

the solution filtered. The filtrate was cooled to 0° and very carefully neutralized with cold hydrochloric acid. The crystalline material so obtained was rapidly filtered off and washed several times with ice water; yield, 60%. The product was purified by crystallization from alcohol or preferably from benzene.

Four g. of ethyl-vinyloxyethyl-barbituric acid, prepared as described above, was placed in 30 cc. of hot water, in which it is insoluble. On the addition of 2 cc. of hydrochloric acid and stirring, the solid dissolved almost immediately while the acetaldehyde formed by the hydrolysis was removed by boiling. The product crystallized on cooling and was purified by crystallization either from alcohol or water; yield of ethyl- $\beta$ -hydroxyethyl-barbituric acid, 70%.

### Summary

1. A method for the preparation of hydroxy-substituted alkyl-barbituric acids has been described.

2. The compounds so prepared may be hypnotics with properties similar to those of veronal and propronal, and should be less toxic. The result of a study of their pharmacology will be reported later.

PITTSBURGH, PENNSYLVANIA

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

## THE MECHANISM OF CARBOHYDRATE OXIDATION. I. *d*-GLUCOSE, *d*-MANNOSE, *d*-FRUCTOSE, *d*- AND *l*-ARABINOSE AND *dl*-GLYCERIC ALDEHYDE

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The work presented in this paper is a continuation of a series of studies being made in this Laboratory on the oxidation of organic compounds.<sup>3</sup>

The principal purpose of these studies is to establish, from exact quantitative data, obtained under definitely chosen experimental conditions, the molecular stages through which some of the organic compounds of the more familiar type pass when they undergo this kind of chemical change. The experiments which are recorded here on the oxidation of *d*-glucose, *d*-mannose, *d*-fructose, *d*- and *l*-arabinose and *dl*-glyceric aldehyde with neutral and alkaline potassium permanganate solutions were carried out with the following definite objectives in view: (a) to determine the effect of alkalinity on the character and amounts of the oxidation products; (b) to ascertain the effect of temperature on the character and amounts of the reaction products; (c) to obtain data which would lead to a better understanding of the oxidation reactions of some of the more common polysaccharides that are wholly or in part composed of some of these well-known hexoses; (d) to learn whether the behavior of *d*- and *l*-

<sup>1</sup> E. I. du Pont de Nemours Fellow, 1922.

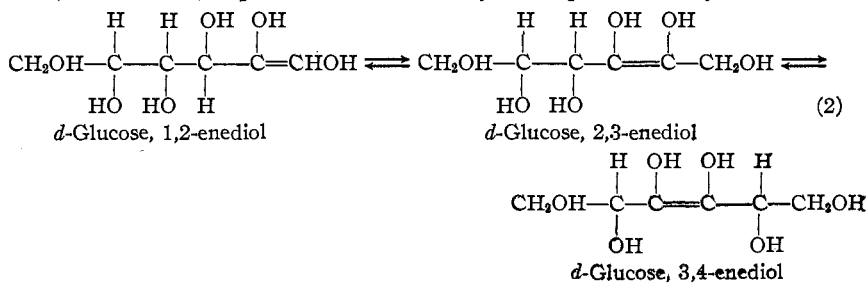
<sup>2</sup> E. I. du Pont de Nemours Fellow, 1923.

<sup>3</sup> THIS JOURNAL, (a) 41, 1267, (b) 1385 (1919); (c) 44, 1730, (d) 2271, (e) 2276 (1922); (f) 45, 171 (1923).

arabinose and *dl*-glyceric aldehyde, compounds that are theoretically possible intermediate substances in the oxidation of these hexoses, is in harmony with the results obtained when they also are oxidized with neutral and alkaline potassium permanganate solutions under the same experimental conditions as those used with *d*-glucose, *d*-mannose and *d*-fructose. (e) The classical experiments of Lobry de Bruyn and Alberda van Ekenstein<sup>4</sup> concerning the action of alkalis on aqueous solutions of *d*-glucose, *d*-mannose and *d*-fructose show that these carbohydrates undergo reciprocal transformations into one another. As is well known the presence of *d*-pseudo-fructose, *d*- $\alpha$ -glucose, and *d*- $\beta$ -glucose in these solutions was also discovered by these workers. These transformations may be represented by Reaction 1<sup>5</sup>. *d*-Glucose, *d*-mannose and *d*-fructose  $\rightleftharpoons$  *d*-pseudo-fructose  $\rightleftharpoons$  *d*- $\alpha$ -glucose  $\rightleftharpoons$  *d*- $\beta$ -glucose (1)

tose did not give rise to systems that were quantitatively identical. This may be due to the fact that the velocity of the transformations is so slow that these particular systems had not reached an equilibrium during the time they were under observation. It also is quite conceivable that such systems may be irreversible equilibria.<sup>6</sup>

Nef<sup>7</sup> and Glattfeld<sup>8</sup> have assumed that in an alkaline solution of these three hexoses there are formed 1,2, 2,3 and 3,4 enediols which give rise to an (beschränkte) equilibrium which may be represented by Reaction 2.



It occurred to us that should the equilibrium consisting of the three enediols of these carbohydrates be an integral part of the de Bruyn and van Ekenstein equilibrium, then certain experimental factors might exist which would cause a shifting in such a system. Indications of such a possibility were seen in the fact that the amount of lactic acid formed

<sup>4</sup> de Bruyn and van Ekenstein, (a) *Rec. trav. chim.*, **14**, 156, (b) 203, (1895); (c) **15**, 92 (1896); (d) **16**, 257, (e) 262, (f) 274 (1897); (g) **19**, 1 (1900); (h) **27**, 1 (1908). Compare (i) Nef, *Ann.*, **403**, 208-213 and note on p. 239 (1914).

<sup>5</sup> Ref. 4 b, p. 214.

<sup>6</sup> Compare (a) Powell, *J. Chem. Soc.*, **107**, 1335 (1915). (b) Armstrong, "The Simple Carbohydrates and the Glucosides," Longmans, Green and Co., 1919, p. 46.

<sup>7</sup> Ref. 5 i, p. 205.

<sup>8</sup> Glattfeld, *Am. Chem. J.*, **50**, 139 (1913).

from alkaline solutions of certain carbohydrates seems to depend on the concentration of the alkali used and also upon the temperature employed. Furthermore, it is clear that the enediols and their subsequent splitting products obtained in turn from Reactions 3, 4 and 5 must also be an integral part of the above general equilibrium system, and as such must also be influenced by such factors as changes in concentration of alkali and in temperature. The behavior of the compounds of fewer carbon atom content thus formed from *d*-glucose, *d*-mannose and *d*-fructose should be in harmony with the oxidation studies of Day, Adkins, Hoover, Sefton and Evans. Hence, we felt that a systematic study of the oxidation phenomena of these carbohydrates would shed much light on such changes if they were taking place as we thought. If the alkaline solutions of these carbohydrates give rise to an irreversible equilibrium, then it is probable that the speed of the slowest reaction in the series of changes involved must be influenced by the concentration of the alkali used and also by the temperature employed. (f) From these considerations, it is also clear that if the equilibrium indicated above (Reaction 2) could be brought to an adjustment through properly chosen experimental conditions such as variation in alkali concentrations and temperature change, then identical systems would probably be the result in each case. Under such circumstances a similarity in the behavior of *d*-glucose, *d*-mannose and *d*-fructose toward given oxidizing agents under the same experimental conditions is to be expected. No exact quantitative study of this question from this point of view has been made hitherto.

a. **Oxidation of *d*-Glucose.**—Smolka<sup>9</sup> oxidized glucose completely to carbon dioxide and water with alkaline potassium permanganate. Witzemann<sup>10a</sup> also oxidized *d*-glucose with potassium permanganate in the presence of various amounts of potassium hydroxide and obtained carbon dioxide and oxalic acid as oxidation products. The yields of carbon dioxide and oxalic acid were 100 and 0%, respectively, from neutrality to 0.028 *N* alkali. At this latter point the formation of oxalic acid began and continued to increase up to 42% in 1.82 *N* potassium hydroxide solution.

b. **Oxidation of *d*-Mannose.**—Only a relatively small amount of work has been done on the oxidation phenomena of *d*-mannose. This condition undoubtedly arises from a number of causes, among which are the cost of this carbohydrate, and the great similarity between its behavior and that of the more easily accessible *d*-glucose. Nef<sup>11</sup> oxidized *d*-mannose with copper acetate, silver oxide, hydrogen peroxide, mercuric oxide, Fehling solution and other oxidizing agents. The nature of the

<sup>9</sup> Smolka, *Monatsh.*, **8**, 26 (1888).

<sup>10</sup> Witzemann, (a) *THIS JOURNAL*, **38**, 150 (1916); (b) **36**, 2223 (1914).

<sup>11</sup> Nef, *Ann.*, (a) **335**, 191 (1904); (b) **357**, 259 (1907).

products varied in character according to the reagent used. Greifenhagen and Scholl<sup>12</sup> also studied the action of alkaline permanganate solutions on *d*-mannose.

c. **Oxidation of *d*-Fructose.**—A study of the literature<sup>13</sup> dealing with the oxidation of *d*-fructose in alkaline solutions discloses, as in the case of *d*-glucose, differences in the behavior of this carbohydrate which may be attributed to the widely different experimental conditions used. However, there is a remarkable similarity in the results obtained with *d*-glucose, *d*-mannose and *d*-fructose when these substances are studied under approximately the same experimental conditions. A very notable example of this is Nef's<sup>11b</sup> work on the oxidation of these compounds with alkaline copper hydroxide. In practically all cases it is difficult to make deductions concerning the influence which such factors as varying the concentrations of reagents and changes in the temperature might have on the final results. The experimental procedure used by us to attain some of these effects is described in the next section. It is obvious that there must be a uniformity in the laboratory methods before sound conclusions with reference to the reaction mechanism can be drawn from the experimental data.

### Experimental Part

**Materials. CARBOHYDRATES.**—The hexoses and pentoses obtained from the Special Chemicals Company were of the highest degree of purity, their identity and properties being checked by the usual laboratory methods. The *dl*-glyceric aldehyde used by us was prepared by Mr. G. P. Hoff, of this Laboratory, according to the method described by Witzemann.<sup>11b</sup> The white solid melted at 138°.

**WATER.**—Water free from carbon dioxide was prepared by passing a stream of air freed from carbon dioxide through distilled water for 12 to 16 hours. One drop of 0.0523 *N* potassium hydroxide solution produced a pink color in 300 cc. of this water to which one drop of phenolphthalein had been added.

**POTASSIUM PERMANGANATE.**—The oxidizing power of this reagent was measured against oxalic acid in the usual manner.

**ALKALI.**—Solutions of alkali were prepared from water free from carbon dioxide and potassium hydroxide purified by alcohol, the carbon dioxide being removed in the usual manner with barium hydroxide.

**PHOSPHORIC ACID.**—The phosphoric acid used in the distillations of volatile acids had an acid content of 85%. Blank analyses of the quantity of this acid used in the distillations yielded volatile components equivalent to 0.25 cc. of 0.0523 *N* potassium hydroxide solution.

**METHOD OF OXIDATION.**—The amount of materials undergoing oxidation in each case was 0.01 mole and the actual weights were as follows: (a) 1.801 g. of the hexoses with 13 g. of potassium permanganate; (b) 1.501 g. of the pentoses with 11.7 g. of the permanganate; (c) 1.5212 g. of *l*-arabitol with 11.7 g. of permanganate; (d) 1.221 g.

<sup>12</sup> Greifenhagen and Scholl, *Biochem. Z.*, **35**, 169 (1911).

<sup>13</sup> (a) Ref. 12 b, pp. 271, 289. (b) Börnstein and Herzfeld, *Ber.*, **18**, 3353 (1885). (c) Hömig, *Ber.*, **19**, 171 (1886). (d) Ruff, *Ber.*, **32**, 3672 (1899). (e) Habermann and Hömig, *Monatsh.*, **3**, 651 (1883). (f) Ssorokin, *J. Russ. Phys. Chem. Soc.*, **1**, 368 (1885).

of erythritol with 10 g. of permanganate; (e) 0.9006 g. of glyceric aldehyde with 7 g. of permanganate. To the potassium permanganate in a 2-liter round bottom flask was added 500 cc. of potassium hydroxide solution of the strength desired, except in the case of glyceric aldehyde where the volume of the alkali used was 250 cc. After the reaction flask had been closed with a stopper fitted with a stirrer A (Fig. 1), the solution of the compound to be oxidized (containing 0.01 mole in 25 cc.) was introduced by means of a buret attached to B at the rate of 0.5 cc. in five minutes until the 25 cc. had been added. This slow rate of addition was necessary because of the local heating occasioned by the interaction of the compound being oxidized and the oxidizing agent. Tube C was closed except when the alkali concentration was below 1 g. in 500 cc., in which cases it was used to admit 3 to 4 liters of air free from carbon dioxide for the purpose of removing that portion of the carbon dioxide that did not unite with the base during the reaction. The necessary absorption train was attached to D. In these cases of low alkalinity, it was found necessary to add 25 cc. of 2 *N* potassium hydroxide solution after the excess of the permanganate had been decolorized with sodium hypophosphite solution, because carbon dioxide was lost when the reaction mixture was filtered as described by Evans and Day.<sup>14</sup>

ANALYSIS.—The methods of analysis previously described<sup>15</sup> were used in the determination of the carbon dioxide, oxalic and acetic acids.

### Experimental Results

**Carbon Dioxide.**—Carbon dioxide was found in all the oxidation experiments. In neutral permanganate solutions the yields were approximately 98% in all cases. This amount decreased very rapidly upon the addition of alkali until a concentration of approximately 0.08 *N* of the base had been reached, at which point the amount of carbon dioxide began to increase gradually.

**Oxalic Acid.**—In the case of the hexoses oxalic acid was an intermediate oxidation product in neutral solutions, but in the presence of added alkali, this acid was found to be a final reaction product. That oxalic acid is an intermediate product in the neutral oxidations of the hexoses was established (by C. A. Buehler and C. W. Kreger, of this Laboratory) as follows. *d*-Glucose was oxidized at 27° in the manner described

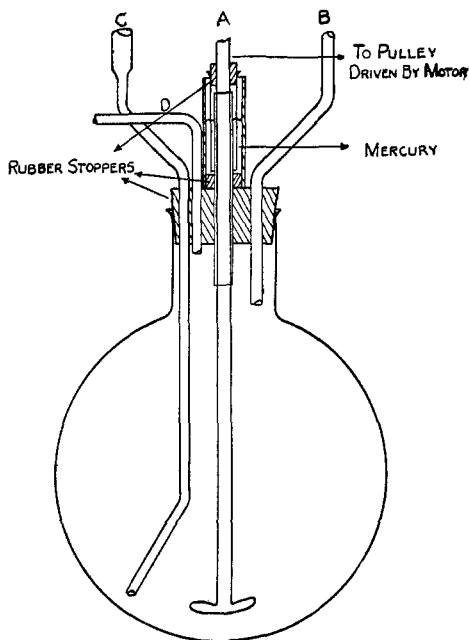


Fig. 1.

<sup>14</sup> Ref. 4 a, p. 1272.

<sup>15</sup> Ref. 4 a, p. 1268.

above. At the end of three hours the reaction mixture was worked up in the usual way. An analysis of the calcium oxalate which had been dried for three hours at  $105^{\circ}$  gave the following data: g. of  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  used: 0.1626, 0.1966.  $\text{CaSO}_4$  calcd.: 0.1515, 0.1832. Found: 0.1500, 0.1818 g. The other hexoses behaved in the same manner.

From these results we concluded that oxalic acid was present as potassium acid oxalate in those solutions to which no alkali had previously been added and also in those to which only small amounts of alkali had been added. It was found that solutions containing equivalent amounts of potassium acid oxalate decolorized potassium permanganate solutions after standing at room temperature for one day.

**Acetic Acid.**—The volatile acid which was found in all the oxidations never exceeded 3% of the total carbon introduced as carbohydrate. The amount of this acid (calculated as acetic) was practically constant for any given carbohydrate at any given temperature, but there seemed to be a slight increase in this amount with an increase in temperature.

Satisfying proof that the volatile acid obtained in our distillations was acetic acid could not be obtained with certainty, since the amounts present were not sufficient to cause the separation of silver acetate and the subsequent analysis of the purified silver salt. Arnold's method<sup>16</sup> for the detection of acetic acid gave a positive result when the phosphoric acid distillate from several oxidation solutions had been concentrated to a small volume.<sup>17</sup>

**Completeness of Oxidation.**—One of the greatest difficulties in these experiments was the failure of the oxidations in neutral solutions and in those of low alkalinity to go to completion at a temperature of  $25^{\circ}$ . In some of these cases, the oxidation was not complete at the end of 20 days' agitation.

**Experimental Data and Curves.**—The results of these experiments are shown in Figs. 2, 3 and 4. The weight of the carbon in carbon dioxide, oxalic and acetic acids obtained in these oxidations is expressed as a function of the initial concentration of the alkali. The lowest return of total carbon was 97.4% and the highest 102%, the general average of all completed experiments being for *d*-glucose 99.8%, *d*-fructose 100.4% and *d*-mannose 99.7%.

### Summary of Experimental Part

Our experimental results may be summarized as follows: (a) when *d*-glucose, *d*-fructose, *d*-mannose, *d*- and *l*-arabinose and *dl*-glyceric aldehyde are oxidized under the conditions of these experiments, the final

<sup>16</sup> Arnold, *Chem. Centr.* [ii] 70, 146 (1899).

<sup>17</sup> In some later experiments carried out in this Laboratory on the action of alkalis on aqueous solutions of *d*-glucose, it has been definitely established that acetic acid is a product of such a reaction.

reaction product in neutral solution is carbon dioxide in practically a quantitative yield, while in alkaline solution oxalic acid and a volatile acid (calculated as acetic) are also present; (b) the yield of carbon dioxide in the case of the hexoses increases with the temperature while that of oxalic acid decreases; (c) the oxalic acid reaches a maximum yield and the carbon dioxide a minimum one in solutions of potassium hydroxide of about 0.08 *N*, after which the yield of oxalic acid gradually diminishes while that of carbon dioxide shows a corresponding increase; (e) at a concentration of potassium hydroxide of 1.7–2.0 *N*, the yield of oxalic acid from the oxidation of *d*-fructose at 100° shows a tendency towards a second maximum, while the carbon dioxide shows a corresponding decrease.

### Theoretical Part

When *d*-glucose, *d*-mannose and *d*-fructose are oxidized at 50° with aqueous solutions of potassium permanganate and potassium hydroxide

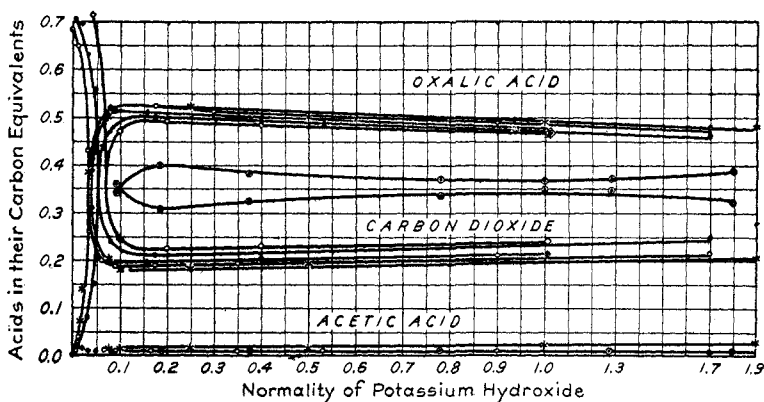


Fig. 2.—Oxidation of *d*-mannose at 50° (\*); *d*-glucose at 50° (◆) and 75°(◇); and *d*-fructose at 50° (○), 75° (●) and 100° (⊙).

of definite concentrations, it is found (Fig. 2) that within the limits of experimental error the oxalic acid-carbon dioxide ratios at given alkalinities are practically the same for each of these hexoses. This identity was also found to be true for *d*-glucose and *d*-fructose at 75° (Fig. 2). These facts lend support to the view that the general equilibrium condition found by de Bruyn and van Ekenstein in alkaline solutions of these hexoses would reach an adjustment by a choice of appropriate experimental conditions. Although the problem has not yet been studied in this Laboratory from this point of view, it is evident that the results obtained by us in the oxidation of *d*- and *l*-arabinose would probably be identical with those which one would obtain by the oxidation of *d*- and *l*-ribose<sup>18</sup>

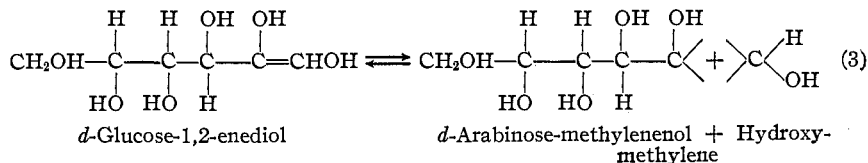
<sup>18</sup> Compare Nef, *Ann.*, **376**, 11 (1910).

and the corresponding 2-ketopentose. Obviously, the same general relationship would also hold true in the xylose and lyxose series, save in this case, however, the 1,2-enediols would be optical antipodes.<sup>19</sup> The results of oxidation of the arabinose-ribose series and those obtained with the xylose-lyxose series would be related to each other as are the oxidation results obtained from *d*-glucose and *d*-galactose, respectively.

These experiments also lend strong support to the views of Nef and his students to the effect that these three different hexoses must undergo a common oxidation mechanism; that is, before they can lose any carbon atoms they must first form the same chemically-active system, a condition which would be realized through the alkaline solution of *d*-glucose, *d*-mannose and *d*-fructose forming the three enediols which subsequently split into components that are common in each case. It should also be emphasized in this connection that alkaline solutions of either *d*-pseudo-fructose, *d*- $\alpha$ -glucose and *d*- $\beta$ -glucose should eventually give rise to the same chemically active components as do these more commonly known carbohydrates if these alkaline systems are reversible equilibria.<sup>20</sup>

Hence, our experimental results, as shown in Fig. 2 are probably the same as those one would obtain if either *d*-pseudo-fructose, *d*- $\alpha$ -glucose, or *d*- $\beta$ -glucose were oxidized under these same experimental conditions. It is to be noted that in this discussion we are considering the *d*- and *l*-glyceric derivatives as identical, since optical antipodes such as *d*- and *l*-arabinose are identical in their behavior when oxidized under these same experimental conditions at 50° (Fig. 3).

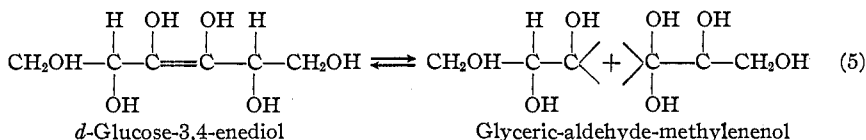
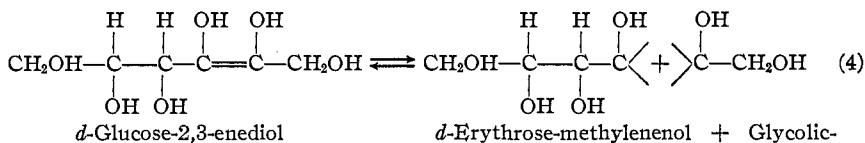
**Influence of the Alkali Concentration.**—An increase in the concentration of the alkali should tend to shift the equilibrium represented by Reaction 2 toward the formation of 2,3- and 3,4-enediols, the extent to which this would take place depending on such experimental conditions as temperature and the concentration of the alkali. These enediols may then split at the double bonds thus giving rise to a series of methylenenols, which, in turn, may undergo one or both of the following reactions: (1) a rearrangement to the corresponding aldehyde, and a subsequent formation of a new enediol (2) an oxidation to the corresponding acid. Such a splitting is represented in Reactions 3, 4 and 5.



<sup>19</sup> Compare Ref. 7 b, p. 80. Also Meyer and Jacobson, "Lehrbuch der Organischen Chemie," Veit and Co., 1913, vol. 1, part 2, p. 950.

<sup>20</sup> Compare Ref. 5 i.





From this point of view, therefore, it becomes clear that the character and amounts of the oxidation products obtained from *d*-glucose, *d*-mannose and *d*-fructose by their oxidation with neutral and alkaline potassium permanganate solutions must depend on the state of the equilibrium in the alkaline solution of the carbohydrates represented by Reactions 2, 3, 4 and 5.

**Carbon Dioxide.**—The carbon dioxide obtained in the oxidation of these hexoses may come from the oxidation of the formic acid resulting

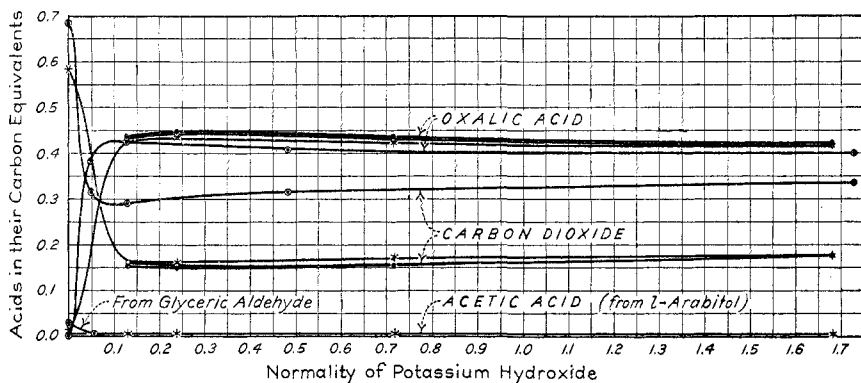


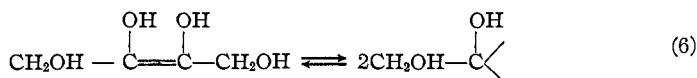
Fig. 3.—Oxidation of *d*- and *l*-arabinose (●, ○); *l*-arabitol (\*) and *dl*-glyceric aldehyde (⊙) at 50°.

from the action of the permanganate on the hydroxymethylene molecules. Our results show that the experimental conditions for realizing the highest yield of carbon dioxide from these compounds is one of low alkalinity, that is, in those regions where the 1,2-hexose-enediol largely determines the character of the products formed. If *d*-arabinose is formed in accordance with Reaction 3, we should expect it also to form 1,2- and 2,3-enediols with a subsequent dissociation into hydroxymethylene, and the methylenenols of *d*-erythrose, glycolic and glyceric aldehydes.<sup>21</sup> The *d*-erythrose would then undergo the same process yielding the methyl-

<sup>21</sup> Compare Ref 12, p. 251.

enol of glyceric aldehyde and two molecules of glycolic aldehyde methylenol. The final products after complete dissociation would be formaldehyde, glycolic aldehyde and glyceric aldehyde. In order that these several reactions of the hexose 1,2-enediol may harmonize with our experimental results, the oxidation of these three final products should be identical with those obtained with these compounds in neutral permanganate solution. It is a well-known fact that formaldehyde is oxidized quantitatively to carbon dioxide and water with neutral permanganate solutions. The same is true for glyceric aldehyde (Fig. 3). The work of Adkins and one of us on the oxidation of glycolic aldehyde indicates that *d*-erythrose would be oxidized to carbon dioxide alone with neutral permanganate solutions. Oxalic acid was obtained in addition to carbon dioxide when potassium glycolate was oxidized with neutral permanganate. Had the free acid been used carbon dioxide would undoubtedly have been the final reaction product. In view of the behavior of glyceric aldehyde with neutral permanganate this result is to be entirely expected from glycolic acid.

**Oxalic Acid.**—As the concentration of the alkali is increased, the equilibrium represented by Reaction 2 is shifted to the right. This general reaction results in the formation of compounds containing two or more carbon atoms with a consequent decrease in the formation of hydroxymethylene molecules. Obviously, this should give rise to an increased yield in the oxalic acid and a corresponding decrease in that of carbon dioxide. Our results show that this is true up to a given region of alkalinity after which there is an interruption in this tendency in consequence of which there results a maximum for carbon dioxide. In studying the cause for these maximum and minimum points, one must ascertain the possible sources from which the oxalic acid is derived. This acid may arise from the oxidation of glycolic acid,<sup>4b</sup> which in turn may be obtained from the following sources: (a) the oxidation of glycolic-aldehyde-methylenol in Reaction 4; (b) that obtained from the 2,3-enediol of *d*-erythrose (Reaction 4) and its subsequent splitting as shown by Reaction 6;



(c) that obtained from the enediol formation and splitting of any glyceric aldehyde which may be formed. That glycolic acid is actually formed in the alkaline oxidation of the hexoses has been abundantly pointed out in the literature.<sup>22</sup>

At the maximum point for the oxalic acid production from these car-

<sup>22</sup> Ref. 12 b, p. 277. Ref. 5 i, p. 256. Spoehr, *Am. Chem. J.*, **43**, 233 (1910). Ref. 9. Anderson, *Am. Chem. J.*, **42**, 401 (1909).

bohydrates at 50°, the oxalic acid-carbon dioxide ratio is 2.74. Adkins and one of us found that a solution of potassium glycolate under somewhat similar conditions gave a value of 3.34, which fact shows that other reactions are also taking place in the oxidation of these hexoses.

Furthermore, from this point of alkalinity, the three hexoses, the pentoses, and arabitol (Fig. 3) show a diminution in the yield of oxalic acid and a corresponding increase in that of carbon dioxide. This must be due in the case of the hexoses to some reactions taking place which will give an oxalic acid-carbon dioxide ratio less than 2.74. The value for the oxalic acid-carbon dioxide ratio of glyceric aldehyde in those regions of alkalinity at 50° where oxalic acid shows a diminution in yield is less than 2.74, and hence the curves for oxalic acid and carbon dioxide should approach each other rather than diverge if the equilibrium in Reaction 2 is strongly shifted to the 3,4-enediol by means of increased concentrations

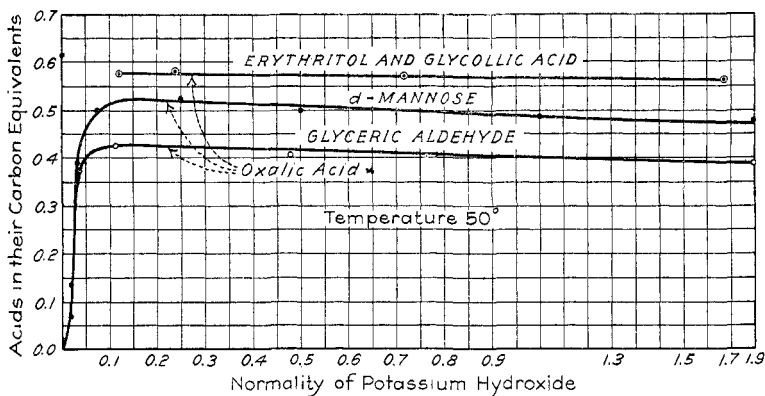


Fig. 4.

of alkali. To test this point of view further we oxidized a mixture of erythritol (1.22 g.) and glycolic acid (0.7604 g.) at 50°, a system intended to simulate, as nearly as we were able to do so, a 2,3-enediol formation of these carbohydrates with a subsequent splitting. Our oxalic acid data together with similar data for glyceric aldehyde are compared with those from *d*-mannose under the same conditions in Fig. 4. Although we had no *d*-erythrose at our disposal, the position and slope of the oxalic acid curve for *d*-mannose seems to support our point of view. While the behavior of glyceric aldehyde has not been exhaustively studied,<sup>23</sup> it is highly probable that the increased concentration of the alkali stabilizes it to the extent that its enediol is oxidized successively at this alkalinity to hydroxypyruvic aldehyde, glyceric acid, hydroxypyruvic acid, formylglyoxalic acid and glyoxalic acid. The oxidation of glyoxalic acid has

<sup>23</sup> At the present time, Mr. H. B. Hass of this Laboratory is studying the action of alkalies on glyceric aldehyde.

been studied<sup>4b</sup> under these conditions at 50°, but not at 75° and 100°. At 0.08 *N* alkali the oxalic acid-carbon dioxide ratio in glyoxalic acid was found to be 2.4 while that for these carbohydrates at the same alkalinity was also 2.4. Were the oxidation of glyoxalic acid the only reaction taking place in the oxidation of these hexoses at this alkalinity, this value would be lower because of the conversion of the third carbon atom of the glyceric aldehyde to carbon dioxide. In view of this fact it seems certain that the reaction giving rise to the first maximum in the oxalic acid production is still taking place to a lesser degree at the higher alkalinities. From the results of the oxidation of glyceric aldehyde it can be shown that the presence of this aldehyde alone in the oxidation of these hexoses at this alkalinity is also not sufficient to account for their oxalic acid-carbon dioxide ratio because that of the hexoses is greater than that for glyceric aldehyde.

Finally, at the higher concentrations of alkali, the glyceric aldehyde will be converted to lactic acid through the intermediate formation of pyruvic aldehyde.<sup>24</sup>

Professor L. F. Weinland, of Otterbein University, has informed us in a private communication that the yield of oxalic acid from lactic acid and alkaline potassium permanganate is an increasing function of the concentration of the alkali. This intermediate formation of lactic acid through the glyceric aldehyde and pyruvic aldehyde stages accounts for the tendency observed in *d*-fructose at 100° to yield a second maximum for oxalic acid and a second minimum for carbon dioxide at yet higher concentrations of alkali. This point of view is in harmony with the experimental results obtained recently in this Laboratory by Miss Rachel H. Edgar on the effect of alkalies and temperature on the production of lactic acid from *d*-glucose.

Owing to the apparently larger temperature coefficient for the carbon dioxide yield between 75° and 100° than that between 50° and 75°, the action of alkaline potassium permanganate on potassium oxalate at these temperatures was also studied. We found that the instability of the potassium oxalate in the presence of alkaline permanganate increases with the temperature, and the concentration of the alkali and the potassium permanganate. At 50° the effect is relatively slight, at 75° it becomes more marked while at 100° there is a rapid decomposition. Owing to the rapidity of carbohydrate oxidation in alkaline potassium permanganate, it is clear that the concentration of the oxidizing agent in these experiments is rapidly reduced to a point where its action becomes inappreciable. The amount of permanganate used was just slightly more than is necessary to oxidize the carbohydrate completely to carbon dioxide, thus leaving

<sup>24</sup> Ref. 12 a, p. 329. Evans, *Am. Chem. J.*, **35**, 116 (1905). Denis, *ibid.*, **38**, 561 (1907).

a very small concentration of permanganate at the close of the neutral oxidation. In all of our studies on the action of alkaline permanganate on potassium oxalate, the carbon in the salt was just equal in weight to two-thirds of that in the carbohydrate oxidized. The lowest concentration of potassium permanganate was 4.5 g. in 500 cc.

This action of alkaline potassium permanganate on potassium oxalate is referred to here because it may be urged that the tendency toward a minimum for oxalic acid and a maximum for carbon dioxide is more apparent than real. The tendency toward a second maximum for oxalic acid and a second minimum for carbon dioxide proves decisively that other factors play a role in this matter. This is clearly evident from the results of the changes taking place at 100° in which the reaction mixtures from the *d*-fructose oxidation were worked up at the end of 16 hours. In a later paper it will be shown by Crawford and one of us that these tendencies at 50°, 75° and 100° also characterize the oxidation of maltose. Looker and one of us also found the same phenomena at 100° with sucrose and also with a mixture of *d*-glucose and *d*-fructose equivalent to the sucrose used.

In conclusion we wish to thank E. I. du Pont de Nemours and Company whose kindly interest and good will have made certain portions of this work possible.

### Summary

In addition to the experimental summary given above we wish to add the following theoretical conclusions.

1. When *d*-glucose, *d*-mannose and *d*-fructose are oxidized in alkaline potassium permanganate solutions, oxalic acid, carbon dioxide and a trace of a volatile acid, probably acetic, are obtained. In neutral solutions (that is, with no added alkali) carbon dioxide is obtained almost quantitatively. The general effect of temperature is to increase the yield of carbon dioxide and decrease that of oxalic acid. Potassium oxalate is not completely stable under the conditions of these experiments at 100°.

2. Within the limits of experimental error, our results at 50° are identical for *d*-glucose, *d*-fructose and *d*-mannose, and at 75° for *d*-glucose and *d*-fructose, thus offering a verification of the classical experiments of de Bruyn and van Ekenstein on the action of alkalis on these three well-known carbohydrates. *d*-Mannose was not studied at 75°.

3. This identity in chemical behavior must be due to the presence of equilibria of the same chemically active components in the solutions of the hexoses. This is best explained by the views of Nef and his students who postulate the existence of a series of enediols which may undergo splitting and subsequent oxidation in the presence of alkalis and oxidizing agents.

4. At 100° and with increasing concentrations of potassium hydroxide,

the yield of oxalic acid from *d*-fructose reaches a maximum, a minimum, and a second maximum, successively, while the yield of carbon dioxide shows correspondingly opposite changes. These variations in the value of the yields of oxalic acid and carbon dioxide are due to the reactions which arise from the shifting of the equilibrium between the three enediols of *d*-fructose, and their subsequent splitting and oxidation. At 50° and 75° a similar tendency towards a minimum was observed.

5. The splitting products obtained from the enediols of the hexoses may also form enediols and subsequently split, a process which results in adding more components to the general equilibrium condition in these carbohydrate systems. Formaldehyde, glycolic aldehyde and glyceric aldehyde, products arising from the splitting of the hexose-1,2-, 2,3-, and 3,4-enediols, respectively, are thought to be the compounds producing the final oxidation products. Formaldehyde is oxidized to carbon dioxide, and glycolic aldehyde and glyceric aldehyde are oxidized to oxalic acid and carbon dioxide with potassium permanganate in the presence of alkali.

6. The tendency of the yield of oxalic acid from *d*-fructose to approach a second maximum at 100° is thought to be due to the formation of lactic acid under the influence of increasing concentrations of alkali and also of increased temperature, both of which factors tend to produce a greater formation of glyceric-aldehyde-methylenol. Lactic acid is formed by the action of alkalis on glyceric aldehyde through the intermediate formation of pyruvic aldehyde.

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

## THE MECHANISM OF CARBOHYDRATE OXIDATION. II. THE OXIDATION OF *d*-GALACTOSE

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The differences which may theoretically exist in alkaline solutions of *d*-galactose and *d*-glucose after these well-known aldohexoses have formed 1,2-, 2,3-, and 3,4-enediols, and then these enediols reacted in the presence of oxidizing agents by undergoing a splitting at the double bonds, pointed to a possible difference in the behavior of these two compounds when they were oxidized under the same experimental conditions. Before oxidation takes place, however, *d*-galactose in undergoing the enediol formation and subsequent splitting at the double bonds should give rise to the methylenols of *d*-lyxose, *l*-threose, glyceric aldehyde, glycolic aldehyde, and formaldehyde, while *d*-glucose, as pointed out

<sup>1</sup> Du Pont Fellow, 1922.