

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

**THE MECHANISM OF CARBOHYDRATE OXIDATION. VI. THE ACTION OF POTASSIUM HYDROXIDE ON *dl*-GLYCERIC ALDEHYDE**

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When a hexose-3,4-enediol undergoes cleavage at the double bond, two molecules of the methylenol of glyceric aldehyde are formed. These, in the presence of oxidizing agents, may be converted into glyceric acid, or they may rearrange into glyceric aldehyde and undergo subsequent enolization, or they may be converted into pyruvic aldehyde.

The studies of Edgar, Hoff and one of us<sup>1</sup> on the behavior of *d*-glucose and *d*-galactose in potassium hydroxide solutions show that the amount of lactic acid formed in these reactions is dependent upon both the alkali concentration and the temperature employed. This relationship is accounted for in the following way: (a) an increase in the concentration of the potassium hydroxide tends to shift the equilibrium existing between the three enediolic forms of these hexoses towards the 3-4 form; (b) the glyceric aldehyde formed loses water thus giving rise to pyruvic aldehyde; (c) the pyruvic aldehyde in the presence of a sufficient concentration of alkali will be changed into lactic acid. If this mechanism for the formation of lactic acid is correct then the following questions suggest themselves; (a) is the amount of lactic acid formed from *dl*-glyceric aldehyde when treated with potassium hydroxide proportional to the alkali concentration and the temperature used; (b) is the amount of pyruvic aldehyde formed from *dl*-glyceric aldehyde a similar function of the same experimental factors? The answers to these questions constituted two of the important purposes of this work.

**Experimental Part**

Many attempts have been made to prepare glyceric aldehyde but the first investigators to obtain this substance pure and crystalline were Wohl and Neuberg.<sup>2</sup> The crystalline form has the same molecular weight as a hexose when the freshly prepared cold solution is examined by the cryoscopic method.<sup>3</sup> Upon standing in the cold or, more rapidly, upon warming, the molecular weight falls to that of a triose. This was explained by Wohl on the assumption that the crystalline form is the half acetal which hydrolyzed in water to the monomolecular form.

Briefly summarized, the method of preparing pure, crystalline *dl*-

<sup>1</sup> Evans, Edgar and Hoff, *THIS JOURNAL*, **48**, 2665 (1926).

<sup>2</sup> Wohl and Neuberg, *Ber.*, **33**, 3098 (1900).

<sup>3</sup> Wohl, *Ber.*, **31**, 2395 (1898).

glyceric aldehyde according to Wohl and modified by Witzemann<sup>4</sup> is as follows: (a) acrolein is dropped fairly slowly into twice its weight of hydrogen chloride in absolute alcohol, saturated at 0°; (b) the  $\beta$ -chloropropionic-aldehyde-diethylacetal thus formed is converted into acrolein-diethylacetal; (c) the acrolein-diethylacetal is oxidized with potassium permanganate solution to glyceric-aldehyde-diethylacetal; (d) the latter compound is hydrolyzed in 0.1 *N* sulfuric acid. Great credit is due Witzemann for the accuracy and reliability of his detailed directions which have made glyceric aldehyde much more available than previously. As a general rule, we found that any deviation from his method resulted in a lower yield. Owing to the very great importance of the compound in organic and biochemical fields we wish to note the only exceptions to this general statement in the following account of our experiences in preparing it and to record some experimental details which were found to make the compound more easily prepared.

**Preparation of Acrolein.**—In order to prepare the acrolein on a larger scale we used a 40-liter (10-gallon) copper still, heated by a gas hot-plate, and connected by means of a short Liebig condenser and an adapter to a 2-liter flask surrounded by a boiling water-bath to prevent the polymerization of acrolein. The bent glass connecting tube leading from the 2-liter flask was constricted at its lower end, and had a hole blown in the side so that the acrolein vapors leaving the flask were not forced through drops of water which continually fell from the tube. The upper end of the connecting tube led into a long condenser, to which was attached another flask surrounded by an ice-bath. An initial charge of about 1200 g. of dehydrated magnesium sulfate and 2000 g. of glycerol was placed in the copper still. Some difficulty was experienced in obtaining lumps of magnesium sulfate because it tended to become a powder when heated. A small amount of water (100–200 cc.), placed in the iron pan in which the salt was dehydrated, facilitated caking. Too much water caused the salt to cake so hard that it could be removed only with difficulty. These cakes were broken into pieces about 1 cm. on an edge. The charge in the copper still was heated to 270° as rapidly as possible. When the acrolein was generated freely, more glycerol was added at about the same rate as that at which it was decomposed until about 3600 g. had been added altogether. This addition was made through a dropping funnel which entered the still through a rubber stopper fitted in a small screw-hole in the still-head. About four to five hours was required for the completion of the reaction. The yield was about 750 g. of acrolein from 3600 g. of glycerol. Witzemann was able to obtain about 900–1000 g. from the same amount, the difference probably being due to the fact that at the temperature of our still (270°) the glycerol (b. p., 290°) was volatile with steam and passed over into the 2-liter flask. In place of a metal retort, which was found to be very advantageous from the standpoint of time, Witzemann used a 3-liter flask with a long neck which acted as a reflux condenser and retained most of his glycerol. Wohl<sup>5</sup> obtained even better yields, using a tube heated to 350–340°.

The still was extremely hard to clean until the procedure was adopted of heating it to a fairly high temperature at the conclusion of each operation. This formed a layer

<sup>4</sup> Witzemann, *THIS JOURNAL*, **36**, 1766, 1908 (1914). Compare Witzemann, *ibid.*, **36**, 2223 (1914).

<sup>5</sup> Wohl and Mylo, *Ber.*, **45**, 2050 (1912).

of soft charcoal between the still and the tar, which allowed the latter to be removed readily with a steel spatula.

**$\beta$ -Chloropropionic Aldehyde.**—The acrolein, previously dried by the use of calcium chloride (made neutral to litmus by saturating with dry hydrogen chloride), was added to the alcoholic hydrogen chloride contained in a 3-liter bottle by means of a dropping funnel that passed through the cork. The bottle was surrounded by chipped ice and water and the contents were agitated vigorously by an electric motor and a glass stirrer. A small glass capillary in the top of the dropping funnel prevented acrolein vapors from escaping after the apparatus was once filled. While the acrolein was being poured into the dropping funnel, however, the vapors escaped quite freely. For this reason the dropping funnel was surrounded by a large funnel and the space between was kept filled with chipped ice and water. In this way the acrolein could be added to the funnel at once and yet not become warm. Regulation of the flow was obtained by means of a rubber tube and screw clamp placed between the dropping funnel and the glass tube leading into the bottle of alcoholic hydrogen chloride. The neutralization of the excess of hydrogen chloride and the purification of the  $\beta$ -chloropropionaldehyde acetal were done according to Witzemann's directions.

**Acrolein-Acetal.**—The conversion of  $\beta$ -chloropropionaldehyde-acetal into acrolein-acetal calls for the use of finely powdered, dry potassium hydroxide. Due to its very hygroscopic nature, this seemed to forecast difficulties. These difficulties were overcome, however, by the use of a small 25cm. disk pulverizer. In this way the potassium hydroxide could be powdered so rapidly (about 225 g. per minute) that very little moisture was taken up from the air. Witzemann warns that the results will be poor if much water is present. The water formed during the reaction, if allowed to exist uncombined, hydrolyzes the  $\beta$ -chloropropionaldehyde-acetal. A small amount of this compound escapes polymerization by the hot alkali (probably being formed in the vapors above the alkali) and passes over with the alcohol, giving to the distillate a lachrymatory odor, even worse than that of acrolein. The boiling point is therefore kept in the neighborhood of 78° (that of alcohol) instead of being allowed to rise to about 120° (that of acrolein-acetal is 123–125°) and affords an index of whether or not the reaction is proceeding favorably. In order to prevent this action, six times the calculated required quantity of potassium hydroxide was used, the excess combining with the water and largely preventing the hydrolysis of the acetals.

In attempting to work with larger quantities than those used by Witzemann (200 g. instead of 50 g.), it was found that the  $\beta$ -chloropropionaldehyde-acetal always began to react before very much of the potassium hydroxide had been added. Since much heat is liberated it is almost impossible to stop the process after large quantities begin reacting. It then proceeds without a large excess of potassium hydroxide being present and poor results are inevitable. Therefore, the procedure was adopted of adding the alkali to the flask in which the reaction was carried out and then pouring in all the acetal for that charge, mixing by a brief, violent shaking and attaching immediately to the Ginsky distilling column. The following comparison of the results obtained from two samples of the same acetal treated in the two different ways justified the change. On adding 400 g. of potassium hydroxide to 200 g. of  $\beta$ -chloropropionaldehyde-acetal, 42 g. (26.9%) of acrolein-acetal was obtained, while the addition of 148 g. of  $\beta$ -chloropropionaldehyde-acetal to 296 g. of potassium hydroxide gave 70 g. (58.8%) of acrolein-acetal. Thus the percentage yield was more than doubled and the yield of Witzemann closely approached.

The very concentrated potassium hydroxide solution heated to about 250–290° (in order to drive all the acrolein-acetal through the distilling column) had such a corrosive action on glass that it was found necessary to make two iron retorts from 10 × 15

cm. gas-pipe nipples and caps, a 2.5cm. hole being made in one of the caps for the admission of a cork fitted with the distilling tube. Since these retorts were too heavy to shake violently, the mixing of the potassium hydroxide with the  $\beta$ -chloropropionaldehyde acetal was accomplished by the use of a heavy glass stirring rod. The heat capacity of such a retort was so great that the temperature could be controlled easily by the use of a free flame. The results, following this procedure, were uniformly good, the yield averaging about 60%. Witzemann's best average was 68.0%. Altogether we prepared 1371 g. of acrolein-acetal.

**Glyceric Aldehyde Acetal.**—The oxidation of acrolein-acetal to glyceric aldehyde acetal is accomplished by allowing a nearly saturated solution of potassium permanganate to drop into a suspension of acrolein-acetal in water. Eleven hundred cc. of an aqueous solution containing 60 g. of potassium permanganate is added to 50 g. of acrolein-acetal and 540 cc. of water contained in a 3-liter flask, the addition being made at the rate of 20 cc. per minute. The mixture is vigorously agitated in an ice-bath. A uniform rate of addition was secured by the use of a piece of glass tubing drawn out at one end. By keeping the potassium permanganate solution at the height of a predetermined mark on the glass tube a very regular rate of flow could be maintained. The control was made possible by means of a dropping funnel which led into the upper end of the constricted tube. Contrary to expectations, very irregular results were obtained at first in this oxidation. When the manganese dioxide precipitate was coarse and black the contents of the flask did not gel and very little glyceric aldehyde acetal was formed. The recovery of unchanged acrolein-acetal was always high in these cases. When the reaction proceeded favorably, the manganese dioxide precipitate was so fine as to be invisible and light brown in color. Soon after stirring ceased, the contents of the flask formed a stiff gel which gradually became darker with time and, upon standing, showed syneresis. In these cases the yield of glyceric aldehyde acetal was always high and very little acrolein-acetal escaped oxidation. This puzzling difference in behavior could always be detected within a few seconds after the potassium permanganate came in contact with the acrolein-acetal, the indication being the color of the manganese dioxide precipitate, brown or black. Almost by accident it was discovered that this difference in behavior was caused by a few degrees' difference in temperature of the suspension of acrolein-acetal and water. When the temperature was allowed to fall below about 5°, the results were poor. Therefore it was not advisable to allow the suspension to assume the temperature of the ice-bath, but the potassium permanganate addition was started almost as soon as the ice-bath was prepared. After this, the heat of the reaction took care of the temperature. Following this discovery eight successive oxidations were made with good results in every case. From the 400 g. of acrolein-acetal used in the last eight oxidations, 337 g. of pure glyceric aldehyde acetal was obtained. Witzemann's best results were in the ratio of 267 g. of glyceric aldehyde acetal from 400 g. of acrolein-acetal, so that his yield was exceeded by this change in detail. From the other 971 g. of acrolein-acetal only 248 g. of glyceric aldehyde acetal was obtained. This is an example of the sensitiveness of this preparation to slight changes in details of manipulation. The working up of the oxidation mixture was carried out as described by Witzemann.

**Glyceric Aldehyde.**—The hydrolysis of glyceric aldehyde acetal by means of 0.1 *N* sulfuric acid involved considerable work but there was nothing especially difficult about it. Witzemann's procedure differs from that of Wohl at this point in that he allowed a week instead of two to three days for the hydrolysis to take place, and in that he added a small amount of acetic acid before neutralizing the sulfuric acid with barium hydroxide. When a slight excess of the latter is present, it forms barium acetate and, therefore, the solution is never alkaline. In this way the triose is not polymerized and crystallizes much better. Witzemann's directions call for warming the hydrolyzing mixture to 35° for about four hours. It is again warmed (temperature not

specified) while being neutralized with barium hydroxide. The same author also states that the results obtained by him in crystallizing glyceric aldehyde were somewhat irregular. It occurred to us that if the sugar is so very sensitive to traces of alkali as Witzemann indicated, it might also be very sensitive to high temperature and, after working up one portion according to Witzemann's directions, the next portion was worked up by *never allowing the temperature to rise above 28°*. This was made possible by the possession of a very good water pump and the fact that the evaporation of the aqueous solution was carried out during December while the tap water was very cold. The distillation was carried out at a pressure of 9–11 mm. Failure to warm the solution results in a very finely divided barium sulfate precipitate which can be readily filtered, using bone black as recommended by Witzemann. After evaporation, the directions call for concentrating the glyceric aldehyde in a vacuum until a water-free sirup is obtained. *This was found to be impossible when the procedure mentioned above was followed because the sugar crystallizes from the water solution.* Apparently crystallization has never before been accomplished. The crystallization was hastened by adding absolute alcohol as recommended by Witzemann. From 584 g. of glyceric aldehyde acetal, a little over 250 g. of glyceric aldehyde was obtained. Witzemann obtained the same amount from about 767 g. of glyceric aldehyde acetal so that the yield is increased by not warming the solution. Otherwise Witzemann's procedure was followed rigidly. The glyceric aldehyde so obtained melted at 141° (corr.). This agrees well with the literature, Wohl<sup>6</sup> giving 130° and 138° and Witzemann giving 132° and 142° as the melting points of different specimens.

Since the experiments to be carried out upon the glyceric aldehyde involved the possible formation of alcohol and acetic acid, great care was taken to remove all traces of these substances. The samples were kept for two weeks in a vacuum desiccator over a mixture of soda lime and calcium chloride at a pressure of 9 mm.

**Chemicals.**—The same precautions were observed with reference to the degree of purity of all reagents used in this work that were taken in previously reported experiments in this series of studies.

**Procedure.**—Owing to the prohibitive difficulty in preparing large quantities of glyceric aldehyde, it was found necessary to work with smaller quantities of this carbohydrate than had been the case with *d*-glucose and *d*-galactose, with the result that we had to change our former general procedure at a number of points. These changes are described here very briefly.

(a) **Action of Potassium Hydroxide on *dl*-Glyceric Aldehyde.**—To a 100cc. round-bottomed flask containing 25 cc. of standardized solution of the alkali at the chosen temperature was added through a large funnel with a shortened stem 2.25 g. of the crystalline *dl*-glyceric aldehyde. The flask was closed with a rubber stopper and then attached to an eccentric-driven agitator placed in a water thermostat. After 48 hours the flask was immersed in cold water while the reaction mixture was being neutralized with a solution of phosphoric acid of the same molar strength as the alkali used, and of such a volume as would make an equal volume of the potassium hydroxide acid to methyl orange. The resulting solution was matched in color with a solution of monopotassium phosphate to which an equal amount of methyl orange had been added. Phosphoric acid was used in the place of hydrochloric acid because it was thought that the proximity of the boiling points of constant-boiling hydrochloric and formic acids was interfering with satisfactory duplicate determinations for these two acids. We established by special experiment that phosphoric acid will liberate 98.5% of the formic acid from solutions of a strength equivalent to our distillates. The residues obtained by such a distillation in this special experiment did not reduce acidified potassium per-

<sup>6</sup> Wohl and Newberg, *Ber.*, **33**, 3100 (1900).

manganate solutions. The distillates obtained throughout this work were tested with ammonium molybdate in the usual way. Only a faint yellow coloration was obtained at any time.

(b) **Determination of Formic and Acetic Acids.**—Nearly all titrations for formic acid based upon the McLeod modification of the Jones method gave results which calculated to more than 100% when compared to the titration for total acidity. Since very consistent results could be obtained when the determinations were made upon pure formic acid samples, this was strong evidence of the presence of one or more reducing agents other than formic acid. Further evidence was found in the fact that the distillates tasted and smelled like caramel and, upon being made alkaline, turned to a pale yellow color, distinctly visible when relatively large amounts of the distillate were present. Since the validity of the Jones method depends upon the absence of reducing agents other than formic acid, a different way of determining formic acid had to be used. None of the methods in the literature seemed applicable to the problem at hand, so a new process was devised. The total acidities of the distillates were determined by titration of 10cc. samples with 0.06221 *N* potassium hydroxide solution. These titrations, with phenolphthalein as an indicator, were run in duplicate and values differing by not more than 0.03 cc. were averaged. Two more 10cc. samples of the distillate were withdrawn and, after they had been boiled gently for 30 minutes on an electric hot-plate with a small excess of mercuric oxide (in order to oxidize the formic acid to carbonic acid), were again titrated with potassium hydroxide. The second value was listed as acetic acid and the difference between this and the value for total acidity was considered to represent the amount of formic acid. To test the accuracy of the above procedure the following special experiments were carried out. (1) Mercuric oxide oxidizes formic acid completely under the conditions of the experiment. Ten cc. of pure formic acid, equivalent to 43.63 cc. of 0.06221 *N* potassium hydroxide, was boiled for 30 minutes with an excess of mercuric oxide. The flask was cooled and a drop of phenolphthalein added. The indicator turned pink when 0.07 cc. of the solution of potassium hydroxide described above had been added. (2) Acetic acid is not oxidized under the conditions of the experiment and mercuric oxide does not interfere with the titration for acetic acid with potassium hydroxide. A sample of acetic acid equivalent to 3.95 cc. of the standard alkali was boiled for one-half hour with an excess of mercuric oxide. The titration then required 3.98 cc. of the alkali. This slight discrepancy was probably due to diluting the solution by the water used to rinse the condenser into the flask. (3) The solution can be manipulated so as to lose all of the carbonic acid formed in the oxidation of formic acid without losing any acetic acid. Four samples were prepared of mixtures of about equal quantities of acetic and formic acids of accurately known concentration and boiled for one-half hour with an excess of yellow mercuric oxide in a 50cc. Erlenmeyer flask with a piece of straight glass tubing of 8 mm. inside diameter and 25 cm. long acting as a reflux condenser. Care was taken that the upper end of the condenser never became warm. The correct value for the acetic acid titration was 3.95 cc. and the following values were obtained in the mixtures after the above treatment: 3.83 cc., 3.83 cc., 4.00 cc., 3.97 cc.; average, 3.91 cc. In titrating the distillates, checks within 0.04 cc. were always obtained before the results were accepted.

(c) **Lactic Acid.**—The lactic acid was removed from the potassium phosphate, caramel and other reducing substances remaining after the distillation, by a 48-hour extraction with ether in the apparatus described previously.<sup>1</sup> The lactic acid was determined as zinc lactate.

(d) **Pyruvic Aldehyde Osazone.**—In the osazone experiments, 25 cc. of standard potassium hydroxide solution was placed in a 100cc. flask to which was then added 17.5 cc. of 95% ethyl alcohol and 5 cc. of phenylhydrazine. After temperature adjustment in the thermostat, 2.25 g. of the crystalline *dl*-glyceric aldehyde was added and the re-

action allowed to take place for 48 hours. The reaction mixture was worked up as previously described.<sup>1</sup>

(e) **Qualitative Reactions.**—The qualitative reactions used in the examination of the distillates were the same as those described in the work on *d*-glucose and *d*-galactose.

**Results.**—The quantitative data obtained in these experiments are shown in Figs. 1, 2 and 3.

### Theoretical Part

The results obtained in these experiments are in complete agreement with those obtained by Edgar, Hoff and one of us<sup>1</sup> on the action of potassium hydroxide on aqueous solutions of *d*-glucose and *d*-galactose. This relationship is at once clear if it is assumed that alkaline solutions of glyceric

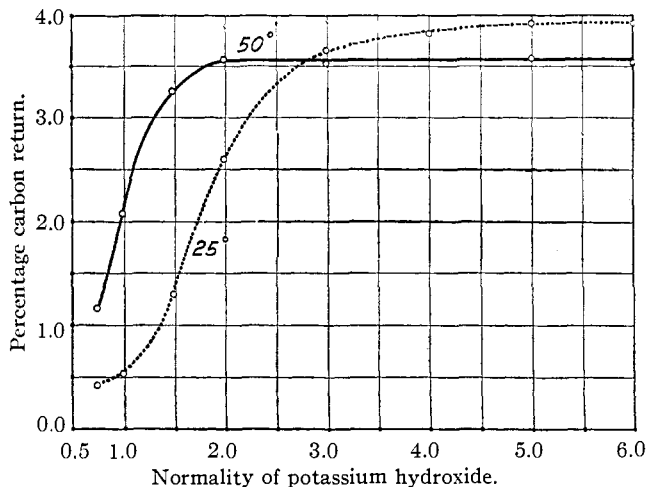
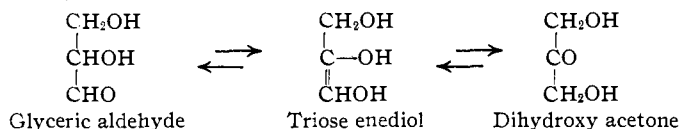


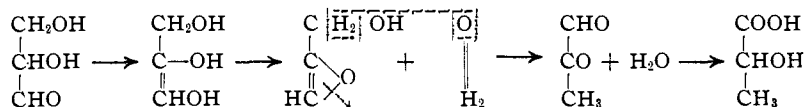
Fig. 1.—Lactic acid from glyceric aldehyde at 25° and 50°.

aldehyde also react in a manner similar to the well-known behavior of hexoses toward alkalis as pointed out in the classical experiments of Lobry de Bruyn and van Ekenstein; that is, alkaline solutions of glyceric aldehyde undergo a transformation into dihydroxy-acetone. Furthermore, it has been pointed out<sup>1</sup> that the character of the reaction products obtained by the action of alkalis on hexoses is best understood if one assumes with Nef and his students that in such solutions a number of enediols are formed. Therefore, it follows that an alkaline solution of glyceric aldehyde may be represented by the following reaction.



That the system represented by this equation will satisfactorily account for the results obtained in this work is shown in the following discussion.

**Lactic Acid.**—Recently, it has been pointed out by Miss Marjorie Pickard Benoy, of this Laboratory, and one of us<sup>1</sup> that the three hexose enediols may be regarded as steps in the mechanism involved in the formation of the several saccharinic acid lactones found by other investigators in alkaline solutions of *d*-glucose and *d*-galactose. That this same explanation may also be used in the case of alkaline solutions of glyceric aldehyde to give the intermediate diketo compound, pyruvic aldehyde, necessary to form lactic acid is evident from the following reaction.



In alkaline solutions of *d*-glucose and *d*-galactose, it is assumed that the 3-4 enediol is split at the double bond with the consequent formation of the

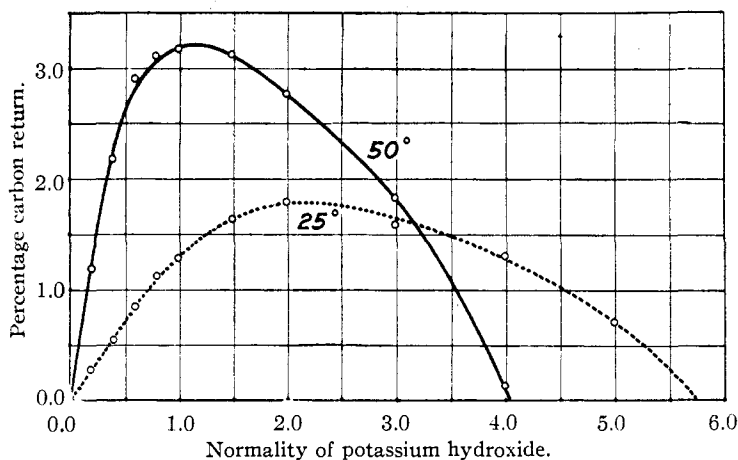


Fig. 2.—Pyruvic aldehyde osazone from glyceric aldehyde at 25° and 50°.

methylenol of glyceric aldehyde, which in turn is then rearranged to glyceric aldehyde, a compound regarded as the forerunner of the lactic acid obtained from these carbohydrates. From Fig. 1, it is seen that the behavior of glyceric aldehyde is in complete harmony with that of the hexoses when studied under the same experimental conditions, that is, temperature changes and changes in alkali concentration. At the higher alkalinities at 50° there was a greater tendency for the glyceric aldehyde to form tar, an action that tended to lower the lactic acid yield. Mr. W. R. Cornthwaite of this Laboratory is at present studying the behavior of dihydroxy acetone to see if its action toward alkalies is in harmony with that expected from the two equations above.

**Pyruvic Aldehyde.**—Since pyruvic aldehyde has been regarded as one of the compounds involved in the formation of lactic acid from the hexoses,



and since glyceric aldehyde formation is regarded as one of the steps in the formation of this acid, it was obviously necessary to show that glyceric aldehyde would also yield a derivative of pyruvic aldehyde when examined under the same experimental conditions as were *d*-glucose and *d*-galactose. The results of our experiments are shown in Fig. 2. It is seen that the effect of varying the temperature and a change in the alkali concentration is of the same general character as that for the two aldohexoses. The maximum point at both 25° and 50° is thought to be the alkali normality at which the rate of osazone formation is just equal to that of lactic acid formation.

**Acetic and Formic Acids.**—The presence of acetic acid and a part of the formic acid in alkaline solutions of *d*-glucose and *d*-galactose has been ascribed to the decomposition of pyruvic aldehyde into acetaldehyde and carbon monoxide,  $\text{CH}_3\text{CO}-\text{CHO} \rightarrow \text{CH}_3\text{CHO} + > \text{CO}$ , which under

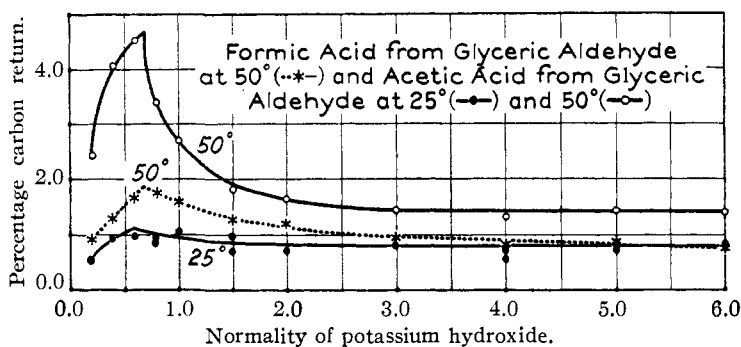


Fig. 3.

the conditions of these experiments yield acetic and formic acids. Since the source of the pyruvic aldehyde is glyceric aldehyde, the latter compound should react in the same manner toward alkalies with respect to acetic and formic acids as do the two aldohexoses. This is shown to be the case in Fig. 3. As in the case of these two well-known carbohydrates, the alkali normality at the maximum yields is regarded as the point at which the rate of formation of these two acids is just equal to the rate of lactic acid formation. As the alkali increases in concentration, the rate of lactic acid formation increases with a concurrent diminution in the yields of acetic and formic acids.

**Formic Acid from Triose Enediol.**—It was pointed out above that the tendency of glyceric aldehyde to form tar caused much difficulty in determining with the desired precision the amount of formic acid present in the distillates. In general, however, our data show more formic acid production than that equivalent to the acetic acid obtained if both acids are considered as being present in equimolecular amounts, which would be the

case if the two acids were being derived only from the decomposition of pyruvic aldehyde. The formic acid in excess of this ratio must come from the cleavage of the triose enediol, a reaction that would yield formaldehyde and glycolic aldehyde. Mr. J. E. Hutchman<sup>7</sup> of this Laboratory has recently shown that glycolic aldehyde under the conditions used in these experiments does not yield either formic or acetic acid, hence the source of this excessive formic acid must be the formaldehyde arising from the cleavage of the triose enediol. Since the amounts of formic and acetic acids at 50° tend to approach an equimolecular ratio with an increasing normality of the alkali, it is clear that the increase in alkalinity tends to bring about the changes shown in the reaction involved in the decomposition of pyruvic aldehyde. This general relationship of these two acids was also observed in the behavior of *d*-glucose and *d*-galactose.

**Order of Reaction of Pyruvic Aldehyde Formation.**—It was thought that a study of the formation of pyruvic aldehyde osazone from glyceric

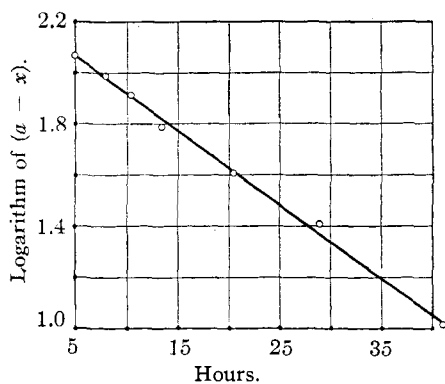


Fig. 4.—Order of reaction.

aldehyde might determine the order of the reaction and consequently shed some light upon its mechanism. A series of experiments was carried out, therefore, using the same technique as previously except that the reaction was interrupted at the ends of various lengths of time as shown in the data. The normality of the solution with respect to potassium hydroxide was 1.053. Allowing  $a$  to stand for the amount of glyceric aldehyde present at the beginning

of the reaction, and  $x$  the amount that has reacted at the end of any definite length of time, then  $(a-x)$  is the quantity left unchanged at the end of that particular length of time. Since there was here no way of knowing directly just how much triose was left unaltered at the end of a certain time, the reaction was allowed to go to completion, that is, until there was no longer a perceptible amount of osazone being formed and, this amount of osazone being proportional to the amount of aldehyde used, it was called  $a$ . Then  $(a-x)$  for any given time is equal to this value  $a$  minus the amount of osazone formed at the end of that particular time. In order that the results might be of value, it was necessary that the entire amount of osazone formed should be discovered, and not merely the amount precipitated. Since the osazone is somewhat soluble in the reaction mixture, there is a difference between these two values. A solubility deter-

<sup>7</sup> Forthcoming publication.

mination was made, therefore, and the value 0.0445 g. (which is the amount of pyruvic aldehyde osazone dissolving in the amounts of reaction mixture used in these experiments) was added to the weights of osazone precipitated. This affected the value of  $a$  without affecting the values of  $(a-x)$ . The data shown in Fig. 4 show this reaction to be of the first order. This result may be explained by assuming that the conversion of glyceric aldehyde to pyruvic aldehyde is a comparatively slow reaction, while the reaction that forms the osazone is much more rapid. These assumptions are necessary on other grounds, the first because the formation of the osazone comes to an end only after about 48 hours, the second because the osazone is formed from methyl-glyoxal before the latter is changed into lactic acid. This result is regarded as evidence for the mechanism involved in pyruvic aldehyde formation from glyceric aldehyde by the loss of water.<sup>8</sup>

Nef and Oppenheimer have shown that glyceric aldehyde forms lactic acid when treated with sodium hydroxide, so that there was every reason to expect its formation when potassium hydroxide was used. No one, however, had made a quantitative study of the reaction using pure, crystalline glyceric aldehyde over a wide range of alkali concentrations or carefully controlled conditions.

We wish to thank Dr. Andrew Irving Andrews, Department of Ceramics, University of Illinois, and Dr. J. F. Lyman, Department of Agricultural Chemistry of this University, for kindly assistance rendered during the progress of this work.

### Summary

1. Certain details are given for the improvement of the method for the preparation of solid glyceric aldehyde which make this compound more accessible.

2. Molar solutions of *dl*-glyceric aldehyde were treated with various concentrations of potassium hydroxide from 0.2 *N* to 6 *N* at 25° and 50°. The resulting formic, acetic and lactic acids were measured quantitatively.

3. The formic acid production at 50° is an increasing logarithmic function of the potassium hydroxide concentration, Fig. 5, until a concentration of 0.7 *N* is reached, after which it is a decreasing logarithmic function of the alkali concentration. The formic acid sources are thought to be two; the decomposition of pyruvic aldehyde, and the triose enediol.

4. The acetic acid production is also an increasing function of the alkali

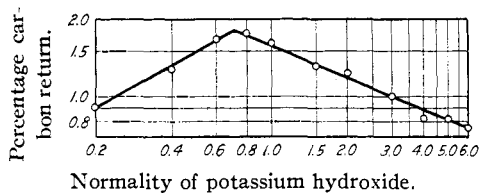


Fig. 5.—Formic acid at 50°.

<sup>8</sup> Wohl, *Biochem. Z.*, 5, 54 (1907).

concentration to 0.6 *N*, after which it is a decreasing function. The source of the acetic acid is believed to be a splitting of pyruvic aldehyde. A new method has been developed that enables one to determine acetic acid quantitatively in the presence of formic acid and non-acid reducing agents. The acetic acid production is an increasing function of the temperature. In general, the formic acid yields are higher than an equimolecular ratio at 50° when referred to the acetic acid yields, but the tendency of the formic acid yields is to approach this ratio as the alkalinity increases.

5. The lactic acid production has been shown to be an increasing function of the alkali concentration, although it rapidly approaches a constant value. At low normalities the lactic acid production is an increasing function of the temperature. At high normalities the constant value approached is slightly higher for the reaction at 25° than at 50°, owing to tar formation at the higher temperature.

6. The glyceric aldehyde is believed to form pyruvic aldehyde before changing to lactic acid. Molar glyceric aldehyde solutions were treated with alcoholic phenylhydrazine solutions in the presence of various concentrations of potassium hydroxide at 25° and 50°. The production of pyruvic aldehyde osazone at 25° is an increasing function of the alkali until a concentration of 1 *N* is reached, after which it is a decreasing function. At 50° the same is true except that the maximum production is at approximately 0.5 *N*. The reaction was found to be of the first order. The lowering in the curve after the peak is reached is believed to be due to increasing conversion of the pyruvic aldehyde into lactic acid.

7. A suggestion is offered for the reaction mechanism of pyruvic aldehyde formation from glyceric aldehyde.

8. The theoretical interpretation of the results is in harmony with that for the behavior of *d*-glucose and *d*-galactose under similar experimental conditions.

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