The Action of Oxygen on Glucose in the Presence of Potassium Hydroxide ¹

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INTRODUCTION

In the presence of strong alkalies, sugars undergo complex transformations which depend upon the various conditions of temperature, concentration of alkali, and the presence or absence of oxidizing agents. Evans (1,2) has presented complete reviews of the progress in this field at two stages in its development; unless cited directly, the earlier work is described in these reviews.

Nef proposed the theory of the formation of intermediate enediols, a mechanism first suggested by Wohl and Neuberg. He postulated that the various enediols formed from the hexoses were split at the double bonds into fragments containing one to five carbon atoms. In the presence of oxidizing agents these fragments would yield a mixture of the corresponding acids.

Evans and co-workers continued the work on the oxidation of compounds related to the carbohydrates in order to establish from quantitative data the molecular stages through which these compounds pass during the course of the reaction; they concluded that the theory of Nef satisfactorily explained the reactions observed.

Schmidt (8), however, pointed out that the bond energy of a carbonto-carbon double linkage is greater than that of a single bond. He proposed his "double-bond rule" which states that the presence of a double bond in a compound strengthens the single bond adjacent to it and weakens the bond next removed. Hence, the compound would be

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expected to split, not at the double linkage as proposed by Nef, but at the carbon-to-carbon linkage α - β to it. The theory of Schmidt, therefore, differs from that of Nef and Evans essentially in the manner in which the molecule is believed to undergo cleavage.

Schmidt's theory received support from the work of Upson and coworkers in their study of the action of barium hydroxide on the aldonic acids in the absence of air. From a quantitative examination of the products obtained they interpreted the fragmentation as occurring in the manner postulated by Schmidt, and submitted strong evidence in opposition to the mechanism of Nef and Evans.

Although the Schmidt theory has been successfully applied to explain the mechanism of the reactions taking place in the absence of air or oxidizing agents, Evans pointed out the difficulty of reconciling the Schmidt theory with the Nef theory where oxidizing agents were used; he suggested that the rupture by alkali in the presence of oxidizing agents may be a different process from that occurring in the absence of oxidizing agents.

The effect of various oxidizing agents such as potassium permanganate, metal oxides and hydrogen peroxide upon the sugars in the presence of alkalies is rather drastic. Hence Nef and collaborators studied the effect of passing a very rapid stream of air through alkaline solutions of various hexoses and pentoses. The products isolated were generally formic acid and some derivative of the aldonic acid of one less carbon content than the parent sugar.

Spengler and Pfannenstiel (9) treated an alkaline solution of glucose with oxygen instead of air. This treatment increased the yield of arabonic acid to 70%. Hudson also found it advantageous to use oxygen.

The present experiments were undertaken to study in more detail the alkaline oxidation of *D*-glucose to determine more completely the products formed and thus to test the relative merits of the Nef and the Schmidt theories as to the mode of cleavage. Oxygen seemed to be the most desirable agent for this purpose because its action is relatively mild and hence fewer products were to be expected in the reaction mixture.

EXPERIMENTAL

Procedure

A shaking cabinet, maintained at a temperature of $30 \pm 1^{\circ}$ C. and equipped with a shaker set at 120 cycles/min., served for mixing the reaction material. The oxygen uptake was measured by means of a calibrated gas buret of 2000-ml. capacity which was connected to a leveling bulb. The top of the buret was connected by rubber tubing to the reaction vessel consisting of a Pyrex bottle with a stopcock sealed at the bottom. This permitted the removal of samples at intervals without opening the bottle. The bottle was closed by means of a Kjeldahl bulb fitted with a stopcock sealed on at one end to prevent the liquid from being shaken out into the connecting rubber tubing. In addition, the stopcock permitted the bottle to be evacuated without the reintroduction of air.

A measured volume of a potassium hydroxide solution was placed in the reaction bottle and shaken in the presence of oxygen for approximately 5 min. to saturate the solution and to allow it to reach the temperature of the cabinet. The weighed sugar sample was then quickly added and, without dissolving the sugar, the bottle was evacuated rapidly and immediately connected to a reservoir which contained the purified and dried commercial oxygen. As soon as the system became saturated, it was connected to the oxygen buret and the shaking was resumed for several minutes to dissolve the sugar. As soon as this had been effected, the shaking was stopped and an aliquot of the sugar solution was withdrawn through the stopcock at the bottom of the reaction vessel. At this point the level of the liquid in the oxygen buret was read by adjusting the meniscus to a level with the connecting leveling bulb. This gave the amount of oxygen in the system at zero time. At intervals, the level of the liquid in the buret was measured and the uptake of oxygen thus followed during the course of the oxidation of the sugar. Aliquots, which were withdrawn from the reacting solution after 0, 1, 4, 9, and 24 hours, were analyzed for total alkalinity, total aldose content, carbon dioxide, and formic acid. The oxygen consumption, as measured, was corrected for the aliquots withdrawn and calculated to standard conditions. In this apparatus it was convenient to use 160 ml. of a solution containing 0.067 moles of anhydrous glucose and one-fifth mole of potassium hydroxide. From the experience of others, three moles of alkali were used for each mole of sugar to allow for the neutralization of the acids produced and to furnish sufficient excess to produce enolization.

METHODS OF ANALYSIS

Carbon dioxide was determined by acidifying an aliquot of the oxidized solution and absorbing the liberated gas in a standard solution of sodium hydroxide. The carbonate was then precipitated with barium chloride and the excess alkali was backtitrated with a standard solution of hydrochloric acid.

Formic acid was determined on the aliquot used for the carbon dioxide determination by oxidation with mercuric acetate in acetic acid to carbon dioxide and water, according to the method of Reid and Weihe (7).

Total alkali was determined by pipetting an aliquot of the alkaline sugar solution into an excess of a standard solution of hydrochloric acid which checked further action of the alkali on the sugar. The excess acid was then back-titrated with a standard solution of sodium hydroxide.

The Goebel modification (3) of the Willstätter-Schudel hypoiodite titration gave satisfactory results when the following conditions were observed. To an alkaline aliquot of the solution was added 20 ml. of 0.1 N iodine; 1.5 equivalents of sodium hydroxide was added drop by drop with stirring over a period of 2-4 min. The mixture was allowed to stand for a total of 13-15 min., acidified, and the excess iodine determined.

Oxalic acid was determined by the permanganate titration of the calcium precipitate from a separate aliquot. Glycolic acid was found to be absent by the method of Rapoport (6).

Lactic acid was estimated by the Hillig (4) spectrophotometric method. Preliminary tests indicated that oxalic acid reduced the color intensity measured at 400 m μ . Consequently the oxalic acid was first removed as its calcium salt, after which the lactic acid was extracted with ether from an acid solution. The lactic acid was converted to its barium salt which was treated with a fresh solution of ferric chloride. The readings were compared with a reference curve obtained upon lactic acid similarly treated.

The benzimidazole derivative described by Moore and Link (5) was adapted to the quantitative determination of arabonic acid. While this derivative has been used to charaterize the aldonic acid, and is relatively easy to prepare, the yield is not quantitative. However, by standardizing the conditions used in its preparation, it was possible to obtain a constant yield $(92 \pm 2\%)$ of the derivative. Relatively large amounts of potassium chloride and potassium formate, which result from the neutralization of the oxidized sugar solution, were found to reduce the yield of the derivative to 56%. Consequently, the procedure was modified so that the benzimidazole derivative was formed from the lactone rather than from the potassium salt; by this procedure it was possible to separate the arabonic acid from interfering substances. For the analysis, 100 ml. of the final solution was brought to a pH of 1-2 with concentrated hydrochloric acid. The solution was reduced to dryness in vacuo at 60°C.; the residue was taken up in 5 ml. water and again dried *in vacuo*. The heating under vacuum was continued for an additional hour at 80°C. to remove the last traces of volatile materials and to produce the γ -lactone. The residue was extracted several times with small portions of absolute ethanol until no additional lactone was removed. The alcoholic extract was reduced in vacuo to a sirup which was dissolved in water and made up to 50 ml. A 10-ml. aliquot was used to prepare the benzimidazole derivative. For this purpose the procedure and the relative amounts of reagents as recommended by Moore and Link were carefully followed. The product was recrystallized (m.p. 234°C.) in the prescribed manner and weighed. A factor of 1.087, based upon the yield of 92%, was used to calculate the content of arabonic acid.

Each of the analytical methods employed in this study was separately tested in the presence of all possible products of the reaction to determine the extent of interference, if any. It was found that none of the compounds formed affected the accuracy of the determinations performed except in the case of analyses of arabonic and lactic acids, for which suitable modifications have been noted.

RESULTS

The rates of consumption of glucose and of potassium hydroxide as well as of the production of formic acid during a run of 24 hr. are illustrated in Fig. 1. The experiment was repeated under the same conditions. The analyses of the original and the final compositions of the two runs are presented in Table I, where headings A and B refer



FIG. 1. Oxidation of glucose.

to the duplicate determinations. The two experiments involved the oxidation of 9.81 and 9.79 g. of glucose, respectively, with the consumption in both cases of 1.393 l. (0.0622 moles) of oxygen. The results show a very satisfactory agreement; consequently, the data employed in the subsequent discussion will refer to the results obtained in the first run.

TABLE I

Summary of Results Obtained in the Oxidation of Glucose^a (Values are given in moles/liter)

	Original solution		Final solution		Difference	
	Ā	B	A	В	A	В
Reactants Glucose	0.418	0.416	0.016	0.016	0.402	0.400
Potassium hydroxide	1.321	1.370	0.413	0.473	0.908	0.897
Products						
Carbon dioxide	0.013	0.010	0.028	0.023	0.015	0.013
Formic acid	0.000	0.000	0.451	0.467	0.451	0.467
Oxalic acid	0.000	0.000	0.007	0.006	0.007	0.006
Lactic acid	0.000	0.000	0.042	0.045	0.042	0.045
Glycolic acid	0.000	0.000	0.000	0.000	0.000	0.000
Arabonic acid	0.000	0.000	0.318	0.322	0.318	0.322

^a The oxygen uptake, when corrected to standard conditions, was 1.393 l., or 0.0622 mole. The headings A and B refer to duplicate runs.

DISCUSSION OF RESULTS

As was anticipated, the major products formed are arabonic and formic acids. Arabonic acid was isolated as the benzimidazole derivative to the extent of 73.4% of the glucose consumed, which is in the order of yield obtained by Spengler and Pfannenstiel (*vide supra*) who isolated the acid. Our attention was then directed to the other products of the reaction.

By comparing the rate of production of formic acid with the rate of consumption of glucose (Fig. 1) it will be noted that the former deviates from the mole for mole ratio expected if the reaction were merely the splitting of one mole of formic acid from each mole of glucose. Thus after 1, 4, 9, and 24 hr. of oxidation, the difference between the glucose consumption and the formic acid production was 0.026, 0.026, 0.032,

TUDDD II

Consumption of Potassium Hydroxide by Glucose Oxidation Products

Constituent	Concentration <i>mole/l</i> .	Potassium hydroxide consumed by each mole/l.
Formic acid	0.451	0.451
Carbon dioxide	0.015	0.030
Oxalic acid	0.007	0.013
Lactic acid	0.042	0.042
Arabonic acid	0.318	0.318
Total		0.854

and 0.049 mole/l., respectively. Apparently a secondary reaction which also results in the production of formic acid is occurring simultaneously. Another indication is seen from the figures for the alkali consumption. The arabonic, formic, lactic, and oxalic acids, together with carbon dioxide, account (Table II) for 0.854 moles of base or approximately 94% of the total alkali neutralized. A residue of 0.054 moles of potassium hydroxide remains to be accounted for.

According to the Nef theory, formic and arabonic acids would be formed from glucose by cleavage and oxidation of the 1:2 enediol. The same products could result from glucose 3:4 enediol through a cleavage at the single bond between C-1 and C-2 (Schmidt's theory). However, the latter scheme would involve a loss of asymmetry of C-3 and C-4 so that p-ribonic, p-lyxonic, and p-xylonic acids would result, in addition to p-arabonic acid. Our results favor the Nef mode of splitting because all the pentonic acid determined was found to be *D*-arabonic acid.

Both theories postulate the possible formation of glycolic acid by enolization at different positions on the carbon chain, which might be further oxidized to oxalic acid. To test this, a sample of glycolic acid was subjected to an alkaline and oxygen treatment similar to that employed with glucose. No oxygen was consumed in the process and no oxalic acid was detected.

There is no reason to believe that one mechanism is involved to the exclusion of the other, and it is suggested that the formation of oxalic acid results from cleavages proposed by both theories. According to this hypothesis, most of the glucose is cleaved to formic and arabonic acids. The remainder undergoes a Schmidt cleavage to form two threecarbon units. A portion of this transient product rearranges in turn to glyceric aldehyde and finally to lactic acid. A smaller portion undergoes a Nef cleavage followed by oxidation to equivalent quantities of formic and oxalic acids.

Glyceric aldehyde is known to rearrange to lactic acid and it was shown that the conditions here employed led to this reaction upon a sirup of glyceric aldehyde prepared by the method of Witzemann (10).

Another degradation path of the sugar molecule should be considered. A portion of the five-carbon fragments from the cleavage of the glucose 1:2 enediol may rearrange to a pentose 1:2 enediol which would be expected to be oxidized to erythronic and formic acids. The erythronic acid was not identified but its formation can be accounted for as follows:

Calculated to the basis of 1 l., 0.402 mole of glucose was consumed. From 0.318 mole of this, an equivalent amount of arabonic acid was produced. A portion of the glucose is postulated as decomposing into glyceric aldehyde, part of which is further oxidized to oxalic and formic acids. The sum of the moles of oxalic and lactic acids produced is equal to twice the number of moles of glucose oxidized in this manner. From the data, this sum is 0.049 mole (0.042 mole lactic acid plus 0.007 mole oxalic acid), which corresponds to 0.025 mole of glucose. The remaining 0.059 mole of glucose can be postulated to yield 0.059 mole of erythronic acid. If erythronic acid is produced in the amount calculated, this would satisfactorily account for the last increment (0.054 mole) of potassium hydroxide consumed in the over-all reaction. In the same manner the reactions can account for the formic acid produced. Thus, 0.318 mole of formic acid is produced with the arabonic acid; 0.007 mole arises from a Nef cleavage of a three-carbon unit, an amount equivalent to the oxalic acid produced; and 0.118 mole (equivalent to twice the number of moles of erythronic acid) from a second

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Distribution of Oxygen Consumed by the Oxidation Products of Glucose

Constituent	Total amount produced mmoles	Oxygen consumed mmoles
Formic acid	65.7	32.9
Arabonic acid	46.4	23.2
Oxalic acid	0.94	0.94
Erythronic acid	8.62	4.31
Total		61.3 or 0.0613 mole

cleavage of the carbon chain. These fractions total to 0.443 mole; the amount of formic acid actually determined was 0.451 mole.

The balance sheet for the distribution of the oxygen consumed is given in Table III. For this computation it was expedient to employ absolute quantities of the compounds produced. Each is the sum of the

TABLE IV

Distribution of Carbon

Substance	Concentration <i>mole/l</i> .	No. C atoms	Gram-atoms C/l.
Formic acid	0.451	1	0.451
Oxalic acid	0.007	2	0.013
Lactic acid	0.042	3	0.126
Erythronic acid	0.059	4	0.236
Arabonic acid	0.318	5	1.590
Total			2.416

amounts removed for analysis during the run plus that of the final solution. On this basis the calculated consumption of oxygen would be 0.0613 mole; the experimental value was 0.0622 mole. Table IV shows that, upon the oxidation of 0.402 mole of glucose containing 2.412 g.-atoms of carbon, the resulting products account for 2.416 g.-atoms of carbon.

Constituent	Amount consumed or produced	Amount account	ted for
	moles/l.	Moles/l.	%
Potassium hydroxide	0.908	0.913	100.5
Formic acid	0.451	0.443	98.2
Carbon content	2.412	2.416	100.2
Oxygen	0.0622 (mole)	0.0613 (mole)	98.6

TABLE V Summary of Materials Accounted for

The formation of 0.059 mole/l. of erythronic acid has been postulated to account for the last small portion of the formic acid. On this postulate the calculated consumption of potassium hydroxide and of oxygen, together with the carbon content of the sugar consumed, are summarized in Table V and compared with the quantities experimentally determined. The agreement is very satisfactory and lends strong indirect support to the postulation that erythronic acid is produced.

SUMMARY AND CONCLUSIONS

Quantitative studies were made of the products from the action of potassium hydroxide and oxygen upon glucose. Standard methods were employed for the determination of the rate of uptake of the reactants and for the production of formic and oxalic acids. The Hillig colorimetric method for lactic acid was modified because the oxalate ion interfered. For the determination of p-arabonic acid, the benzimidazole derivative was prepared under empirical conditions which involved, at one stage, the preparation and separation from interfering substances of the γ -lactone. No glycolic acid was found, nor could this acid be oxidized under the conditions employed. However, it was shown that glyceric aldehyde would yield lactic acid. From the quantity of formic acid produced it was postulated that erythronic acid was formed; this compound was not isolated, but the quantities of oxygen, base, and carbon (from glucose) consumed are in keeping with its formation.

A mechanism is proposed which involves both the Nef and the Schmidt mode of cleavage of enediols. The chief products are formic and p-arabonic acids which could arise by a Nef cleavage of the 1:2 enediol. The formation of lactic and oxalic acids is best explained by a Schmidt cleavage of the same enediol to two glyceric aldehyde fragments, part of which could produce the lactic acid and part could undergo a Nef cleavage to formic and oxalic acids.

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