made up to 50 cc. in water gave $\alpha_D^{22} = -1.72°$ in a 2-dm. tube. Hence $[\alpha]_D^{22} = -50.3°$; $[M]_D = -223°$.

l-*iso*-**Diphenylhydroxy-ethylamine** was obtained from the pure *l*-B-*dl*-A salt and purified as described for the *d*-base. It melted at 115.2° (corr.) and had $[\alpha]_D^{22} = -125.6°$ in absolute alcohol; values in agreement with those of the *d*-base.

d-*iso*-**Diphenylhydroxy-ethylamine** *dl*-**Camphorsulfonate.**—For comparison this salt was made from the pure components. It is similar in all respects to the enantiomorphous *l*-B-*dl*-A salt.

Rotation (Hydrated salt). Subs., 1.0242: made up to 50 cc. in water gave $\alpha_D^{22} = +1.98°$ in a 2-dm. tube. Hence $[\alpha]_D^{22} = +48.2°$.

dl-*iso*-**Diphenylhydroxy-ethylamine** *dl*-**Camphorsulfonate.**—From the mother liquors from the separation of the *l*-B-*dl*-A salt there was obtained a much more soluble salt in masses of poorly formed prisms. It still showed a faint negative rotation. For comparison the pure salt was made from the pure components. It is soluble in about 26 parts of water at 25° and is hydrated. The anhydrous salt melts at 198–199° (corr.).

Anal. Subs., 3.6527: lost 0.1392 g. at 105°. Calcd. for $C_{24}H_{31}O_5NS \cdot H_2O$: H_2O, 3.88. Found: H_2O, 3.81.

The author is indebted to the University of Illinois for part of the chemicals used and for laboratory facilities during part of the work.

Summary

A second new method for the complete resolution of externally compensated compounds has been described in connection with a related method previously described. The method was applied to the resolution of inactive *iso*-diphenylhydroxy-ethylamine with *d*- and *dl*-camphorsulfonic acids.

NASHVILLE, TENNESSEE

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

THE MECHANISM OF CARBOHYDRATE OXIDATION. IX. THE ACTION OF COPPER ACETATE SOLUTIONS ON GLUCOSE, FRUCTOSE AND GALACTOSE[1]

BY W. L. EVANS, W. D. NICOLL, G. C. STROUSE AND C. E. WARING[2]

RECEIVED MAY 19, 1928 PUBLISHED AUGUST 4, 1928

The mechanisms proposed in some of the more recent literature dealing with the oxidation of carbohydrates in alkaline solutions rest on one or more of the following ideas: (*a*) that carbohydrates in alkaline solutions are converted into equilibrated systems consisting of a number of isomeric carbohydrates;[3] (*b*) that these carbohydrates give rise to a series of three

---

[1] Presented to the Second National Symposium on Organic Chemistry, Columbus, Ohio, December 29, 1927. References have been made in this communication to papers which have appeared subsequently.

[2] E. I. DuPont de Nemours Fellow, 1927.

[3] (a) De Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 156, 203 (1895); (b) **15**, 92 (1896); (c) **16**, 257, 262, 274 (1897); (d) **19**, 1 (1900); (e) **27**, 1 (1908); (f) Wolfrom with Lewis, THIS JOURNAL, **50**, 842 (1928).

enediols which form a restricted equilibrium;[4] (*c*) that the general equilibrium consisting of the components in (*a*) and (*b*) may be influenced by certain experimental factors, such as a change in both the temperature of the reaction and also the concentration of the alkali used.[5] Nef[6] states that these enediols do not exist in solutions of an alkalinity less than $N/30$, and that they begin to decompose in systems above an alkalinity of $N/20$. In view of the data obtained in this Laboratory with reference to the behavior of glucose, mannose and fructose toward aqueous solutions of potassium hydroxide, the question arises whether one or more of these enediols may not exist in alkaline solutions of even lower normality than $N/30$ and possibly in solutions of less P_{H} value than 7; that is, would the molecular mechanism involved in the oxidation of these sugars in an acid solution be in harmony with the concept which postulates the presence of enediols as intermediate substances in reactions of this character?

Experimental Part

The following is an account of the experimental methods used by us in this investigation.

Oxidizing Agent.—Copper acetate was chosen as our oxidizing agent by reason of the following distinct advantages. (*a*) A saturated solution of this salt is acid. (*b*) Since the cupric ion is reduced to the cuprous condition during the progress of the reaction, the cuprous oxide thus formed becomes an accurate measure of the oxygen consumed in the oxidation. (*c*) Although preliminary experiments showed that the rate of oxidation of fructose with copper acetate is greater than that of either glucose or galactose under the same conditions, yet the reaction proceeds slowly enough for the purposes of measurement and it may be stopped at any desired time. (*d*) Certain compounds formed in the oxidation of carbohydrates in acid solutions may escape detection when the same sugars are oxidized in alkaline solutions by reason either of their very rapid rearrangement to other compounds or a further oxidation of the intermediates. (*e*) The unused copper ion is easily removed from solution with hydrogen sulfide.

Oxidation Procedure.—Nine-tenths g. of either glucose, fructose or galactose was placed in a 250-cc. Florence flask containing 25 cc. of a copper acetate solution pre-

---

[4] (a) Nef, *Ann.*, **403**, 208–213 and note on 239 (1915); (b) compare Powell, *J. Chem. Soc.*, **107**, 1335 (1925), also Armstrong, "The Carbohydrates and the Glucosides," Longmans, Green and Co., **1924**, p. 59; (c) Anderson, *Am. Chem. J.*, **42**, 407 (1909); (d) Spoehr, *ibid.*, **43**, 241 (1910); (e) Upson, *ibid.*, **45**, 458 (1911); (f) Glattfeld, *ibid.*, **50**, 135 (1913); (g) Jensen and Upson, THIS JOURNAL, **47**, 3023 (1925); (h) Gustus with Lewis, *ibid.*, **49**, 1512 (1927).

[5] Evans and co-workers, *ibid.*, **47**, 3086 (1925); **48**, 2666 (1926); **50**, 1496 (1928); Amick, *J. Phys. Chem.*, **31**, 1473 (1927).

[6] Nef, *Ann.*, **403**, 224 (1915).

viously saturated at 50°. To this mixture an excess of copper acetate was added, the amount of it depending on the duration of the experiment. For all experiments of less than one hundred hours 10 g. was added, while 15 g. was sufficient for the longer periods. The flask was closed with a tightly fitting rubber stopper and was then shaken by means of a mechanical agitator in a thermostat kept at 50°.

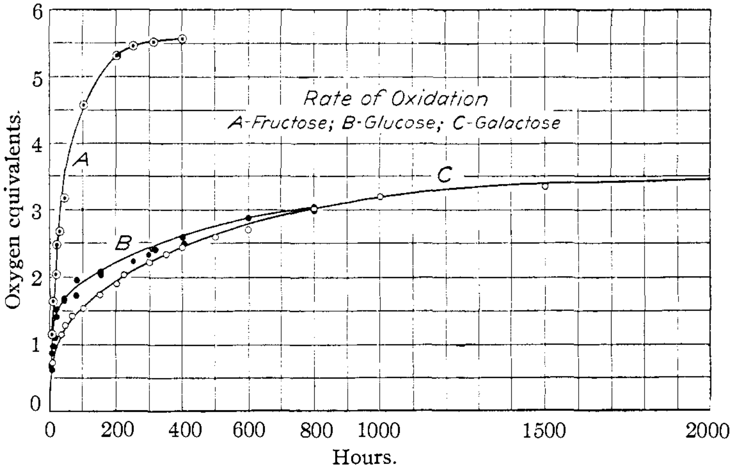


Fig. 1.—Rate of oxidation.

Determination of Consumed Oxygen.—When the sugar had been oxidized for the desired length of time, the flask was removed and cooled with tap water before taking out the stopper in order to avoid the loss of volatile products, for instance, formic acid. The cuprous oxide and unchanged copper acetate were collected in a weighed Gooch crucible, the excess of the acetate being removed by washing the residue with about 50 cc. of cold water. The crucible contents were dried in a vacuum oven at 70–80° without any appreciable oxidation of the cuprous oxide. The rate at which oxygen is used up by these carbohydrates in solutions of copper acetate at 50° is shown in Fig. 1. In Fig. 2, it is seen that the relation between the logarithm of the oxygen equivalents and that of the experimental period is a linear one.

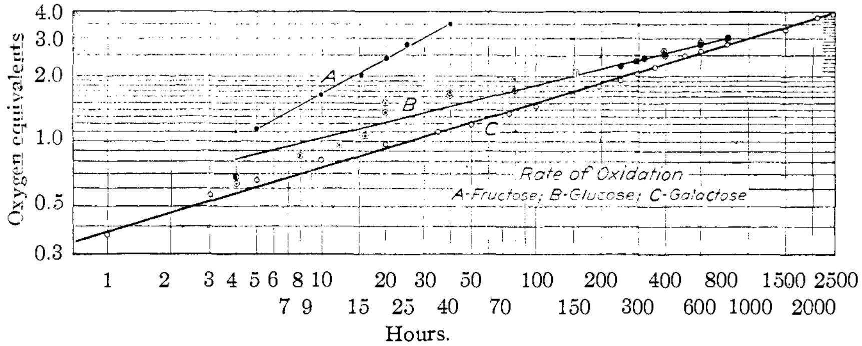


Fig. 2.—Rate of oxidation.

Reaction Products.—Our reaction mixtures were examined for oxalic, formic, glyoxylic and carbonic acids, and glucosone. The percentages of the hexose carbon converted into each of these compounds is expressed as a function of the time in Figs. 3, 4 and 5. The number of oxygen equivalents used per gram molecular weight of the hexose was calculated by dividing the weight of the cuprous oxide by the factor 0.716.

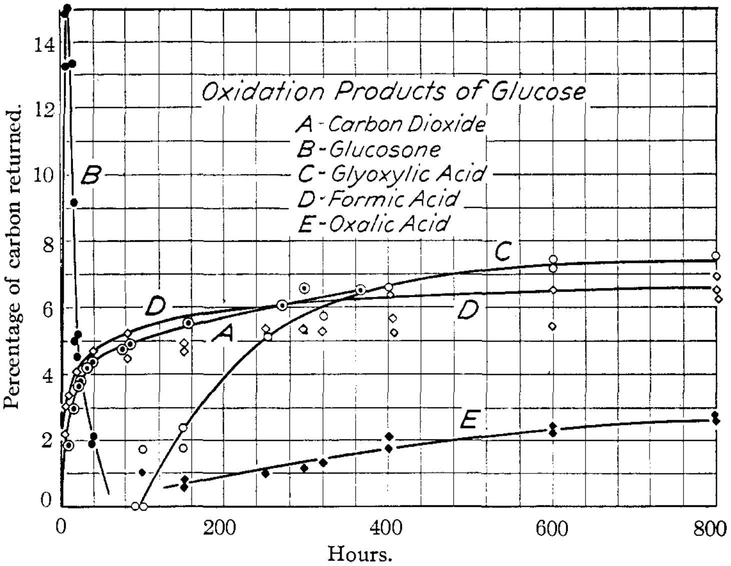


Fig. 3.—Oxidation products from glucose.

The percentages of the hexose carbon converted to the reaction products for which our mixtures were examined is expressed as a function of the oxygen equivalents in each case in Figs. 6, 7 and 8.

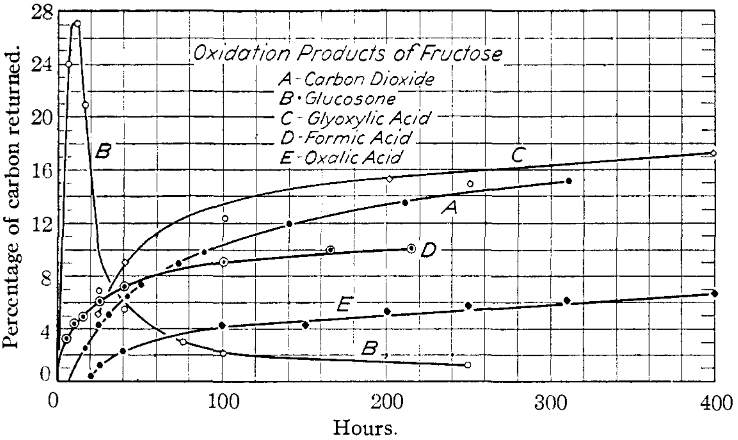


Fig. 4.—Oxidation products from fructose.

Oxalic Acid. (a) **Qualitative Identification.**—When the cuprous oxide and the unused solid copper acetate from the oxidations of longer duration had been filtered and washed with water, a greenish-white, scaly material was found associated with the residual cuprous oxide. From the work of McLeod[7] it was thought that this substance might be copper oxalate, although this investigator states that the cuprous oxide obtained by him contained no copper oxalate. Our suspected oxalate was soluble in dilute ammonium hydroxide solution. Its identity was established by its conversion to the calcium salt, the composition of which was determined by titrating with potassium permanganate in the presence of sulfuric acid, and also by converting a weighed portion dried *in vacuo* at 120° into the sulfate. This specimen of calcium oxalate contained 27.3% of calcium, while the monohydrate contains 27.43%. Furthermore, the oxalic acid present in the above ammoniacal solution was also converted into the highly insoluble phenylhydrazine oxalate.[8]

(b) **Quantitative Determination.**—The oxalic acid in these oxidations was determined quantitatively essentially as just described. The cuprous oxide residue was treated with 25 to 30 cc. of dilute ammonium hydroxide (1:19). By this procedure small amounts of the cuprous oxide as well as the cupric oxalate were dissolved. In all of the quantitative data throughout this report, the necessary corrections for the solubility of cuprous oxide have been made at the appropriate places. After acidifying the ammoniacal filtrate with acetic acid, the oxalic acid was precipitated as calcium oxalate, which was measured in the way described above.

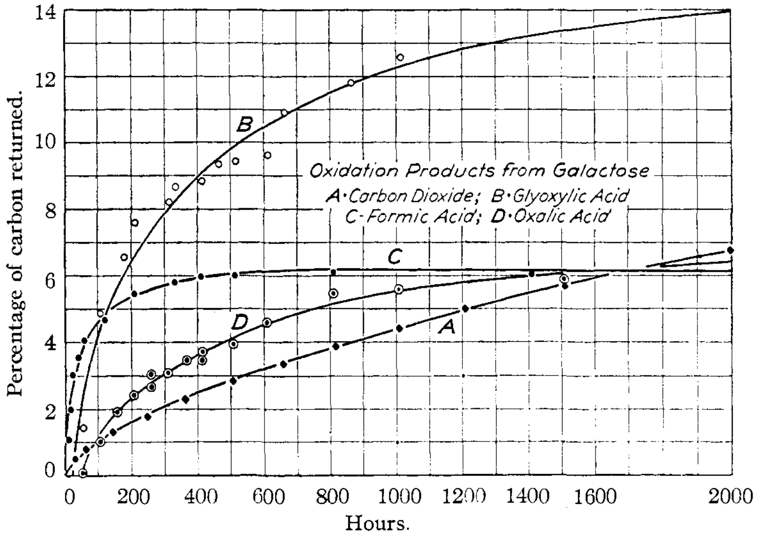


Fig. 5.—Oxidation products from galactose.

The formula given by Schaefer[9] for cupric oxalate is $CuC_2O_4 \cdot {}^1/_2H_2O$. This formula was used in determining the correction to be applied to the weight of the cuprous oxide obtained in our oxidation curves. The solubility of the oxalate, as determined by Schaefer by conductivity methods at 25°, is 0.02364 g. per liter. It was conclusively proved that all of the oxalic acid obtained in these oxidations was present as the in-

---

[7] McLeod, *Am. Chem. J.*, **37**, 20 (1907).

[8] Fischer, *Ann.*, **190**, 85 (1878). See Evans, Mong and Sinks, THIS JOURNAL, **39**, 1724 (1917).

[9] Schaefer, *Z. anorg. Chem.*, **45**, 310 (1905).

soluble oxalate, since the original filtrates showed no evidence of the presence of this acid.

Formic Acid.—The original filtrate obtained in the procedure described above under "Determination of Consumed Oxygen" was made copper ion-free in the cold by means of hydrogen sulfide. The addition of 1 g. of sodium chloride to this filtrate was found to be fairly effective in preventing the formation of colloidal cupric sulfide. After removing the copper sulfide by suction and washing with water, the filtrate usually had a volume of approximately 150 cc. The presence of formic acid was established by distilling a portion of this copper ion-free filtrate in a vacuum apparatus through which a small amount of natural gas instead of air was allowed to pass. This distillate was examined for formic acid by Fincke's method.[10] It was also found to decolorize dilute alkaline potassium permanganate solutions in the cold. Formic acid was determined quantitatively by using a modification of Jones' well-known method.[11]

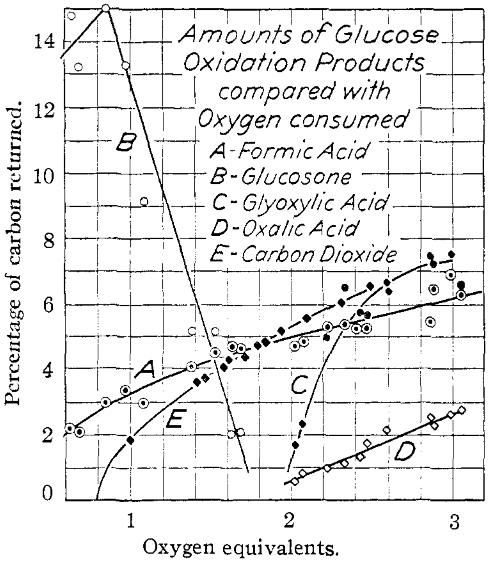


Fig. 6.

Glyoxylic Acid. Qualitative Identification.—(*a*) The solution obtained by the removal of the copper ions from the filtrate from the cuprous oxide, unused solid copper acetate and the copper oxalate resulting from the oxidation of 0.90 g. of fructose for 165 hours was evaporated to dryness, thus removing formic and acetic acids. On theoretical grounds, we had reason to think that glyoxylic acid should be one of the reaction products in these oxidations. The solution obtained by dissolving the residue resulting from this evaporation in 100 cc. of water was treated with a calcium acetate solution in the hope that calcium glyoxalate would precipitate. By reason of the known insolubility of copper oxalate, we were much surprised to learn that our fine, white precipitate was calcium oxalate. In the filtrate from this precipitate, there began a deposition of comparatively large crystals which were shown by microscopic examination to be different from calcium oxalate. A solution obtained by the same procedure from an experiment with 0.90 g. of fructose for 215 hours yielded almost 0.30 g. of these same crystals. When 0.2806 g. of the crystals, previously dried in a desiccator, was fumed with sulfuric acid, 0.1775 g. of calcium sulfate was obtained. This corresponds to 18.62% of calcium, while calcium glyoxalate dihydrate ($Ca(C_2HO_3)_2{\cdot}2H_2O$) contains 18.02% of calcium. In a similar procedure, 0.90 g. of these crystals was obtained from the 200, 300 and 400 hour experiments with galactose. On analysis these were found to contain 18.35% of calcium. When the solution from a 200-hour experiment with galactose was treated with barium acetate instead of calcium acetate, a white and fairly crystalline compound

---

[10] Fincke, *Z. Nahr. Genussm.*, **21**, 1 (1911); **22**, 88 (1911); see Bender, *Bur. of Chem. Bull.*, **162**, 78 (1912).

[11] Jones, *Am. Chem. J.*, **17**, 539 (1887); see Evans, Edgar and Hoff, THIS JOURNAL, **48**, 2667 (1926).

was obtained after twenty-four hours' standing. When filtered immediately after precipitation and dried at 100°, this substance had a barium content of 49.9% when fumed with sulfuric acid. An air-dried specimen obtained by slow precipitation was found to contain 47.06% of barium. Anhydrous barium glyoxalate contains 48.48% of barium, the monohydrate 45.59%, and the oxalate monohydrate 56.54%. (*b*) Positive identification of glyoxylic acid was made as follows. After the original filtrates from a 200-hour experiment with fructose and a 300-hour experiment with galactose had been made copper ion-free, they were treated without previous distillation with 3 cc. of phenylhydrazine. If the slow-forming precipitate contained glyoxylic acid phenylhydrazone, then according to Fischer's[12] observations, the latter should be soluble in alkali and be reprecipitated on treatment with hydrochloric acid. When so treated the lemon-yellow colored precipitate was found to melt at 143° (uncorr.). Fischer gave 138°, Pechmann[13] found 143–145°.

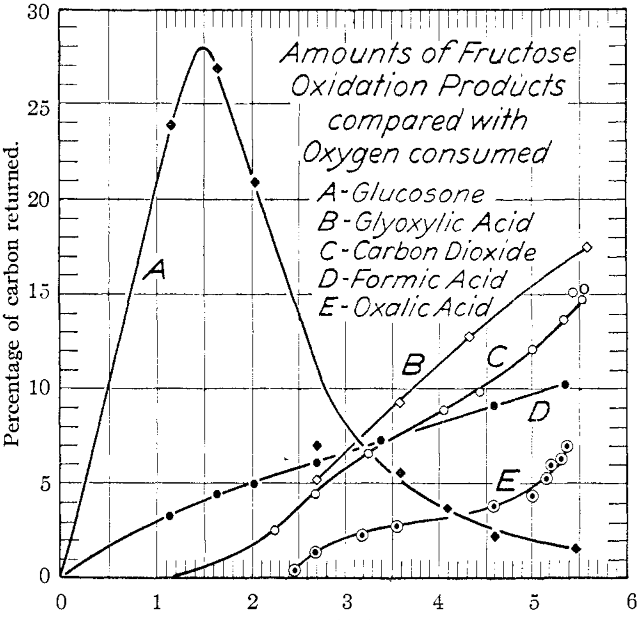


Fig. 7.

Qualitative Identification of Glucosone.—When the original filtrates from the glucose and fructose oxidations of five to ten hours' duration were made copper ion-free and were then treated with phenylhydrazine in the cold, glucosazone was immediately precipitated. This behavior corresponds with that of glucosone, which is known to form the osazone in the cold immediately. A control test on glucose and fructose under similar conditions showed that it took several hours for the osazone to precipitate. No galactosone was found in our experiments.

Presence of Unchanged Glucose and Fructose.—If the glucosazone formed on standing at room temperature for about an hour is filtered, and the resulting filtrate is

---

[12] Fischer, *Ber.*, **17**, 577 (1884).

[13] Pechmann, *Ber.*, **29**, 2163 (1896).

heated at 100° for an hour, the amount of precipitated glucosazone is so large that it can hardly be accounted for on the ground that it resulted only from the osone still remaining in the solution.

Determination of Glyoxylic Acid, Glucosone and Unchanged Carbohydrate.—The following method was adopted for the estimation of glyoxylic acid, glucosone and unchanged carbohydrate. The solution which had been made copper ion-free was made up to 100 cc. and then treated with 3 cc. of phenylhydrazine. The mixture was allowed to stand at room temperature for three hours. Parallel experiments with fructose and glucose showed no reaction during this time.[14] At the end of this period, the osazone was filtered, allowed to remain in contact with 25 cc. of a saturated sodium carbonate solution for two or three hours, again filtered, washed with water, dried *in vacuo* at 60° and weighed. About 25 cc. of cold benzene was allowed to percolate through the glucosazone in the crucible, which was again dried and weighed. This weight was considered as that of the osazone of glucosone. The sodium carbonate extract was now acidified with hydrochloric acid, which caused a precipitation of any glyoxylic acid phenylhydrazone which might have been present. This yellow-colored precipitate was transferred to a Gooch crucible, dried in a vacuum oven and weighed. From this weight the amount of glyoxylic acid formed in these oxidations was calculated. Fischer[15] states that phenylhydrazine will detect one part of glyoxylic acid in 300 parts of water.

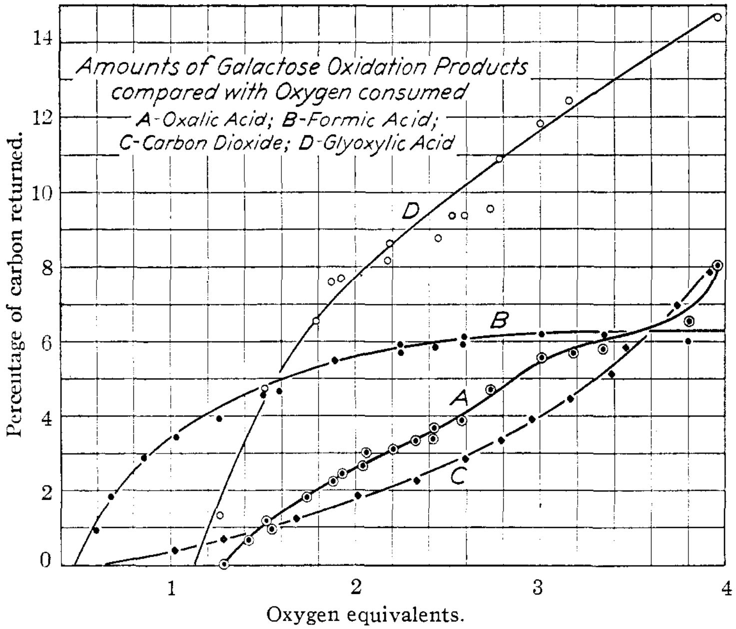


Fig. 8.

The filtrate from the above osazone was now heated on a boiling water-bath for an hour, after which it was cooled in an ice-bath and filtered. After treatment with sodium carbonate, the residue was washed, dried and weighed as described above, in order to determine the osazone of the unchanged sugar. In all but one case it was found that all

---

[14] See Morrell and Crofts, *J. Chem. Soc.*, **75**, 787 (1899).

[15] Fischer, *Ber.*, **17**, 577 (1884).

of the glyoxylic acid phenylhydrazone was completely removed in the first precipitation with phenylhydrazine. The osazone thus obtained was regarded as that of the unoxidized carbohydrate. The first melting points of the osazones prepared in this way were usually low, 185–190°, but percolation of the crucible contents with two crucible volumes of cold benzene to remove traces of tar resulted in obtaining a pure product, m. p. 206–208°. Previous tests on pure glucosazone had shown that the loss in weight on treatment with cold benzene was well within the limits of error of our method.

With reference to the accuracy of the separation of glucosone and unchanged sugar, our method leaves much to be desired, but every attempt was made to make it relatively accurate. Morrell and Crofts[14,16] found that both glucose and glucosone were precipitated only very incompletely by phenylhydrazine. From this it is to be concluded that our glucosone was not completely precipitated in the cold and that the remainder would be found with the osazone of the unchanged sugar. However, the formation and the partial estimation of the glucosone present as a reaction product, together with the very accurate measure of the oxygen consumed, have aided us very greatly in the interpretation of the mechanism involved in the oxidation of these carbohydrates with copper acetate solutions. Mr. E. F. Schroeder, of this Laboratory, is at present studying the properties of glucosone.

Carbon Dioxide.—During these oxidations gas pressure developed within the reaction flasks. The gas was found to be carbon dioxide. In some cases, especially that of fructose, the pressure became so great that the stoppers were blown from the flasks with considerable force. The apparatus used for the quantitative determination of carbon dioxide consisted of a sufficiently large flask (500–1000 cc.), closed with a stopper fitted with an inlet tube for the admission of carbon dioxide-free natural gas, an outlet tube attached to a small Liebig condenser inclined at an angle of 45° and a mercury seal through which a mechanical stirrer passed. The condenser was connected to two ordinary gas washing bottles arranged in series. These contained barium hydroxide solution. To the outlet of the last wash bottle a soda lime tube was attached. The natural gas used to sweep the carbon dioxide to the absorption train was passed very slowly through a large soda lime tower attached to the inlet tube of the reaction flask. All stoppers were coated with shellac. The reaction flask was immersed in the thermostat. The following oxidation charges were used for the carbon dioxide determinations in these experiments. (*a*) Eighteen grams of glucose was dissolved in 500 cc. of copper acetate solution saturated at 50°. To this solution, 300 g. of solid copper acetate was added, the total reaction mixture being contained in a 1000-cc. Pyrex flask. (*b*) Nine grams of fructose and 27 g. of galactose were used, the other agents and size of reaction flask being in the same ratio as that given for glucose. The barium carbonate formed in the absorption train was removed by filtration through a porous Alundum crucible, then thoroughly washed with recently boiled water, dried in a vacuum oven and weighed. The crucible was freed from the barium carbonate with dilute acetic acid, carefully washed with water, dried and weighed. The difference between the two weights was regarded as that of barium carbonate.

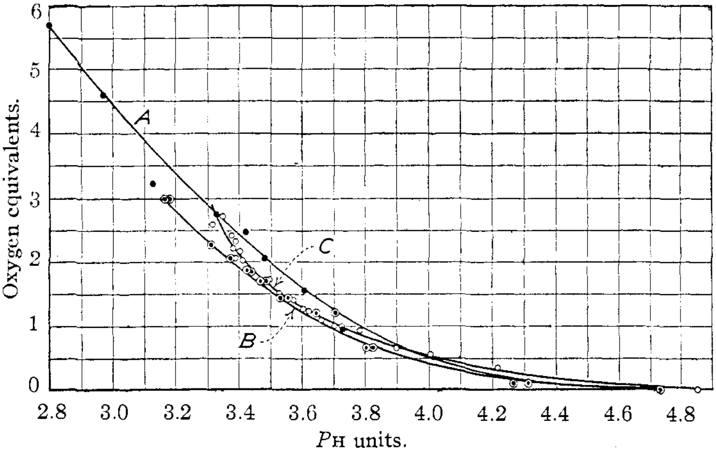
Hydrogen Ion Concentration.—Preliminary work had shown that the hydrogen electrode could not be used for this work because the platinized platinum electrode soon became coated with copper. However, the quinhydrone electrode gave satisfactory results if the temperature was not too high.[17] We used two general methods in actual practice. (*a*) In one, 3.6 g. of glucose was dissolved in saturated copper acetate

---

[16] See Fischer, *Ber.*, **21**, 2632 (1888).

[17] (a) Mathews, *Trans. Faraday Soc.*, July 6 (1925); (b) Eucken, Jette and LaMer, "Fundamentals of Physical Chemistry," McGraw-Hill Book Company, New York, **1925**, p. 396.

solution. The reaction mixture was kept under exactly the same conditions as described for the oxidation procedure. At suitable intervals 5cc. samples were withdrawn and the voltages measured with the saturated calomel electrode and the quinhydrone electrode, using the standard potentiometric method. (*b*) Our second method, employed with fructose and galactose, consisted in using individual reaction mixtures for each reading. In both cases the P_H values were calculated from the equation $P_H = 7.68-(E/0.059)$, in which E is the observed voltage.[18] The change in the hydrogen ion concentration throughout these oxidations is shown in Fig. 9.



A, fructose; B, glucose; C, galactose.

Fig. 9.—Change of hydrogen ion concentration.

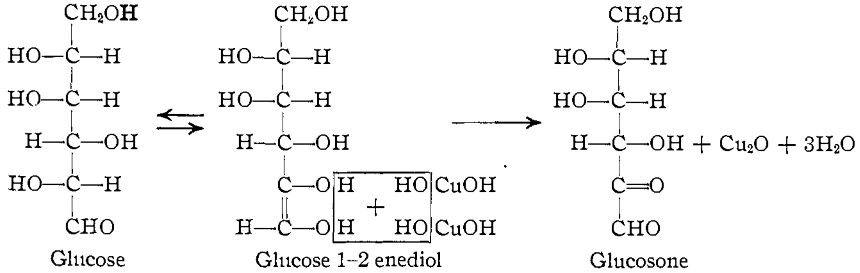
Theoretical Part

Glucosone.—It has been pointed out above that one of the main objectives in these experiments was to determine whether the general principles underlying the mechanism of carbohydrate oxidation in alkaline solutions were sufficient to explain the course of such oxidations in acid solutions. Our experimental data show that 13.25% of the glucose carbon and 23.83% of that of fructose were recovered from the reaction mixture of each carbohydrate as glucosone at the end of the first five-hour period. These facts and the relative rate of formation of the hexosone from these two sources are shown in Fig. 10. Galactosone was not detected in the reaction mixtures of galactose. Its absence may have been due to its being oxidized at a greater velocity than that at which it was formed.[19] The presence of glucosone in these reactions is easily understood on the assumption that the common 1–2 hexose enediol of glucose and fructose is present in these acidic solutions, and that this enediol is oxidized in accordance with the following reactions.

---

[18] LaMer and Rideal, This Journal, **46**, 224 (1924).

[19] See Morrell and Crofts, *J. Chem. Soc.*, **77**, 1220 (1900).



Such an explanation would regard the enediol as being an acid. In addition to the well-known behavior of these hexoses in alkaline solutions of varying concentrations, the following evidence seems to support this point

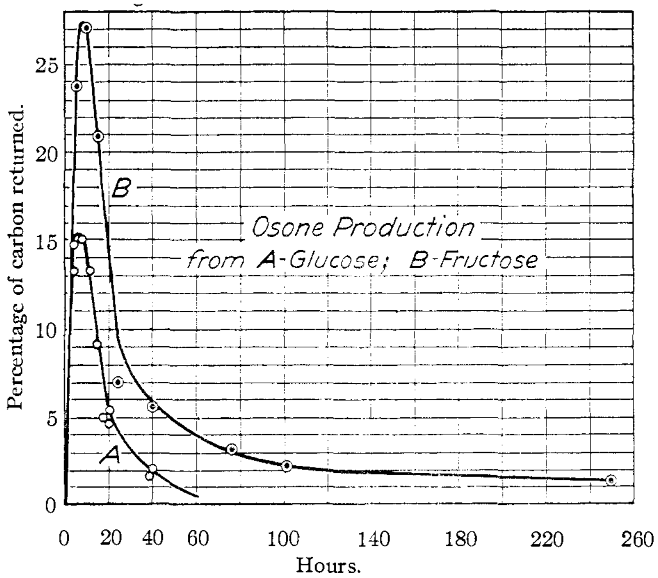


Fig. 10.—Osone production.

of view. (*a*) Michael and Rona[20] determined the dissociation constants of some very weak acids, particularly the carbohydrates, and found that the value for fructose was 8.8×10^{-13}, for glucose 6.6×10^{-13} and for galactose 5.3×10^{-13}. (*b*) Powell[21] has shown both by conductivity methods and also by the change of rate in the hydrolysis of ethyl acetate in the presence of fructose and glucose that these carbohydrates form definite compounds with the base, and in the case of sodium hydroxide the reaction takes place in the ratio of one molecule of each. (*c*) Kling[22] found that

[20] Michael and Rona, *Biochem. Z.*, **49**, 232–248 (1913); *C. A.*, **7**, 2715 (1913).

[21] Powell, *J. Chem. Soc.*, **107**, 1335 (1915).

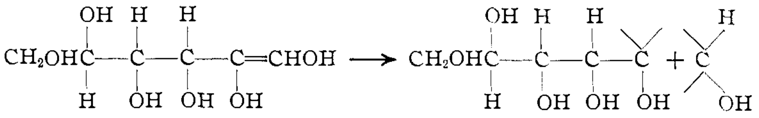
[22] Kling, *Compt. rend.*, **140**, 1256 (1905); see Evans and Hoover, THIS JOURNAL, **44**, 1739 (1922).

acetol gives an acid reaction when dissolved in water and that the acidity returns even after neutralization. Acetol may undergo the following reaction

$$\mathrm{CH_3COCH_2OH} \rightleftharpoons \mathrm{CH_3C(OH){=}CHOH}$$

This observation of Kling with reference to the behavior of acetol toward aqueous solutions of alkalies suggests an analogous behavior in the carbohydrates, especially fructose. (*d*) Wolfrom and Lewis[23] have shown recently that the P_H value of one liter of lime water to which had been added one mole of *d*-glucose is changed from 12.6 to 10.6. In a separate experiment it was established that gluconic acid is not oxidized with copper acetate under the same experimental conditions as those employed with fructose, glucose and galactose. The same observation was made with galactonic acid lactone. Therefore, it is clear that these oxidations proceed by some mechanism other than through the formation of these acids.

Existence of Hydroxymethylene. Formic Acid.—If the hexose-1,2-enediol of these carbohydrates exists in these solutions, the presence of formic acid as an oxidation product shows that the common 1,2-enediol of fructose and glucose and that of galactose are suffering decomposition according to the following equation.



$$\mathrm{CH_2OH}\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\mathrm{C}}{=}\mathrm{CHOH} \longrightarrow \mathrm{CH_2OH}\underset{\mathrm{H}}{\overset{\mathrm{OH}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}-\underset{\mathrm{OH}}{\overset{\diagdown\diagup}{\mathrm{C}}} + \underset{\diagup\diagdown\mathrm{OH}}{\overset{\diagdown\diagup\mathrm{H}}{\mathrm{C}}}$$

(Galactose-1,2-enediol) (Lyxose—active form) + (Hydroxymethylene)

The active form of the pentose and that of formaldehyde may be oxidized to the pentonic and formic acids, respectively. This interpretation is strongly supported by the recent convincing experiments of Gustus and Lewis on the oxidation of 2,3,4,6-tetramethyl glucose.[4h] The percentages of the hexose carbon converted to formic acid through oxidation with copper acetate is expressed as a function of the time in Fig. 11.

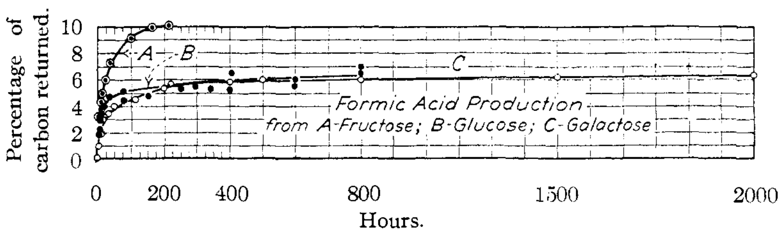


Fig. 11.—Formic acid production.

The postulated existence of hydroxymethylene as an intermediate product in these carbohydrate oxidations made it necessary to ascertain

---

[23] Wolfrom with Lewis, This Journal, 50, 842 (1928).

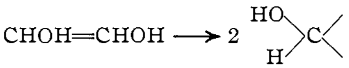
whether, if formed, it could be oxidized with copper acetate directly to formic acid. In order to obtain information on this very important point, we carried out the following experiments.

(a) **Hydroxypyruvic Acid and Copper Acetate.**—The action of hydroxypyruvic acid toward copper acetate was first studied. It was prepared by the following modification of Wills'[24] method. A solution of 40 g. of collodion in 160 g. of ether added to 500 cc. of 10% sodium hydroxide solution was allowed to stand for thirty-six hours. The solution obtained by acidifying the final lower layer of this mixture was evaporated to 200 cc., boiled with animal charcoal, made just alkaline with ammonium hydroxide and then treated with lead acetate. After filtration, the thick, white precipitate was suspended for an hour in water through which a current of air was passing to remove any free ammonia. A portion of the light yellow filtrate obtained after decomposing the suspended white precipitate with hydrogen sulfide gave an osazone which melted at 200–201° without purification. Wills found 205° for the pure substance. Aliquot portions (25 cc.) of a 200cc. solution containing 0.1544 g. of hydroxypyruvic acid were treated with 2 g. of solid copper acetate in the thermostat at 50° for 360 hours. The mixtures gave positive reactions when examined qualitatively for formic acid by the methods of Fincke and Bender. Another reaction mixture containing 0.193 g. of hydroxypyruvic acid was found to contain 0.0482 g. of cuprous oxide (1.81 oxygen equivalents per one g.m.w. of hydroxypyruvic acid), and 0.0162 g. of formic acid (6.66 cc. of 0.1 N $KMnO_4$). The carbon dioxide evolved in one sample gave 0.0365 g. of barium carbonate (0.008 g. of CO_2). If hydroxypyruvic acid should undergo the following reaction in the presence of copper acetate

$$CH_2OHCOCOOH \longrightarrow CH_2OHCHO + CO_2$$

the glycol aldehyde thus formed should in turn undergo an enolization to ethenediol and this tautomer then subsequently decompose according to the following equations

$$CH_2OHCHO \rightleftharpoons CHOH{=}CHOH$$



$$CHOH{=}CHOH \longrightarrow 2\ {}^{HO}_{H}\!\!>\!C\!<$$

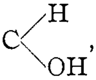
two molecules of formic acid and one molecule of carbon dioxide should be found in the reaction products. Our experimental data are compared with the demands of these assumptions in the following table.

	$CH_2OHCOCOOH$ used, g.	Oxygen equivalents	CO_2	HCOOH
Found	0.193	1.81 (90.5%)	0.008	0.0162 (95%)
Theoretical	.193	2.00	.008	.0171

---

[24] Wills, *Ber.*, **24**, 401 (1891).

(b) **Glycol Aldehyde and Copper Acetate.**—Dihydroxymaleic acid (3.4 g.) was converted into glycol aldehyde according to the directions of Nef,[25] a procedure which should yield 1.2 g. of the desired compound. In this experiment we removed the carbon dioxide with carbon dioxide-free natural gas instead of air. On treating 25 cc. of a 200cc. aqueous solution of this product with *p*-nitrophenylhydrazine hydrochloride and sodium acetate, 0.5058 g. of the hydrazone was obtained,[26] thus showing that our solution contained 0.736 g. of the aldehyde instead of 1.2 g. Since the hydrazone is somewhat soluble, this figure may be a little too low. Six 25cc. portions of our standard solution were treated with 2 g. of solid copper acetate and agitated on the thermostat at 50°. Their distillates, prepared as described above, were examined for formic acid. Cold alkaline potassium permanganate and Fincke's test gave positive reactions. Denigès' test for hydroxy acids gave only a faint response. A quantitative determination showed that 25% of the aldehyde had been converted into formic acid. Neither of the final reaction mixtures in (*a*) and (*b*) reduced Fehling's solution nor did they affect Schiff's reagent. (*c*) In this connection we confirmed the observations of McLeod with reference to the fact that aqueous solutions of formaldehyde do not reduce copper acetate.[27] This investigator used a temperature of 100°, while ours was 50°.

From these experimental results it is clear that *the active form of formaldehyde must be hydroxymethylene.* Baly[28] in his lecture on "Photosynthesis" said, "In view of the remarkable reactivity of activated formaldehyde it seems to me that it is necessary to picture a different formula from that given to ordinary formaldehyde. This abnormal activity is well expressed by the Nef formula,  $C\begin{matrix}\diagup H\\ \diagdown OH\end{matrix}$, with the carbon atom bivalent." Nef himself had given expression previously to a similar opinion in the statement,[29] "Die physiologischen Wirkungen des Formaldehyds beruhen zweifellos auf einer Dissociation desselben in Oxymethylen." ("The physiological activities of formaldehyde doubtless depend upon a dissociation of it into oxymethylene.")

We feel that our experimental results given above in (*a*), (*b*) and (*c*) are convincing evidence for this general point of view, and that the hydroxymethylene obtained from these sugars must be oxidized to formic acid under the conditions of our experiments.

---

[25] Nef, *Ann.*, **376**, 40 (1910). The recent experiment of Fischer, Taube and Baer, *Ber.*, **60**, 479 (1927), appeared after our work was completed. (W. L. E.)

[26] Wohl and Neuberg, *Ber.*, **33**, 3108 (1900).

[27] McLeod, *Am. Chem. J.*, **37**, 50 (1907); see Zimmerli, *Ind. Eng. Chem.*, **19**, 524 (1927).

[28] Baly, *The Rice Institute Pamphlet*, **12**, 93 (1925).

[29] Nef, *Ann.*, **298**, 303, reference 147 (1896).

Carbon Dioxide.—Carbon dioxide was being evolved from galactose in decreasingly smaller amounts at the end of 2000 hours and in similar amounts from glucose and fructose at the end of 366 hours and 315 hours, respectively. Our quantitative data show that this gas begins to appear as one of the reaction products of fructose and glucose at about the same time that the glucosone production has reached a maximum. In view of this fact, we concluded that α-ketogluconic acid was being formed by the direct oxidation of the osone, and that this compound was undergoing decomposition into carbon dioxide and arabinose. Since α-ketogluconic acid is unknown as a stable substance,[30] hydroxypyruvic acid was chosen for our study as being representative of hydroxy-α-keto acids. This acid under the same experimental conditions decomposes with an evolution of carbon dioxide, as pointed out above. As the time of oxidation of these carbohydrates is increased, it is seen from Figs. 3, 4 and 5 that the glucosone gradually is being used up. The percentage of the hexose carbon being converted to carbon dioxide is expressed as a function of the time in Fig. 12. It is seen that fructose is the most sensitive of the three sugars to copper acetate. Ruff found that gluconic acid on being oxidized with bromine was converted into α-ketogluconic acid. In our experiments with fructose and glucose we found no derivative of α-ketogluconic acid. By the following special experiments it was conclusively proven that carbon dioxide was not a decomposition product of the following compounds.

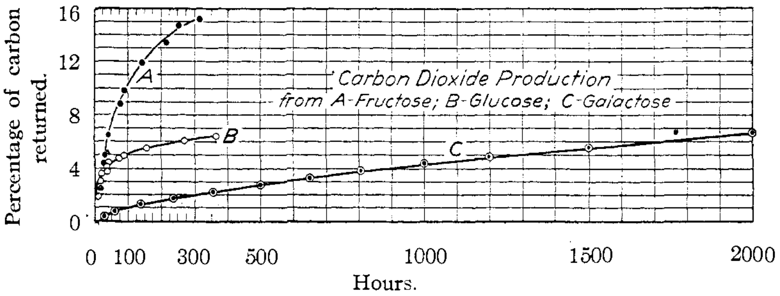


Fig. 12.—Carbon dioxide production.

(a) **Galactonic and Gluconic Acids.**—One gram of gluconic acid and of galactonic acid lactone were each treated for one week at 50° with copper acetate. Only a trace of cuprous oxide was formed. This was probably due to some slight impurity. The solid copper acetate disappeared and the solution took on the well-known dark blue color.

(b) **Glyoxal.**—No trace of cuprous oxide was found when glyoxal was treated with copper acetate.

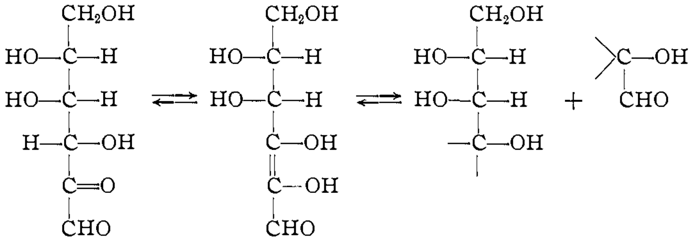
---

[30] Ruff, *Ber.*, **32**, 2269 (1899).

(c) **Glycolic Acid.**—This acid gave the merest trace of reduction.

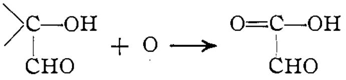
(d) McLeod made the same kind of observations with formic acid, formaldehyde, glycolic acid and oxalic acid.

Hexosone-2,3-enediol. Glyoxylic, Oxalic and Glycolic Acids.—(*a*) The hexosones formed in these oxidations may also undergo an enolization. The hexosone-2,3-enediol thus formed may then split at the double bond, thus giving rise to a tetrose and glyoxal. These changes may be represented by the following reactions.



Glucosone Glucosone-2,3-enediol Active form of erythrose Half active form of glyoxal

Although Fischer[31] does not record the nature of the insoluble lead compound of glucosone which he prepared by the interaction of lead hydroxide and the hexosone, it is quite probable that it is a lead salt of the hexosone-2,3-enediol. No compounds were formed in our reaction products which indicated that the hexosone-2,3-enediol itself had undergone oxidation with copper acetate. (*b*) In quite the same manner as hydroxymethylene is oxidized to formic acid, so also is the half active form of glyoxal converted to glyoxylic acid according to the following reaction.



$$\begin{matrix} >C{-}OH \\ | \\ CHO \end{matrix} + O \longrightarrow \begin{matrix} O{=}C{-}OH \\ | \\ CHO \end{matrix}$$

In connection with this point of view concerning the half active form of glyoxal, it is of importance to emphasize that glyoxal itself is unacted upon by copper acetate under the experimental conditions of this work. The oxalic and glycolic acids obtained in these experiments are undoubtedly derived from the glyoxylic acid, a compound which Debus[32] observed would rearrange into these two acids. He also noticed that its calcium salt was converted into calcium oxalate and glycolate in the presence of lime water. Debus furthermore observed that glyoxylic acid does not reduce copper acetate. In solutions of copper acetate containing this acid, we found that copper oxalate was being formed without the formation of any cuprous oxide. With reference to the decomposition of the hexosone-2,3-enediol, it is of much interest to note that Morrell and Crofts[33]

[31] Fischer, *Ber.*, **21**, 2631 (1888); **22**, 87 (1889).

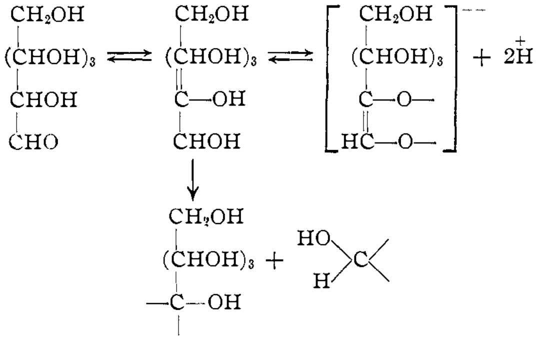
[32] Debus, *Ann.*, **338**, 336 (1905).

[33] Morrell and Crofts, *J. Chem. Soc.*, **81**, 67 (1902).

oxidized glucosone with bromine and isolated the erythronic acid so formed as the calcium salt.

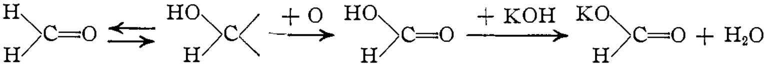
Hydrogen Ion Concentration.—It has been pointed out previously that an increase in the alkali normality of aqueous solutions of the hexoses and potassium hydroxide causes a shifting in the equilibrium existing between the three isomeric enediols in the direction of the 3,4-form. The relation existing between the amounts of lactic acid formed from these sugars and the alkali normality favors this point of view, as do also the character and amounts of oxidation products obtained when these aldohexoses are oxidized with alkaline potassium permanganate. The observations of Amick concerning the influence of alkalinity on the copper number of glucose is especially significant in this connection. Therefore, it is clear that an increase in the hydrogen ion concentration of the oxidizing mixtures containing copper acetate should have the opposite tendency and that the degree of ionization of these enediols should be diminished through the mass action effect of the common ion. The recent work of Wolfrom with Lewis on the de-enolization of an equilibrated mixture obtained from 2,3,4,6-tetramethyl glucose supports this point of view.

The equilibrium represented by the following equation would be shifted to the left by an increase in the hydrogen ion concentration.

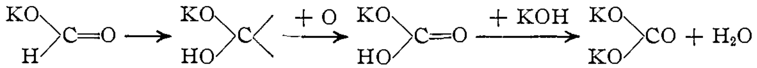


Since this would tend to diminish the concentration of the hexose-1,2-enediol, it follows that the amount of hydroxymethylene would diminish accordingly. This would result in the yield of formic acid being a decreasing function of the time. That this is so is seen in Fig. 11. That the oxidation of the hexose to a hexosone still continues after the cessation of formic acid is evidenced by the fact that glyoxylic and carbonic acids are still forming. In this connection it is of interest to point out that the oxidation of these hexoses with aqueous solutions of copper sulfate proceeds very much more slowly than with copper acetate. Furthermore, when copper acetate solutions are treated with highly ionized acids, the oxidation of the sugars practically stops. We found no evidence for the oxidation of the hexosone-2,3-enediol, since phenylhydrazine did not yield a trihydrazone.

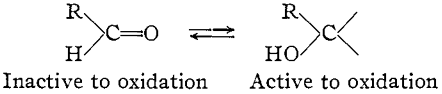
Applying this point of view to the following equation it is seen that alkaline oxidizing agents should yield alkali metal formates.



The conversion of formates into carbonates in alkaline potassium permanganate oxidations must be preceded by a further enolization before oxidation takes place. These changes may be represented by the following equation.



In this general interpretation it is obvious that the ionization of hydroxymethylene after the same manner as the sugar enols might be considered, but we have assumed that the monose is probably oxidized instantly to formic acid. From this discussion one must conclude that the *direct* oxidation of aldehydes in general seems to involve an equilibrium condition in which a certain portion of the aldehyde molecules is in the activated condition. This may be expressed by the following equation



In harmony with this point of view with reference to the experiment of Upson and Jensen in which these investigators obtained only 0.5 g. of gluconic acid from 200 g. of glucose is the observation of Amick to the effect that "enolization must occur before oxidation."

Summary

1. Aqueous solutions of fructose, glucose and galactose have been oxidized with copper acetate at 50° for the purpose of ascertaining whether the general principles underlying the mechanism of carbohydrate oxidation in alkaline solutions are sufficient to explain the course of such oxidations in acid solutions. The advantages in the use of copper acetate are outlined.

2. The reaction mixtures were examined for glucosone, formic, carbonic, glyoxylic and oxalic acids. The presence of glycolic acid was proved qualitatively but it was not measured quantitatively. The quantities of these are expressed both as functions of the time and the oxygen consumed. The oxygen consumed by each sugar is expressed as a function of the time. The hydrogen ion concentration is expressed as a function of the oxygen consumed.

3. The hexose-1,2-enediols are regarded as being oxidized to the

hexosones. The glucosone is obtained as the osazone. The galactosone was not obtained as a reaction product. It is probably used up as rapidly as it forms.

4. The formic acid arises from the oxidation of the hydroxymethylene obtained by the decomposition of the hexose-1,2-enediols. That hydroxymethylene will undergo this change was shown by a study of the behavior of copper acetate toward glycol aldehyde and hydroxypyruvic acid.

5. It is thought that carbon dioxide arises from the decomposition of the unstable α-ketohexonic acids. Hydroxypyruvic acid loses a molecule of carbon dioxide when oxidized with copper acetate.

6. The hexosones may form hexosone-2,3-enediols, which are thought to decompose into the active form of the tetrose (tetrose methylenol) and glyoxal (half active form). The latter is oxidized to glyoxylic acid, which in part is converted into oxalic and glycolic acids. Glyoxal does not reduce copper acetate solutions, hence it is thought that the glyoxylic acid comes from an activated form (glyoxal-half-methylenol).

7. The increase in the hydrogen ion concentration tends to reverse the equilibrium between the enediols in the direction of suppressing enediol formation. This would lead to a reduction in the formic acid yields with increasing time. The increase in the hydrogen ion concentration would tend to suppress the enolization of the hexosone-2,3-enediol. This harmonizes with the fact that no derivatives were found of 2,3-diketohexoses when the reaction mixtures were treated with phenylhydrazine.

8. The view is expressed that aldehydes exist in two forms, those active toward oxidizing agents and those not. The former are derivatives of methylenols; that is, the hydrogen is bound to oxygen and the carbon is bivalent. In the inactive molecules the carbon is tetravalent. An equilibrium exists between these two forms which may be shifted by a change in the hydrogen ion concentration. The conversion of aldehydes to carbonates in alkaline solutions is explained on this basis.

COLUMBUS, OHIO