added to the sugar-iodine solution. It was observed by the author that if sodium hydroxide be added immediately to the glucose-iodine solution, or even over an interval of 30 to 60 seconds, one does not obtain quantitative oxidation of glucose. The success of the method depends therefore primarily on the rate of addition of sodium hydroxide to the mixture of glucose-iodine. These observations are summarized in Table I.

It is clearly seen that the rate of addition of alkali has a distinct influence on the success of the reaction glucose→gluconic acid in reaching completion. If solutions of glucose-iodine, to which alkali has immediately been added, as in Experiments 6 to 8, are permitted to stand longer than 15 minutes, the oxidation of the sugar fails to come to completion, and at the end of a prolonged period of time, an hour or more, no hypoiodite can be demon-

strated in the reaction mixture. Apparently the oxidizing re-
action has reached completion with some 4 per cent of glucose
still unchanged. In the preparative method found in the following
paper, this effect is shown to be greatly magnified. It is the
nature of this phenomenon which will be discussed.

TABLE I.

Effect of Rate of Addition of 2 Equivalents of 0.1 N Sodium Hydroxide on Oxidation of 10 Cc. of 0.9 Per Cent Glucose and 20 Cc. of 0.1 N Iodine-Potassium Iodide Solutions at 23°.

Experiment No.	Glucose.	Time over which 2 equivalents of 0.1 N NaOH were added.	0.1 N iodine utilized in oxidation of glucose.	Glucose oxidized as calculated from iodine consumption.
	mg.	*sec.*	*cc.*	*per cent*
1	90	240	10.00	100
2	90	120	10.00	100
3	90	60	9.94	99.4
4	90	30	9.87	98.7
5	90	15	9.78	97.8
6	90	Immediately.	9.65	96.5
7	90	"	9.64	96.4
8	90	"	9.65	96.5

The method of analysis in Table I was carried out as follows: To 10 cc.
of a 0.9 per cent glucose solution were added 20 cc. of 0.1 N iodine-potassium
iodide solution and to this were added 2 equivalents of 0.1 N sodium hy-
droxide (40 cc. + 5 cc. to neutralize the gluconic acid formed). When the
alkali was added over an interval, it was run from a burette at constant
rate. When added "immediately" the required amount was quickly poured
from a cylinder into the well stirred glucose-iodine solution. The whole
operation required about 3 seconds. After standing a total of 15 minutes,
when the reaction had come to completion, the mixture was acidified with
6 cc. of 1.0 N hydrochloric acid and was titrated with 0.05 N sodium thio-
sulfate, using starch as an indicator. The method is accurate to about
± 0.15 per cent. The temperature variation was not greater than ± 0.25°.
The sodium hydroxide used in these and subsequent experiments was exactly
0.1 N and was prepared from carbonate-free sodium hydroxide from sodium.
The iodine-potassium iodide solution contained 25.0 gm. of the potassium
iodide per liter.

Until the appearance of Schönbein's (3) "Beiträge zur nähern
Kenntniss des Sauerstoffs und der einfachen Salzbildner" it was
believed that iodine and potassium hydroxide in solution reacted
instantaneously to form potassium iodate. Schönbein showed this

reaction to take place in two stages, and from the analogy between the chemical similarity of iodine and chlorine, assumed that an intermediate product, potassium hypoiodite, was formed. Schön-bein's experiments showed that the first stage of the reaction, the formation of hypoiodite, is incomplete and reversible, and that an equilibrium between alkali, iodine, iodide, and hypoiodite existed. With regard to the second reaction, the formation of iodate from hypoiodite, he says little except that the velocity is increased as the temperature is raised. This second reaction has been studied in great detail by many investigators and an excellent review of the literature is to be found in the articles of Skrabal (4) which appeared in 1907. Most of these studies have been carried out in solutions of high dilution, but Taylor (5) has demonstrated that in solutions of decinormal strength the formation of iodate from hypoiodite progresses at a remarkable rate.

In an attempt to obtain a more accurate knowledge of the rate of formation of iodate from hypoiodite in solutions of the same concentration as those employed in the Willstätter-Schudel method, a short series of experiments was carried out and the results are given in Table II. A second series of experiments was also carried out concerning the effect of the rate of addition of alkali on the rate of oxidation of glucose and on the rate of the formation of iodate from hypoiodite in these sugar-containing solutions. These results are found in Table III.

From the data presented in these tables there are two facts worthy of note: first, in the absence of glucose the rate of addition of 0.1 N sodium hydroxide to 0.1 N iodine solution has no influence on the speed of formation of iodate from hypoiodite over the time intervals studied; and second, the rate of addition of alkali has a marked influence on the speed of formation of iodate from hypoiodite in a solution containing glucose. It is to be borne in mind that in an alkaline glucose-iodine solution in addition to the reaction (1) $RCHO + 3NaOH + I_2 = RCOONa + 2NaI + 2H_2O$ there is going on simultaneously a second and equally important reaction (2) $3NaOI = NaIO_3 + 2NaI$. In order to explain the phenomenon of the failure of glucose to be oxidized quantitatively when alkali is immediately added to glucose-iodine solution, it becomes necessary to take into consideration the reaction velocity of this second reaction.

Before going on with this discussion, however, an important factor which determines the success of reaction (1) must be pointed out. This factor is the necessity for the presence, as the

TABLE II.

Rate of Formation of Iodate from Hypoiodite in Solution of 20 Cc. of 0.1 N Iodine, 10 Cc. of Water, and 40 Cc. of Sodium Hydroxide at 23°.

Series No.	Time of reaction.	Hypoiodite-iodide in solution.	Iodate-iodide in solution.
	min.	*per cent*	*per cent*
I	2	17.8	82.0
	7	5.8	94.2
	12	3.7	96.1
II	2	18.0	82.0
	7	4.5	95.6
	12	3.7	96.2

The method of procedure in Table II was as follows: In Series I 2 equivalents (40 cc.) of 0.1 N sodium hydroxide were immediately added to 20 cc. of 0.1 N iodine-potassium iodide solution and 10 cc. of water. The mixture was permitted to stand the required length of time, then to it were added 2 gm. of solid sodium bicarbonate and 100 cc. of water. A blast of carbon dioxide was passed through to neutralize the free alkali. In this manner the iodine from the hypoiodite and unaltered iodine was liberated. 15 cc. of 0.1 N sodium arsenite were added and the excess arsenite was titrated with 0.1 N iodine-potassium iodide solution, using starch as indicator. The back titration with 0.1 N iodine in cc. minus 15 cc. is equal to the cc. of iodine in the form of hypoiodite and iodine in equilibrium. The iodate was then determined by adding small portions of HCl to this solution and titrating with 0.05 N sodium thiosulfate. In Series II 2 equivalents of 0.1 N alkali were added at constant rate from a burette over an interval of 2 minutes, and the analyses made exactly as above. The time of reaction was counted from the first addition of sodium hydroxide. The temperature variation, as in all these experiments was not greater than ± 0.25°. Since iodine in sodium hydroxide exists in equilibrium form, $2I + NaOH \rightleftharpoons NaOI + HI$, and $3NaOI = NaIO_3 + 2NaI$, the so called "hypoiodite" determination represents all of the iodine in the first equation; the "iodate" determination represents the right hand member of the second equation.

reaction approaches completion, of a concentration of hypoiodite sufficient to drive it to the end. If the concentration falls below a certain optimum, reaction (1) will never reach completion. The condition which determines this factor is the rate of addition of sodium hydroxide.

A sufficiently high concentration of hypoiodite is possibly not attained when alkali is added immediately to a glucose-iodine

TABLE III.

Influence of Rate of Addition of 2 Equivalents of 0.1 N Sodium Hydroxide on Rate of Formation of Iodate from Hypoiodite and Rate of Oxidation of Glucose in Alkaline Hypoiodite Solution at 23°.

Series No.	Glucose.	Time.	0.1 N iodine in form of:		0.1 N iodine consumed by glucose.	Iodine-hypo-iodite in solution.	Hypo-iodite converted to iodate-iodide.	Glucose oxidized.
			Iodine-hypo-iodite.	Iodate-iodide.				
	mg.	min.	cc.	cc.	cc.	per cent	per cent	per cent
I	90	2	1.45	10.00	8.55	12.7	87.3	85.5
		5	0.48	10.16	9.36	4.5	95.5	93.6
		8	0.42	10.16	9.42	4.0	96.0	94.2
		11	0.22	10.26	9.52	2.1	97.9	95.2
		15	0.12	10.27	9.61	1.1	98.9	96.1
		30	0.05	10.27	9.68	0.5	99.5	96.8
		120	None.	10.35	9.65	0.0	100.0	96.5
		240	"	10.35	9.65	0.0	100.0	96.5
II	90	2	3.10	7.60	9.30	29.0	71.0	93.0
		5	1.57	8.61	9.82	15.5	84.5	98.2
		8	0.84	9.21	9.95	8.4	91.6	99.5
		11	0.65	9.36	9.99	6.5	93.5	99.9
		15	0.64	9.38	9.98	6.4	93.6	99.8
		30	0.30	9.69	10.01	3.0	97.0	100.1
		120	0.07	9.93	10.00	0.7	99.3	100.0
		240	0.03	9.97	10.00	0.3	99.7	100.0

The method of procedure in Table III was as follows: To 10 cc. of 0.9 per cent glucose and 20 cc. (2 equivalents) of 0.1 N iodine-potassium iodide solutions were added 2 equivalents (40 cc. + 5 cc. to neutralize the gluconic acid formed) of 0.1 N sodium hydroxide, either immediately, as in Series I, or over an interval of 2 minutes at constant rate from a burette, as in Series II. At the end of the time interval (tabulated in the third column) counted from the first addition of alkali, the analyses for "hypoiodite" and "iodate" were made as under Table II. The iodine consumed in the oxidation of glucose represents the sum of the hypoiodite-iodate titration subtracted from 20. The percentages of hypoiodite and iodate are those calculated for iodine unconsumed by glucose.

solution for the reason that reactions (1) and (2) start out simultaneously. Under these conditions reaction (1), though it has a

greater *initial* speed than reaction (2), will be overtaken by the second as the two near completion with the result that at this point the concentration of hypoiodite falls below the necessary optimum and both reactions end with the hypoiodite completely converted to iodate and the glucose in an incomplete state of oxidation. If, on the other hand, the alkali be added over an interval of 2 minutes, the major part of the hypoiodite will enter into the first reaction as it is formed, since the *initial* speed of the reaction glucose → gluconic acid is greater than that of the reaction hypoiodite → iodate. In other words, during the addition of the first increments of alkali the concentration of hypoiodite in the solution would be kept low, a condition unfavorable for the reaction hypoiodite → iodate.

A second interpretation might explain the phenomenon of the failure of quantitative oxidation when alkali is immediately added to glucose-iodine solutions; namely, the initial high concentration of hydroxyl ions would favor any process of enolization of glucose to unoxidizable ketose. If this be the case, then if glucose and alkali be first mixed and iodine be added to the mixture over an interval of 2 minutes, one should obtain 96 per cent oxidation. As a matter of fact, one obtains some 98 per cent oxidation. The discrepancy is even more striking if one permits glucose and 0.1 N alkali to stand 12 hours before adding iodine, for nearly 25 per cent of glucose will remain unaccounted for. It may be assumed therefore, that a small amount of glucose is actually converted to ketose under these conditions, and that the rapid conversion of hypoiodite to iodate does not completely explain the phenomenon of incomplete oxidation.

If potassium iodide be added in excess to iodine-glucose solutions (thus tending to increase the concentration of hypoiodite by repressing the reaction $3NaOI = NaIO_3 + 2NaI$), and then 2 equivalents of sodium hydroxide be added at one time, the oxidation progresses approximately to the same point as when iodine is added over an interval of 2 minutes to glucose-alkali solutions. This observation confirms the assumption that a high initial hydroxyl ion concentration hinders in part the reaction glucose → gluconic acid from going to completion despite a high hypoiodite concentration at the end of the reaction. Whether this hindering effect can be attributed entirely to a process of enolization of the

sugar, to an anticatalytic effect, or to some other remote cause is of course difficult to say when one deals with highly complex alkaline glucose-iodine solutions in which rapid concentration changes are taking place.

SUMMARY.

An explanation, based on the necessity of a proper hydroxyl ion concentration, for the stoichiometrical progression of the reaction glucose $\rightarrow$ gluconic acid in alkaline solutions of iodine, has been offered, and found to agree with the observed reaction curves.

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