exert a specific influence on its activity, such as the nitrate and chloride ions, is intermediate between the activity of the enzyme in the presence of each taken separately. The activity of the enzyme in the presence of such a mixture of salts is dependent on the relative concentrations of the ions. Anions which alone do not influence the activity of the enzyme seem to have comparatively small influence on the activity of the enzyme in the presence of anions which exert a decidedly specific influence.

Reducing the concentration of enzyme by one-half or using 4% instead of 2% starch does not appreciably influence the concentration of sodium chloride necessary for complete activation of the enzyme or the optimal hydrogen-ion activity.

The presence of neutral salt is essential to the activity of pancreatic amylase. The influence exerted by different salts appears to be very specific.

The anion is far more influential than the cation, although the latter seems to have some slight influence on the activity of the enzyme.

Of the ions studied, chloride is the most efficient ion and the salts may be placed in the following order in their influence on the hydrolysis of starch by pancreatic amylase: sodium and potassium chlorides, lithium chloride, sodium bromide, sodium nitrate, sodium chlorate and sodium sulfocyanate, sodium fluoride. Sodium sulfate and phosphate were found to be without influence on the activity of pancreatic amylase.

A consideration of the influence of the different anions on the activity of pancreatic amylase indicates that these have specific effects on the enzyme or substrate.

NEW YORK CITY

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

THE MECHANISM OF CARBOHYDRATE OXIDATION. X. (a) THE ACTION OF POTASSIUM HYDROXIDE ON MANNOSE. (b) A COMPARISON WITH THAT OF GLUCOSE AND FRUCTOSE

BY WILLIAM LLOYD EVANS AND DAVID CHARLES O'DONNELL[1]

RECEIVED JUNE 29, 1928 PUBLISHED SEPTEMBER 5, 1928

It has been shown[2] that glucose, fructose, mannose and galactose may be oxidized with alkaline potassium permanganate solutions into carbon dioxide, oxalic acid and small amounts of acetic acid. At lower alkali normalities and at a temperature of 25°, it was observed that the oxalic acid–carbon dioxide ratios obtained from both glucose and galactose were not of the same value. However, as the normality of the alkali was in-

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[1] Read at the Detroit Meeting of the American Chemical Society, September 6, 1927.

[2] (a) Evans and co-workers, THIS JOURNAL, **47**, 3085 (1925); (b) Evans and Buehler, *ibid*, **47**, 3098 (1925).

creased in both cases, there seemed to be a tendency for the amounts of oxalic acid and carbon dioxide derived from these two carbohydrates to become more nearly the same. As the temperature of these oxidations was increased, this tendency toward identical yields became so marked that at 75° it was found that the amounts of these reaction products were practically the same. In the case of glucose, mannose and fructose at 50°, and at the lower alkali normalities, there were observable differences in the oxalic acid–carbon dioxide ratios, but these differences tended to disappear in the region of higher alkali normalities. At 75°, the yields of reaction products from glucose, fructose and galactose were practically the same. It has been suggested that alkaline solutions of the carbohydrates contain equilibrated systems which are easily influenced by a change in these experimental factors—a condition which ought to give rise to the differences observed in the amounts of the reaction products obtained in these oxidations.

By reason of this suggestion, it has become of much interest to study the behavior of these carbohydrates toward aqueous solutions of potassium hydroxide of various concentrations and at different temperatures for the purpose of observing whether the same general tendencies observed in the presence of oxidizing agents would also be observed in their absence. The general methods employed in this phase of the problem have been given in much detail in previous papers from this Laboratory. Because the earlier results on glucose and galactose and the more recent ones on fructose[3] have been reported, the two principal purposes in this study were these: (*a*) to learn if the behavior of mannose toward changes in alkali concentration and temperature were the same as those to be expected on the basis of the above suggestion; (*b*) to make a comparative summary of the results obtained by us and other workers[4] in this Laboratory to see if, by reason of the structural relations, certain predicted similarities in the behavior of the mannose–glucose–fructose group of hexose sugars could be detected.

Experimental Part

Chemicals.—The same precautions were taken with reference to the degree of purity of all reagents and chemicals used throughout this work as were observed in the previously reported experiments in this series of carbohydrate studies.[4]

Treatment of Mannose with Potassium Hydroxide.—The procedure adopted in the treatment of mannose with aqueous solutions of potassium hydroxide of appropriate normality was exactly the same as that used by Evans and Hass[5] in their work on glyceric aldehyde.

The amount of hexose sugar used in each experiment was 2.25 g. This was dissolved in 25 cc. of potassium hydroxide solution of the desired normality.

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[3] Evans and Hutchman, THIS JOURNAL, **50**, 1496 (1928).

[4] Evans, Edgar and Hoff, *ibid.*, **48**, 2665 (1926).

[5] Evans and Hass, *ibid.*, **48**, 2703 (1926).

Determination of Formic, Acetic and Lactic Acids.—Formic, acetic and lactic acids were identified and determined by methods previously described. By reason of some later work on fructose, we redetermined the amount of lactic acid obtained from glucose and potassium hydroxide solutions.

Determination of Pyruvic Aldehyde Osazone.—Owing to the fact that mannose differs from the sugars previously studied in this series, in that it forms an insoluble phenylhydrazone, the method for determining the amounts of pyruvic aldehyde osazone had to be modified as follows. The general procedure used in the case of fructose by Evans and Hutchman[3] was followed in every detail in our experiments with both mannose and glucose. It was found necessary to separate the pyruvic aldehyde osazone from the insoluble mannose phenylhydrazone by treating the mixture with acetone, a reagent in which the pyruvic aldehyde osazone was found to be completely soluble. The acetone solution was evaporated to dryness, the residue being washed with 30% alcohol, dried in a vacuum oven at 65° and then weighed. The osazone thus obtained was identified by its melting point (144–146°) and by the color test with sulfuric acid, a reagent which causes it to give an olive green color, which changed into a slate blue and finally to a violet.

Qualitative Reactions.—The reaction mixtures were qualitatively examined at appropriate places during the progress of a given experiment for hydroxy acids, acetol and pyruvic acid.[4]

Our quantitative data have been expressed in the form of graphs.

Theoretical Part

Mannose and Potassium Hydroxide.—The views[2a,4] which have been used in the interpretation of the results obtained from the alkaline oxidation of certain carbohydrates and also those resulting from the behavior of aqueous solutions of potassium hydroxide toward these same sugars are summarized at this point. (*a*) The classical experiments of Lobry de Bruyn and Alberda van Ekenstein[6] show that mannose, as well as either glucose or fructose, gives rise to an equilibrated system which may be represented by the following

$$d\text{-Mannose} \rightleftarrows d\text{-Glucose} \rightleftarrows d\text{-Fructose} \rightleftarrows d\text{-Pseudo-fructose} \rightleftarrows$$
$$d\text{-}\alpha\text{-Glucose} \rightleftarrows d\text{-}\beta\text{-Glucose}$$

The equilibria arising from the use of each of these three sugars taken individually are not quantitatively identical.[7,6b] (*b*) Through an elaboration of the views of Wohl and Neuberg,[8] and Fischer,[9] Nef[10] concluded from the results of oxidation experiments that mannose would yield a system of enediols in alkaline solutions which he represented by the following reaction.

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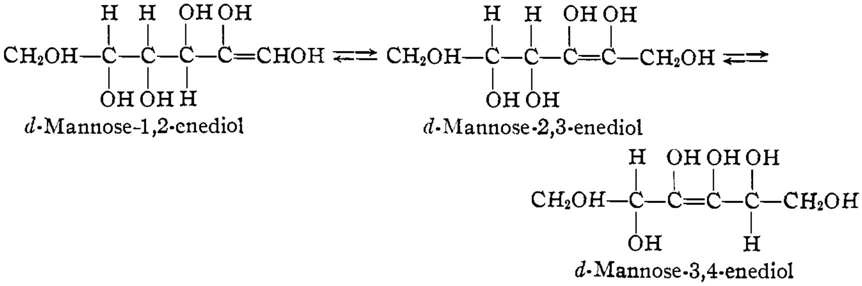
[6] de Bruyn and van Ekenstein, (a) *Rec. trav. chim.*, **14**, 164 (1895); (b) **14**, 203, 214 (1895); (c) **16**, 257 (1896); (d) compare Nef, *Ann.*, **403**, 239, note (1914).

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

[8] Wohl and Neuberg, *Ber.*, **33**, 3099 (1900).

[9] Fischer, *Ber.*, **28**, 1149 (1895).

[10] Nef, *Ann.*, **403**, 208–213 (1914).



$$CH_2OH-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\overset{OH}{C}=CHOH \rightleftharpoons CH_2OH-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\overset{OH}{C}=\overset{OH}{C}-CH_2OH \rightleftharpoons$$

d-Mannose-1,2-enediol *d*-Mannose-2,3-enediol

$$CH_2OH-\underset{OH}{\overset{H}{C}}-\overset{OH}{C}=\overset{OH}{C}-\underset{H}{\overset{OH}{C}}-CH_2OH$$

d-Mannose-3,4-enediol

It is clear that glucose and fructose should also yield the same enediols as does mannose. When these enediols are ruptured at their respective double bonds, the resulting systems will still be qualitatively identical. The fission products of such an action will give rise to an extension of the above system through the formation of other enediols containing a smaller number of carbon atoms. In the presence of appropriate oxidizing agents, these bivalent carbon atom intermediates will be oxidized to their respective acids. Therefore, it follows that the alkaline oxidation of mannose, glucose and fructose should yield products which are *qualitatively* the same but not necessarily *quantitatively* so. This point of view is supported by the literature in this field of investigation. (*c*) In offering an explanation for the quantitative differences observed in the oxidation of these well known carbohydrates in alkaline potassium permanganate solutions, the suggestion was made that these reactions are best understood when it is assumed that a more general equilibrium, consisting of reactions (*a*) and (*b*), is present in the alkaline solutions of these sugars. Changes in the experimental factors, such as concentration of alkali and temperature, should give rise to changes in an equilibrium of this kind which, in the presence of oxidizing agents, would become evident through a variation in the *amounts* of the reaction products formed. Furthermore, it was pointed out that should these alkaline solutions give rise to irreversible equilibria, then it is probable that the speed of the slowest reaction in the series of changes taking place must be the factor that is influenced by the two experimental conditions, temperature and alkali concentration.

Mannose-1,2-enediol. Formic Acid.—When mannose-1,2-enediol is ruptured at the double bond, the active forms of formaldehyde and arabinose are produced. Formic acid should be found in alkaline solutions in which such an action takes place. Our results are shown in Fig. 1. It is obvious that the amounts of formic acid are a function of the alkali normality and the temperature employed. This behavior of mannose is in complete accord with that of glucose and fructose when these carbohydrates are studied under exactly the same experimental conditions. Upson[11]

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[11] (a) Power and Upson, THIS JOURNAL, **48**, 195 (1926); (b) Jensen and Upson, *ibid.*, **47**, 3019 (1925).

and his collaborators found that formic acid is a product of the oxidation of glucose in the presence of calcium hydroxide and also when Soldiani's reagent is used. These investigators also isolated the arabonic derivatives in each case. Gustus and Lewis[12] have shown that 2,3,4,6-tetramethyl-*d*-glucose yields formic acid when it is oxidized with alkaline hydrogen peroxide solutions. There are many similar cases in the literature.[13]

Mannose-3,4-enediol. (a) **Pyruvic Aldehyde.**—(I) When one molecule of the 3,4-enediol common to mannose, glucose and fructose is ruptured at its double bond, two molecules of the active form of glyceric adehyde are produced. In the absence of oxidizing agents, the glyceric aldehyde so formed will then give rise to the equilibrated system represented by the following reaction.

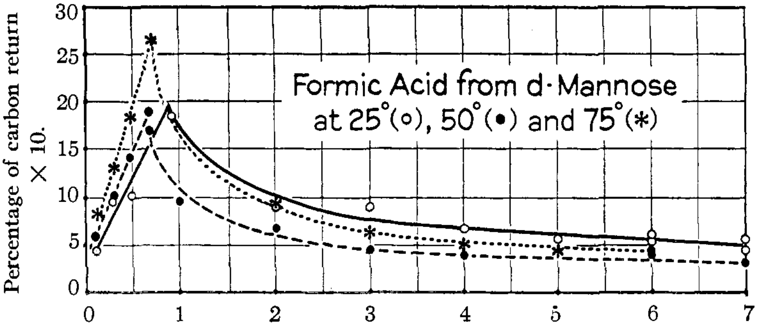
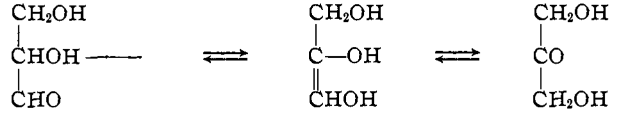


Fig. 1.



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CHO \end{array} \rightleftharpoons \begin{array}{l} CH_2OH \\ | \\ C{-}OH \\ \| \\ CHOH \end{array} \rightleftharpoons \begin{array}{l} CH_2OH \\ | \\ CO \\ | \\ CH_2OH \end{array}$$

Glyceric aldehyde Triose enediol Dihydroxy-acetone

It has been shown previously that pyruvic aldehyde is formed when glyceric aldehyde and dihydroxy-acetone are treated with aqueous solutions of potassium hydroxide. The amounts of the aldehyde thus formed are a function of the alkali concentration, the temperature and the speed with which it is converted into lactic acid. If glyceric aldehyde is present as a fission product in alkaline solutions of mannose and glucose, then it follows that these two carbohydrates should also give rise to pyruvic aldehyde whose amounts would be dependent upon the same factors as those

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[12] Gustus with Lewis, This Journal, **49,** 1512 (1927).

[13] (a) Cross, Bevan and Smith, *J. Chem. Soc.*, **73,** 463 (1898); (b) Meisenheimer, *Ber.*, **41,** 1009 (1908); (c) McLeod, *Am. Chem. J.*, **37,** 20 (1907); (d) Anderson, *ibid.*, **42,** 401 (1909); (e) Spoehr, *ibid.*, **43,** 227 (1910); (f) Glattfeld, *ibid.*, **50,** 163 (1913).

observed when the aldehyde is obtained from either glyceric aldehyde or dihydroxy-acetone. This was found to be the case. Our results are shown in Figs. 2 and 3. (II) According to the demands of the theory, alkaline solutions of all the hexose sugars should show exactly the same qualitative behavior as that of the trioses in this respect because the active form of glyceric aldehyde is a fission product of the 3,4-enediol, a tautomer which is common to all of this series of carbohydrates. In addition to the pyruvic aldehyde data which we have obtained from mannose and glucose in these experiments, we have assembled for the purposes of comparative study all similar data obtained in this Laboratory that have a

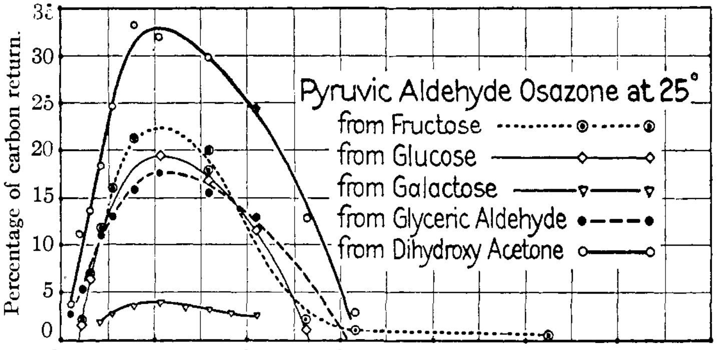

Fig. 2.

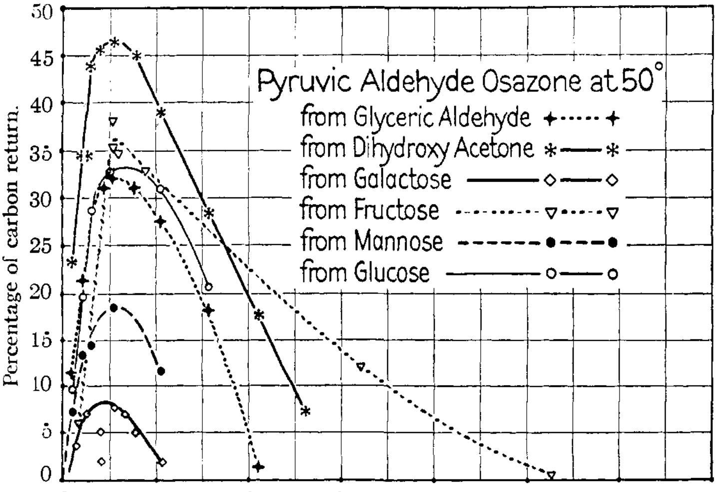

Fig. 3.

bearing on this point in carbohydrate chemistry. It has been shown previously that alkaline solutions of fructose and galactose, during a period of forty-eight hours as well as glucose for ninety-six hours, give rise to the formation of pyruvic aldehyde. (III) Owing to the fact that glucose, mannose and fructose do not form identical equilibria at the temperatures used, it was to have been expected that the yields of pyruvic aldehyde osazone from this series of sugars should not be the same. It does not follow from this fact, however, that there may not be experimental conditions under which this might not be true. At the only temperature beyond 50°, namely, 75°, at which work on this point has been carried out, it has been found that tarry materials seriously interfere with the demands of precise work. From the data in Figs. 2 and 3, it is seen that the maximum yields of pyruvic aldehyde osazone derived from the glucose, fructose and mannose at 25° (except mannose) and 50°, occur at approximately 1.0 *N* and 0.50 *N* alkali, respectively. This is also true for galactose. It is a very significant fact that both glyceric aldehyde and dihydroxy-acetone also yield their maximum return of pyruvic aldehyde osazone at these two temperatures and at practically the same alkali normality as do these hexose sugars—an observation which seems to us to be most convincing evidence for the theoretical views expressed above to the effect that the hexose carbohydrates in alkaline solutions of appropriate

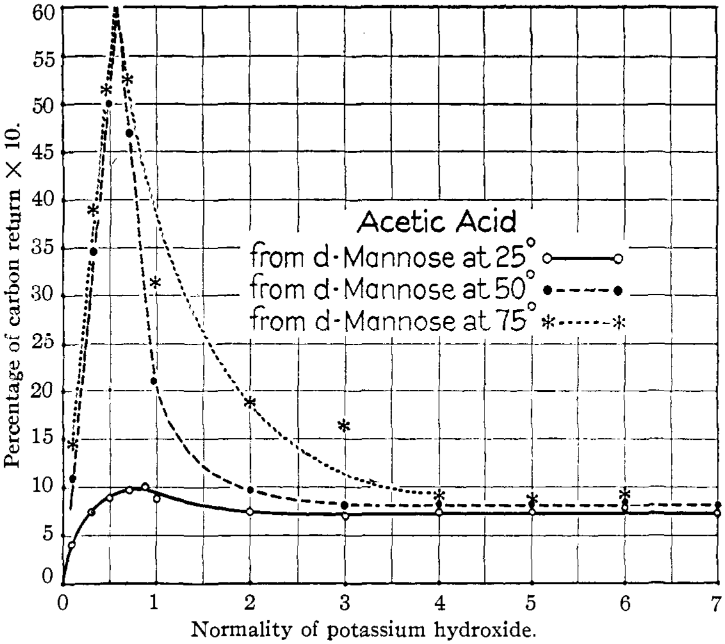

Fig. 4.

normality will give rise to glyceric aldehyde as an intermediate compound in the formation of pyruvic aldehyde, which, in turn, is converted into lactic acid. Although we had no other hexose sugars at our disposal, it may be safely predicted that the other carbohydrates of this series will also show the same behavior. (Dr. D. C. O'Donnell and Karl B. Nordstrom of this Laboratory are at present studying the pentose sugars from this point of view.)

(b) **Acetic Acid.**—In previous papers it was pointed out that the acetic acid and a portion of the formic acid obtained from the carbohydrates thus far studied must arise from the action of the potassium hydroxide on the dissociation products of pyruvic aldehyde shown in the following reaction

$$CH_3COCHO \longrightarrow CH_3CHO + CO$$

The results obtained with mannose (Fig. 4) are of the same general character as those obtained previously with glucose and fructose.

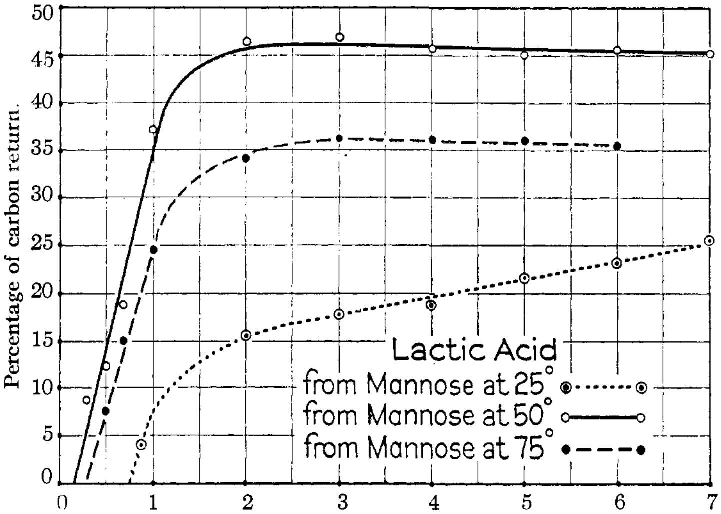

Fig. 5.

(c) **Lactic Acid.**—In the action of aqueous solutions of potassium hydroxide on mannose, as well as its action on fructose and glucose, it has been observed that a maximum point of production was found for both acetic and formic acids. As previously pointed out,[4] this must be due to the fact that pyruvic aldehyde at that alkali normality and temperature is rearranging to lactic acid with the same speed that it is dissociating after the manner of the above equation. When the speed of rearrangement of the aldehyde to lactic acid becomes greater with the increased alkali normality, the yields of acetic and formic acids should decrease accordingly and that of the lactic acid should increase. Our results on lactic acid from mannose and glucose are shown in Figs. 5 and 6, respectively. They are essentially of the same general character as those obtained previously with fructose. In all three cases the amounts of lactic acid obtained at 75° are less than those obtained at 50°, a fact which is probably due to the pronounced tar formation at the higher temperature. In this connection it should be noted that we did not examine these tars for the presence of saccharinic acids.

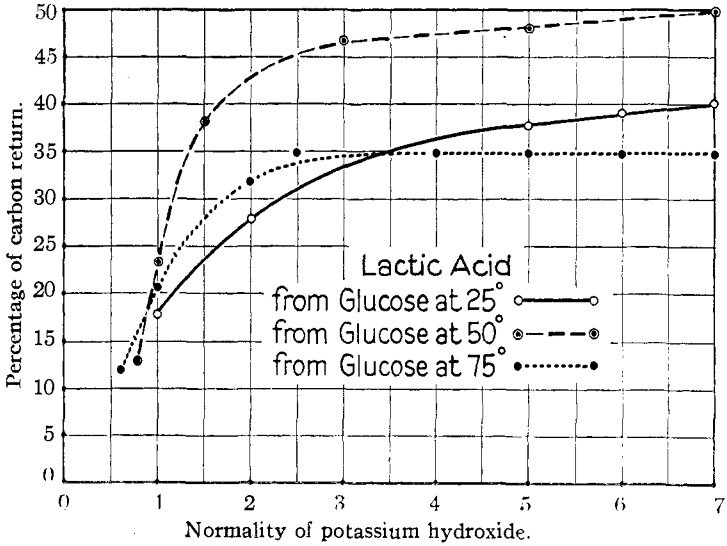

Fig. 6.

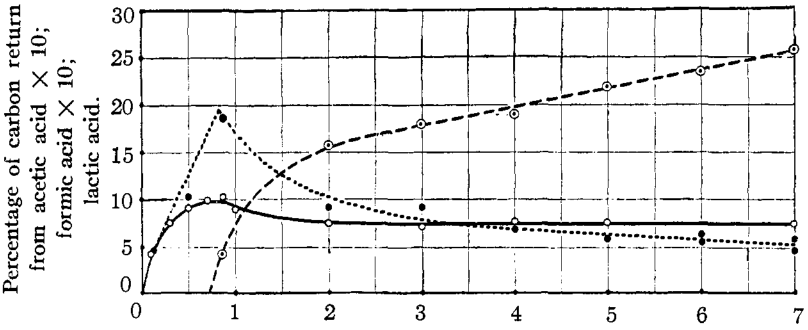

Fig. 7.—Production relation of acetic acid, ○; formic acid, ●; and lactic acid, ⊙ from mannose at 25°.

In the pyruvic aldehyde osazone curves (Figs. 2 and 3), the maximum yield in each case is regarded as the point at which the velocity of rearrangement of pyruvic aldehyde to lactic acid is just equal to that of the osazone formation at that temperature and alkali concentration. According to the explanation given above for the sources of pyruvic aldehyde, acetic, formic and lactic acids, the same interdependence should exist in the yields of these compounds from mannose as was observed previously with fructose.[3] This is shown in Figs. 7 and 8. For the purposes of this comparative study we have assembled in Figs. 9 and 10 our lactic acid and pyruvic aldehyde osazone results from glucose and also those for acetic and formic acids obtained by Evans, Edgar and Hoff from the same carbohydrate. It is thus seen that this same interdependence is found in this sugar also. The fact that this same interdependence which is common to mannose, glucose and fructose is also present in the trioses, seems to us most convincing evidence for the point of view expressed above with reference to the important role played by pyruvic aldehyde when these three carbohydrates are acted upon with aqueous solutions of potassium hydroxide. It may be safely predicted that all the hexose sugars will show this important interdependence in the yields of pyruvic aldehyde, lactic, acetic and formic acids.

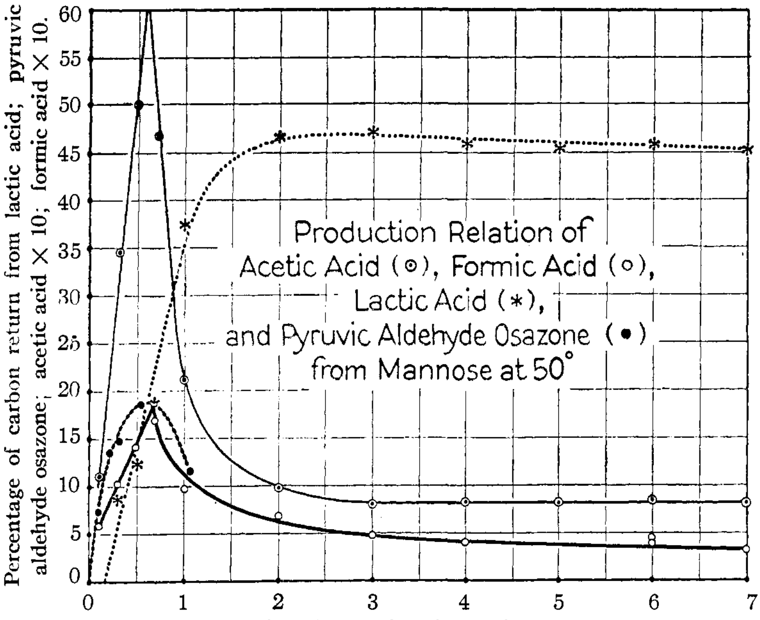

Fig. 8.

Influence of Alkali Concentration and Temperature on Mannose, Glucose and Fructose.—It has been shown that the temperature and the alkali concentration exercise a marked effect on the products obtained in the oxidation of these carbohydrates with potassium permanganate. The quantitative differences observed at the lower alkali normalities and at the lower temperatures have been attributed to the fact that these three sugars taken individually do not give rise to identical equilibria. However, in the alkaline oxidation of fructose and glucose with potassium permanganate at 75°, it was found that the amounts of oxalic acid and carbon dioxide obtained from these two carbohydrates were practically the same. This must have been due to the fact that both experimental factors, the alkali concentration and the temperature (or some temperature between that and 50°) influenced the general equilibrium in each case to

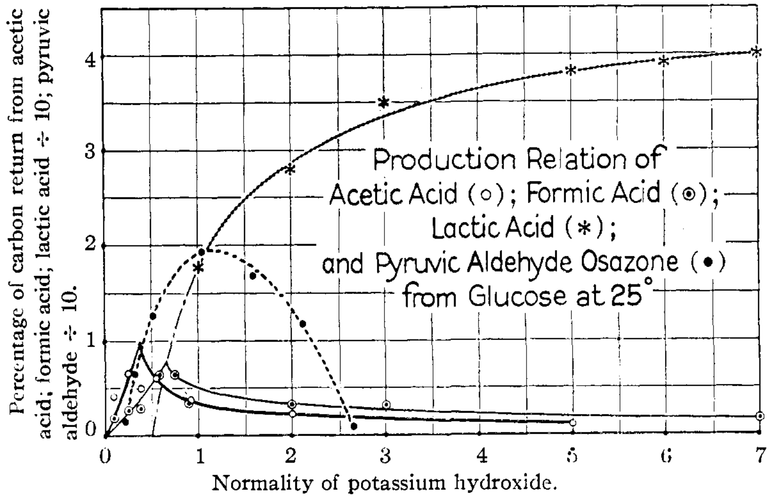

Fig. 9.

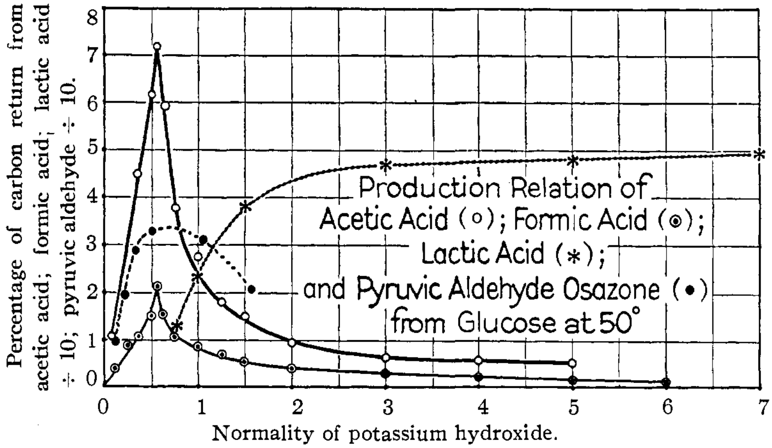

Fig. 10.

such an extent that the resulting solutions had become practically identical both qualitatively and quantitatively. If this is so, then the amounts of lactic acid obtained by the action of alkalies, in the absence of potassium permanganate, on mannose, glucose and fructose should also be the same in each case. In Fig. 11 we have shown that within the limits of experimental error this is true. In this connection it is of much interest to note that Nef[14] obtained closely agreeing results when he oxidized mannose, glucose and fructose with copper hydroxide in the presence of sodium hydroxide, a fact which undoubtedly depended on the close relationship existing between the equilibria in the alkaline solution of each of these carbohydrates. In a recent study of the methods for determining the copper number of glucose, Amick[15] came to the important conclusion that the amount of oxidation is dependent on the alkalinity of the solution.

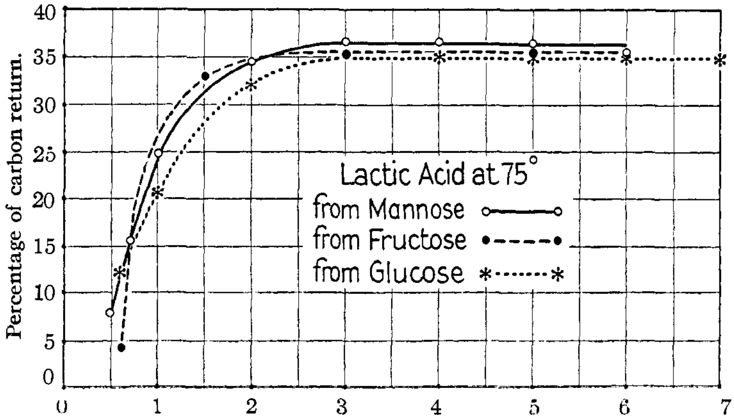

Fig. 11.

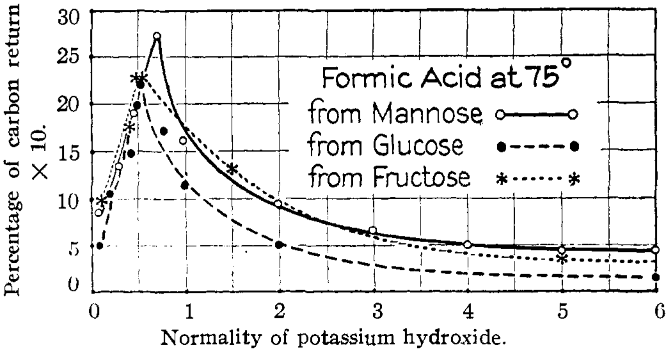

Fig. 12.

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[14] Nef, *Ann.*, **357**, 271, 277, 282 (1907).

[15] Amick, *J. Phys. Chem.*, **31**, 1476 (1927).

Finally, the yields of formic and acetic acids obtained from these three sugars at 75° are shown in Figs. 12 and 13 to be practically the same. Since the amounts of these two acids are relatively small, it has been necessary to multiply our results by 10 in order to show this point. This has magnified the experimental errors of our quantitative methods, which already in themselves have inherent errors. However, the comparison of the data for these two acids at 75° seems to be in complete harmony with the views set forth above with reference to the general condition of the equilibrium existing in alkaline solutions of these three well-known carbohydrates. On the basis of one of the statements above, it may be safely assumed that the relationship of the yields of acetic and formic acids obtained from the other hexose sugars will be approximately the same as that shown here for the mannose–glucose–fructose group.[16]

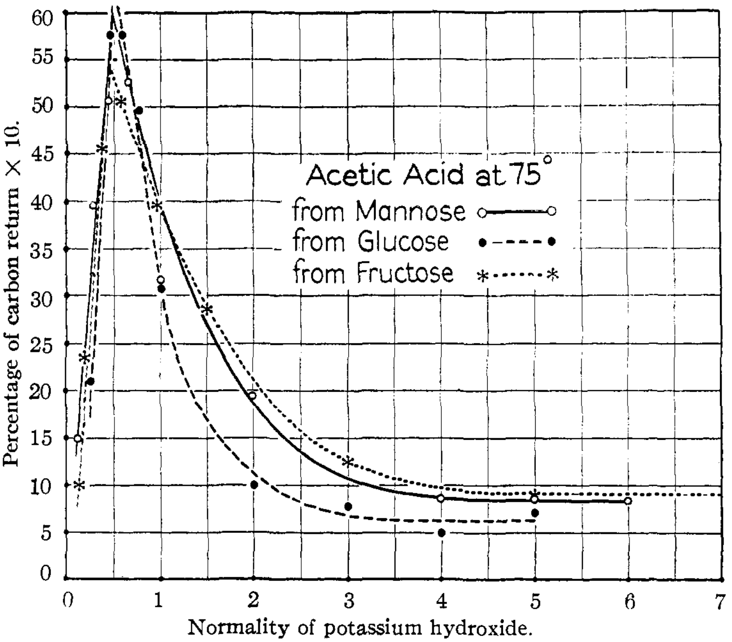

Fig. 13.

Summary

1. The action of aqueous solutions of potassium hydroxide of various concentrations on mannose was studied at 25, 50 and 75° in order to ascertain whether this hexose, like fructose and glucose, reacts in accordance with the suggestion that the alkaline solutions of these well-known carbohydrates contain an equilibrated system which can be disturbed by changes in the experimental conditions, temperature and alkali normality.

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[16] Ref. 4, p. 2672–2673.

2. The reaction mixtures were examined quantitatively for pyruvic aldelyde, lactic, acetic and formic acids. Changes in temperature and alkali concentration produced changes in the yields of these compounds which were of the same general character as those obtained under exactly the same conditions from fructose and glucose.

3. The interdependence of the reactions producing pyruvic aldehyde, lactic, acetic and formic acids in alkaline solutions of glyceric aldehyde and dihydroxy-acetone is also found to exist between the reactions producing these same products when they are obtained from alkaline solutions of mannose, glucose and fructose under the same experimental conditions. This fact is regarded as evidence for the assumption that the formation of glyceric aldehyde, a fission product of 3,4-hexose enediols, is an intermediate step in the production of these compounds from the hexose sugars. It is predicted that this interdependence will be found to exist in the products of the interaction of aqueous solutions of potassium hydroxide and of all those hexose sugars which are not available for laboratory experimentation at the present time.

4. At the lower temperatures and the lower alkali normalities, the amounts of reaction products obtained from mannose, glucose and fructose are not the same. This is understood on the ground that the equilibria formed in each individual case with the hexose and the alkali are not quantitatively identical.

5. At 75° the amounts of lactic, acetic and formic acids obtained from mannose, glucose and fructose are practically the same in each case. These facts seem to support the view that the equilibrated systems in the alkaline solutions of these hexose sugars are identical at this temperature.

COLUMBUS, OHIO

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE STATE UNIVERSITY OF IOWA]

NEW BROMINE SUBSTITUTION PRODUCTS OF VANILLIN AND SOME OF THEIR DERIVATIVES[1]

BY L. CHAS. RAIFORD AND W. C. STOESSER

RECEIVED JUNE 30, 1928 PUBLISHED SEPTEMBER 5, 1928

One purpose of this work was to complete the list of possible bromine substitution products of vanillin and to use these compounds in studying the effect of the ortho substituents of an aldehyde in its reaction with an amino compound. Kauffmann and Franck[2] were unable to convert 2-aminoresorcinoldimethyl ether into either a thiocarbanilide or a benzyli-

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[1] Condensed from a portion of the thesis submitted by W. C. Stoesser to the faculty of the Graduate College of the State University of Iowa in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Kauffmann and Franck, *Ber.*, **40**, 3999 (1907).