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

The Mechanism of Carbohydrate Oxidation. XXIV.[1] The Action of *aldehydo-d*-Glucose and of *aldehydo-d*-Galactose in Alkaline Solutions[2]

BY ROY JOSEPH PLUNKETT AND WILLIAM LLOYD EVANS

When *d*-glucopyranose and *d*-galactopyranose are treated with aqueous solutions of potassium hydroxide, lactic acid is formed in amounts which are dependent upon the concentration of the alkali used and the temperature employed. Shaffer and Friedemann[3] have found that the yields of lactic acid obtained from alkaline solutions of dihydroxyacetone are also a function of the concentration of the triose.

The reaction mechanism, which has been used to account for lactic acid formation in alkaline solutions of certain hexoses and other carbohydrates, involved a rupture of the oxidic structure with the resulting intermediate formation of the corresponding aldehydo structure. The next stage of the reaction assumed the fragmentation of the hypothetical hexose enediols[4] derived from the *aldehydo* sugar thus formed, an action which has been postulated as the third one in the series of changes resulting in lactic acid formation. This latter step should yield pyruvic aldehyde, the precursor of lactic acid, and, in addition, other products of fewer carbon atoms than the original carbohydrate. This has been found to be true. Nef[4] and Schmidt[5] have postulated mechanisms for the rupture of the hypothetical enediols formed by alkaline solutions of reducing sugars.

The discovery of *aldehydo-d*-glucose and *aldehydo-d*-galactose pentaacetates by Wolfrom[6] has made it possible to test the validity of the postulate that the *aldehydo* sugars, if formed as intermediates in the alkaline fragmentation of reducing carbohydrates, should yield lactic acid as one of the reaction products. To establish the validity of this assumption experimentally was the principal objective of this study.

Experimental Part

1. ***aldehydo-d*-Glucose Pentaacetate and *aldehydo-d*-Galactose Pentaacetate.**—These compounds were prepared according to the methods of Wolfrom.[6]

2. **Reagents.**—All reagents used throughout these experiments were examined for their purity by appropriate physical and chemical methods.

3. **Apparatus.** **(a) Volatile Acid Distillation.**—A 500-ml. round-bottomed flask, bearing a small separatory funnel and an aspirator, was connected through a Kjeldahl bulb and an adapter to the top of an upright coil condenser. The delivery end of the condenser dipped into a layer of water about 1 cm. in thickness contained in a 750-ml. suction flask. Heat was supplied through a water-bath and the whole system was evacuated by means of a water pump.

(b) Extraction Apparatus.—Modified Soxhlet[7] apparatus was used to extract the lactic acid.

(c) Volumetric Apparatus.—All volumetric apparatus was carefully calibrated by the usual well-known methods.

4. **Analytical Procedures.** **(a) Determination of Lactic Acid.**—The procedure for lactic acid was essentially the same as that reported by Nadeau, Newlin and Evans.[8] Only the procedure used for *β-d*-glucopyranose pentaacetate will be described since the same procedure with slight modifications was used throughout this work.

A small glass vial (18 × 50 mm.) was charged with *β-d*-glucopyranose pentaacetate (2.4385 g. = $^1/_{160}$ mole) and placed upright in a 150-ml. carbon dioxide flask containing 12.5 ml. of potassium hydroxide solution of the desired concentration. The flask was closed with a rubber stopper bearing two outlet tubes and a copper wire. The wire was used to hold the vial and its contents in an upright position until the time of mixing. The flask was then evacuated and filled with nitrogen gas, which had been scrubbed with ferrous sulfate and alkaline pyrogallol to remove any oxygen. The evacuation and introduction of nitrogen was repeated twice. The flask and its contents were then placed in the thermostatically controlled water-bath and then allowed to come to the temperature equilibrium. After ten minutes the copper wire was pulled out and the contents of the vial spilled into the potassium hydroxide solution. The flask and contents were agitated in the thermostat for a period of forty-eight hours (except in the case of the time runs) after which the flask was removed, a 5-ml. sample of the contents pipetted into a tared weighing bottle and the density determined. After the density determination the sample was returned to the flask, the stopper, pipet and bottle washed with distilled water, and the potassium hydroxide neutralized with 12.5 ml. of phosphoric acid of the appropriate molarity.

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(1) Number XXIII of this Series, Harry Gehman, Leonard C. Kreider and Wm. Lloyd Evans, THIS JOURNAL, **58**, 2388 (1936).

(2) Abstracted from a thesis presented by Roy Joseph Plunkett to the Graduate School of The Ohio State University in partial fulfilment of the requirements for the degree, Doctor of Philosophy.

(3) P. A. Shaffer and T. E. Friedemann, *J. Biol. Chem.*, **86**, 345 (1930).

(4) J. U. Nef, *Ann.*, **335**, 191 (1904); **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1913).

(5) Otto Schmidt, *Chem. Rev.*, **17**, 137 (1935); *Ber.*, **68**, 60, 795 (1935); *cf.* C. Neuberg, *ibid.*, 505.

(6) M. L. Wolfrom, THIS JOURNAL, **51**, 2188 (1929); **52**, 2464 (1930).

(7) W. L. Evans, R. H. Edgar and G. P. Hoff, *ibid.*, **48**, 2665 (1926).

(8) G. F. Nadeau, M. R. Newlin and W. L. Evans, *ibid.*, **55**, 4957 (1933).

The vial was then removed and the neutral solution treated with carboraffin, filtered, transferred to a 500-ml. round-bottomed flask and evaporated to dryness under a water pump vacuum at 50°. Forty ml. of water (in 10-ml. portions) were added to the residue and evaporated off to effect the completeness of the removal of acetic and formic acids. The residue was dissolved in a small amount of water (50–100 ml.) and transferred to the Soxhlet extractor, after which the aqueous solution was extracted for forty-eight hours with ether. The Sy flask contained a few grams of zinc carbonate and 50 ml. of water in addition to the ether. At the end of the forty-eight hour period the ether was evaporated off and the aqueous solution treated with carboraffin and boiled for a few minutes to complete the reaction between the zinc carbonate and lactic acid. The carboraffin and excess zinc carbonate were filtered off and the filtrate transferred to a 4-inch (10-cm.) evaporating dish and evaporated to dryness on a water-bath maintained at a temperature of about 50°. The crystallized zinc lactate trihydrate was treated with a minute amount of cold water and about 2 ml. of cold 95% ethanol and cooled to 0°. The crystalline salt was transferred to a tared Gooch crucible and washed with a few ml. of anhydrous ether. The salt was dried at 40–45° in a vacuum oven and examined quantitatively for its water of hydration and zinc oxide content. The filtrate and washings were evaporated for a second crop of zinc lactate trihydrate.

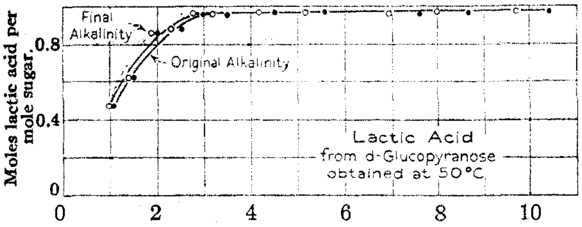

Fig. 1.—Lactic acid yields from *d*-glucopyranose expressed as a function of the original alkali normality and the final normality obtained after applying the necessary correction due to the expansion of the reaction mixture.

(b) **Saccharinic Acids.**—The aqueous solutions remaining after the extraction of lactic acid were evaporated to dryness and the residue freed from moisture by drying in a vacuum oven at 65°. The dried residue was placed in a bottle, covered with about 50 ml. of absolute ethanol, the bottle stoppered, and then allowed to stand for several days at room temperature. The mixture was next digested on the hot-plate for two to three hours and filtered through a Büchner funnel, the phosphate residue being washed with absolute ethanol. The filtrate and washings were transferred to a 250-ml. beaker, water added, and the alcohol evaporated off. An excess of zinc carbonate was added and the mixture digested on the hot-plate for several hours. This was necessary because it was found by titration that the saccharinic acids were present in the form of their lactones, which hydrolyzed slowly. After the completion of the reaction between the saccharinic acid and zinc carbonate the excess zinc carbonate was filtered off, the filtrate concentrated to low volume and transferred to a tared crucible. The liquid in the crucible was evaporated to dryness, the crucible weighed and the zinc saccharinate roasted to zinc oxide and the crucible reweighed. The ratio of zinc oxide/zinc saccharinate indicated that the saccharinic acids were largely $C_6H_{12}O_6$, *i. e.*, rearranged hexoses.

5. **Effect of Expansion on Alkali Normality.**—In comparing the lactic acid yields obtained from the pentaacetates of *d*-glucopyranose and *d*-galactopyranose with those of *d*-glucopyranose and *d*-galactopyranose themselves, it was desired to plot the amount of lactic acid produced as a function of the normality of the alkali. To make the data comparable it was necessary to apply a correction factor to the alkali concentration used with the pentaacetates to account for the potassium hydroxide used up in neutralizing the acetic acid formed by the hydrolysis of the acetates.

In this manner, using equivalent quantities of the various sugars, it was thought that the amount of sugar and the alkali concentration in the solutions were comparable. However, in some preliminary experiments by Mr. L. C. Kreider,[9] of this Laboratory, and ourselves, it was noted that when β-glucopyranose pentaacetate was dissolved in potassium hydroxide there was an appreciable expansion in volume. Thus it became necessary to determine experimentally the magnitude of this expansion for the acetylated sugars and for the sugars themselves and to make suitable corrections for the change in alkali normality before strict comparisons could be made.

From the density of the solutions and the weight of materials originally used, the volume and the potassium hydroxide concentration of the reacting solution were calculated easily. This value is only as accurate as our method used in determining the density. The density measurements could be reproduced within 1%, which is sufficiently accurate for this work. The expansion in all cases for the pentaacetyl derivatives was found to be approximately 18%, while that of *d*-glucopyranose was approximately 8%. Our experience shows that the volume change was inappreciable during the time of the experiment.

In Fig. 1 are shown the effects of the correction for such expansion on the character of the lactic acid curve obtained from *d*-glucopyranose at 50°, while in Fig. 2 similar data are plotted for β-*d*-glucopyranose pentaacetate. The curves for *d*-glucopyranose and β-*d*-glucopyranose pentaacetates are not greatly changed, except in the points being shifted in a direction parallel to the potassium hydroxide axis. However, the curve for *aldehydo-d*-glucose pentaacetate is altered quite appreciably (Fig. 3). The same relationships are true at least qualitatively for all of the experimental data herein reported.

In connection with the density determinations it should be pointed out that Powell[10] in studying the velocity of decomposition of dextrose and fructose by alkalies, measured the change in volume during the reaction by means of a very delicate dilatometer. He noted that for a reaction volume of about 15 ml. (sugar concn. = 0.4 *M*) the expansion was of the order of magnitude 0.15 ml. This expansion would lie within our experimental error.

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[9] Harry Gehman, L. C. Kreider and W. L. Evans, This Journal, **58**, 2388 (1936).

[10] C. W. R. Powell, *J. Chem. Soc.*, **107**, 1335 (1915).

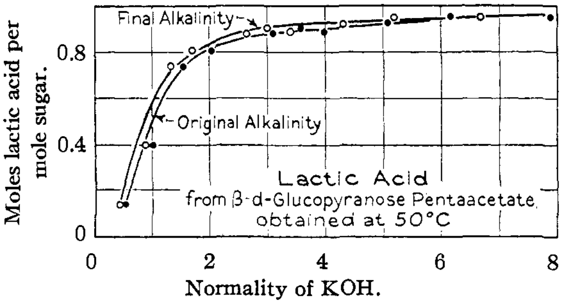

Fig. 2.—Lactic acid yields from *β-d*-glucopyranose pentaacetate expressed as a function of the original alkali normality and the final normality obtained after applying the necessary correction due to the expansion of the reaction mixture.

The increase in volume of our solutions caused a decrease in the sugar concentration. We were interested to learn if this decrease would have any effect on the lactic acid production. To check this point, varying amounts of *d*-glucopyranose (2.0000–2.5000 g.) were treated with 25 ml. of 3.221 *N* potassium hydroxide. The amount of lactic acid produced is 0.95 mole per mole of *d*-glucopyranose over this range of concentration. Thus the lowering of the sugar concentration by the increase in volume had no effect on lactic acid production, under our experimental conditions. These results are in quantitative agreement with those of Shaffer and Friedemann,[3] who found that the yield of lactic acid from 5 *N* sodium hydroxide solutions containing 100–500 mM. equivalents of glucopyranose is 1.05–1.01 moles. The concentration of sugar in our experiments was in no case far removed from 500 mM. equivalents.

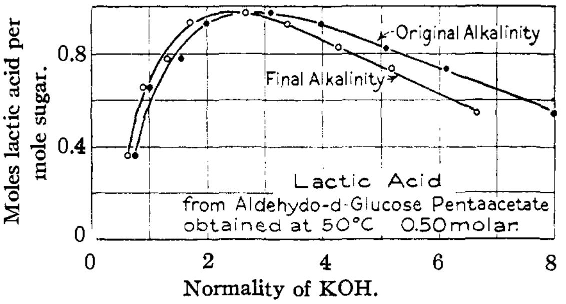

Fig. 3.—Lactic acid yields from *aldehydo-d*-glucose pentaacetate expressed as a function of the original alkali normality and the final normality obtained after applying the necessary correction due to the expansion of the reaction mixture.

The Effect of Time on the Lactic Acid Production.—In a comparative study of this type it is necessary that the reactions proceed to completion. At first it was thought that the differences in lactic acid production might be due to different reaction rates. This point was cleared up by running *d*-glucopyranose, *β-d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate in approximately 3 *N* potassium hydroxide for various periods of time from five minutes to forty-eight hours. Reference to Fig. 4 shows that the yield of lactic acid increases rapidly with time, reaching a constant value in about four hours. The quantitative differences between the three curves are obvious in Fig. 4. The data show definitely that the reaction was completed in the forty-eight-hour period.

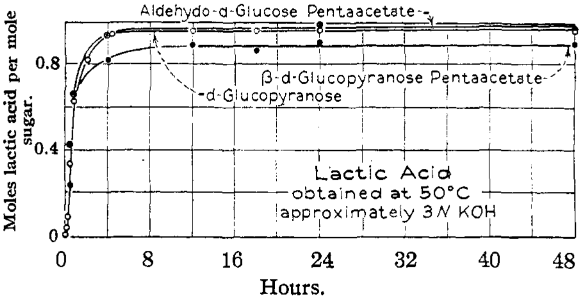

Fig. 4.—Lactic acid yields obtained from alkaline solutions of *d*-glucopyranose, *β-d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate expressed as a function of time.

Discussion

Lactic Acid Formation from *aldehydo*-Hexoses.—The data obtained in this experimental study concerning the behavior of *aldehydo-d*-glucose pentaacetate and *aldehydo-d*-galactose pentaacetate in alkaline solutions show conclusively that lactic acid is formed in these reactions under the same conditions as were used in obtaining this acid from *d*-glucopyranose and *d*-galactopyranose, respectively. *Therefore, it is clear that the mechanism which postulates the intermediate formation of aldehydo structures from the respective pyranoid structures in the production of lactic acid from the sugars seems to be well founded.*

Marchlewski[11] and collaborators found that alkaline solutions of glucose, galactose, maltose and arabinose give strong absorption bands in the ultraviolet, but after neutralization the original continuous spectrum is restored. The band in the region of about 2700 Å. is characteristic for alkaline solutions of the above carbohydrates and is attributed to the formation of an aldehyde group.

Effect of Temperature and Alkali Normality on Lactic Acid Formation.—Since it was necessary to use *aldehydo-d*-glucose pentaacetate and *aldehydo-d*-galactose pentaacetate in these experiments, it followed that one each of the *d*-glucopyranose pentaacetates and *d*-galactopyranose pentaacetates should be employed throughout for comparative purposes. We chose *β-d*-glucopyranose pentaacetate and the *β-d*-galactopyranose

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[11] (a) Gabryleski and Marchlewski, *Biochem. Z.*, **261**, 393 (1933); *C. A.*, **27**, 4218 (1933); (b) Marchlewski and Urbanczyk, *Biochem. Z.*, **262**, 248 (1933); *C. A.*, **27**, 5004 (1933).

pentaacetate in each case by reason of their more favorable solubilities.

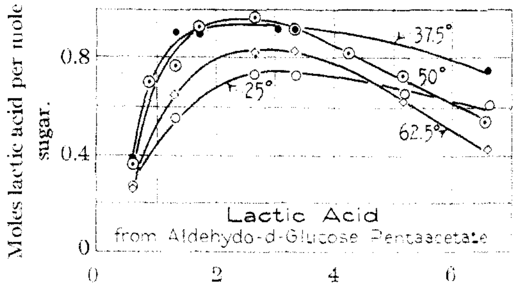

Fig. 5.—Lactic acid yields obtained from alkaline solutions of *aldehydo-d*-glucose pentaacetate at 25, 37.5, 50 and 62.5°.

(a) The yields of lactic acid obtained from the *aldehydo-d*-glucose pentaacetate under varying alkali normalities at 25, 37.5, 50 and 62.5° are shown in Fig. 5, while the comparative data obtained from *β-d*-glucopyranose pentaacetate under the same experimental conditions are shown in Fig. 6.

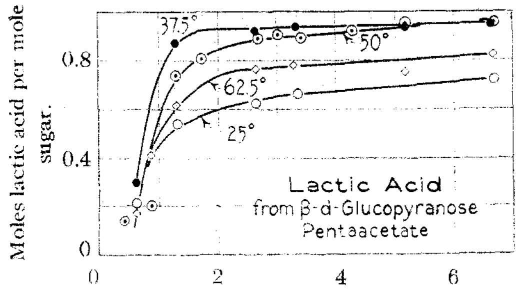

Fig. 6.—Lactic acid yields obtained from alkaline solutions of *β-d*-glucopyranose pentaacetate at 25, 37.5, 50 and 62.5°.

From these two figures it is seen that in some respects *d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate differ quite markedly in their behavior in alkaline solutions of the same alkali normality and the same temperature. The diminishing yields of lactic acid from the aldehydo form of the sugar after the range 2–3.5 N has been passed are in marked contrast to those obtained from the pyranoid form. From limited experimental data available in this Laboratory it has become increasingly evident that these diminishing yields of lactic acid from *aldehydo-d*-glucose pentaacetate are compensated for by the increasing yields of saccharinic acids, while the increasing yields of lactic acid obtained from *d*-glucopyranose pentaacetate are on the contrary accompanied by diminishing yields of the saccharinic acids.[3] This relationship is shown in Fig. 7 for which the data have been gathered by a method which leaves much to be desired. This same general relationship was observed by Gehman, Kreider and Evans[9] in a study of the products obtained by the action of mixtures of cellobiose octaacetate and dihydroxyacetone monoacetate in alkaline solutions. In this connection it also should be noted that Hoff[7] obtained crystalline *d*-galacto-*α*-saccharinic acid lactone from alkaline solutions of *d*-galactopyranose in yields which increased with the temperature and the alkali normality.

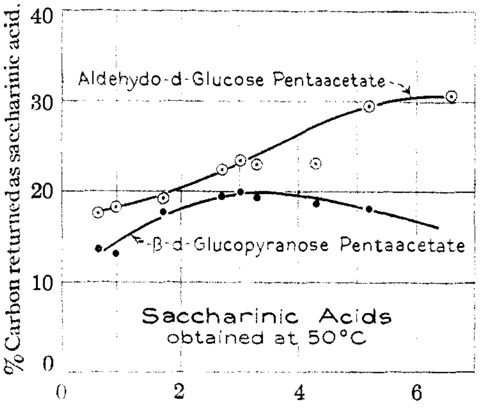

Fig. 7.—Saccharinic acids obtained from alkaline solutions of *aldehydo-d*-glucose pentaacetate and *β-d*-glucopyranose pentaacetate.

We may classify the reactions of reducing sugars in alkaline solutions into the following general groups: (1) they may undergo rearrangement to form saccharinic acids; (2) they may undergo the Lobry du Bruyn and Alberda van Ekenstein rearrangement to form other reducing sugars; (3) they may rearrange to form hypothetical enediols; (4) they may mutarotate; (5) they may form polymers; (6) they may fragment. The general relationship existing between the yields of lactic and saccharinic acids may be regarded as due to differences or equalities in the rates of the two reactions here shown

$$\text{Fragmentation} \longleftarrow \text{Reducing sugar} \longrightarrow \text{Rearrangement}$$

The fragments may undergo rearrangement to give acetic, lactic, C_4 and C_5 saccharinic acids, while the sugar itself may at the same time rearrange to the C_6 saccharinic acids. It has been pointed out by Hockett[12] that glycol aldehyde, a

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[12] W. L. Evans and R. C. Hockett, This Journal, **52**, 4065, ref. 14 (1930).

theoretically possible fragmentation product of the saccharides, must be regarded as one of the possible sources of acetic acid when this simplest of all carbohydrates is reacted upon by alkalies, *i. e.*, acetic acid must be considered the saccharinic acid of glycol aldehyde in quite the same way as lactic acid may be regarded as the saccharinic acid of glyceric aldehyde and dihydroxyacetone.

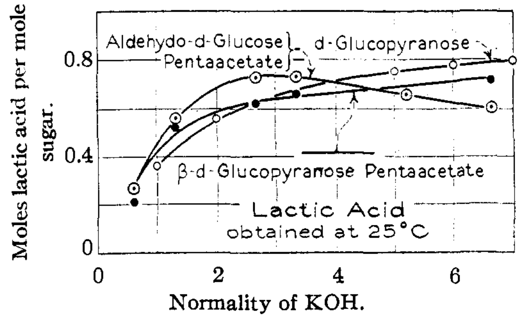

Fig. 8.—Comparison of lactic acid yields obtained from alkaline solutions of *d*-glucopyranose, β-*d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate at 25°.

From the above discussion it is evident that the relationship between the yields of C_6 saccharinic acids and those containing less carbon atoms must depend on many experimental factors among which are temperature, alkali normality and concentration of sugar studied. From Figs. 5 and 7 it is clear that approximately 73% of the *aldehydo-d*-glucose pentaacetate carbon has been recovered at 50° between 2–3 *N* potassium hydroxide as lactic and saccharinic acids. This is our maximum value in this series of studies. These reactions are accompanied by tar formation. Work is now in progress in this Laboratory to extend our knowledge of these complicated systems. (b) From the data in Figs. 5 and 6 it is seen that the lactic acid yields from *aldehydo-d*-glucose pentaacetate and *d*-glucopyranose pentaacetate are not necessarily at a maximum at the highest temperatures. At 37.5° and in the region of lower alkali normalities, the two compounds seem to be more sensitive than at the other temperatures studied and tend to give as high yields of lactic acid as under more drastic conditions of alkalinity.[8] This is more especially true with the pyranoid structure, while with the aldehydo form the yields at 37.5 and 50° from 0.6 *N* to approximately 4 *N* are almost the same. It is to be noted that the yields of lactic acid are lower from both compounds at 62.5°, a fact in harmony with previous studies[13] on *d*-glucopyranose, *d*-mannopyranose and *d*-fructopyranose at 75°. The lactic acid data obtained by a comparative study of *aldehydo-d*-glucose pentaacetate, *d*-glucopyranose, and β-*d*-glucopyranose pentaacetate at 25° are shown in Fig. 8.

Although no extensive experimental studies have been made of the effect of temperature and different alkali normalities on the formation of saccharinic acids containing six carbon atoms from the hexoses except that of Hoff,[7] it may be concluded safely that the data obtained by such an inquiry will be found in general to be in accord with the views expressed above with reference to lactic and saccharinic acid formation at 50° with various alkali normalities. (c) The yields of lactic acid obtained from *aldehydo-d*-galactose pentaacetate, *d*-galactopyranose pentaacetate and *d*-galactopyranose at 25 and 50° are shown in Figs. 9 and 10, respectively. It is seen that the *aldehydo* structure seems to be more reactive than either of the two pyranoid structures which were used for comparative purposes. The *aldehydo* structure reaches a maximum production of lactic acid at a lower alkalinity at 50° than it does at 25°.

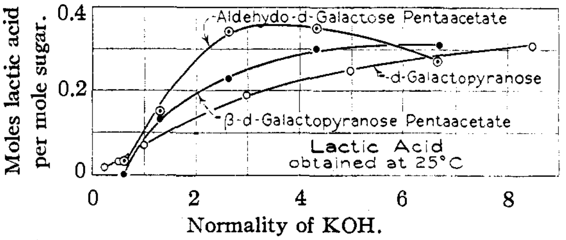

Fig. 9.—Comparison of lactic acid yields from alkaline solutions of *d*-galactopyranose, *d*-galactopyranose pentaacetate and *aldehydo-d*-galactose pentaacetate at 25°.

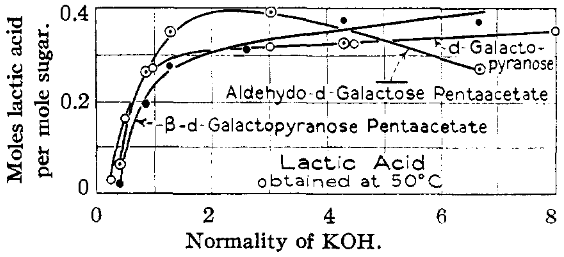

Fig. 10.—Comparison of lactic acid yields from alkaline solutions of *d*-galactopyranose, *d*-galactopyranose pentaacetate and *aldehydo-d*-galactose pentaacetate at 50°.

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[13] (a) W. L. Evans and J. E. Hutchman, THIS JOURNAL, **50**, 1496 (1928); (b) W. L. Evans and D. C. O'Donnell, *ibid.*, **50**, 2543 (1928).

(d) Although the galactose series does not yield as much lactic acid as the glucose series under our experimental conditions the data obtained by Hoff[7] show that the formation of *d*-galacto-α-metasaccharinic acid at 50° increases rapidly with increasing alkali normalities, a fact in harmony with the views expressed above. Whether there is a higher concentration of the *aldehydo* form of galactose under these conditions has not been established.

Effect of Potassium Acetate on Lactic Acid Formation.—Since potassium acetate is formed in all of these reaction mixtures, it became necessary to determine the extent of its influence on the formation of lactic acid. This was done by studying the yields of this acid obtained from *d*-glucopyranose, *aldehydo-d*-glucose pentaacetate, *β-d*-glucopyranose pentaacetate, and a mixture of *d*-glucopyranose and potassium acetate equal to that produced by the hydrolysis of the pentaacetates, at 50° and at various alkali normalities. The data obtained in these experiments are shown in Fig. 11. An examination will show that in general there is a close correspondence in the results obtained in the pyranoid structures, especially in the case of the two pentaacetates. At the higher alkalinities the yields of lactic acid from the pyranoid structures tend to become more nearly identical, *i. e.*, the data are within the limits of the experimental error.

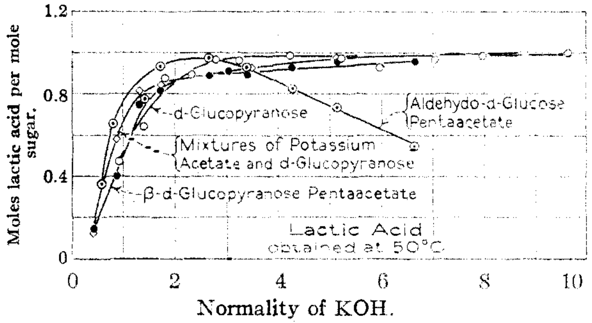

Fig. 11.—Comparison of the yields of lactic acid obtained from alkaline solutions of *d*-glucopyranose, *β-d*-glucopyranose pentaacetate, *aldehydo-d*-glucose pentaacetate, and mixtures of potassium acetate and *d*-glucopyranose.

Acknowledgment.—The authors wish to express their indebtedness to Drs. Leonard C. Kreider and Harry Gehman for valuable assistance rendered during the progress of this work.

Conclusions

1. (a) A comparative study has been made of the yields of lactic acid obtained from *d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate at 25, 37.5, 50 and 62.5° in potassium hydroxide solutions ranging from 0.5–6 *N*. (b) A similar study was made with *d*-galactopyranose pentaacetate and *aldehydo-d*-galactose pentaacetate at 25 and 50°.

2. Lactic acid was obtained as a reaction product from the *aldehydo* structures as well as from the pyranoid structures, a fact which supports the postulate that pyranose sugars are converted into *aldehydo* ones as intermediate compounds in the mechanism involved in the formation of lactic acid in alkaline solutions.

3. The most striking difference in the yields of lactic acid obtained from the pyranoid and *aldehydo* structures of both glucose and galactose lies in the maximum point reached by the *aldehydo* structures after which there is a marked decrease in the yield of this acid.

4. (a) In the glucose series the difference in lactic acid yields between the *aldehydo* and pyranoid structures has been attributed to a concurrent production of the isomeric saccharinic acids at such rates as to produce this effect. (b) At 50°, it was found that the yields of lactic acid obtained from *d*-glucopyranose pentaacetate and *aldehydo-d*-glucose pentaacetate bear an inverse relation to their respective yields of the saccharinic acids.

5. At 50°, the *aldehydo-d*-glucose pentaacetate yielded the maximum amount of its carbon as lactic and saccharinic acids, namely, approximately 73% between 2–3 *N* potassium hydroxide.

6. At 37.5° and the lower alkali normalities, *d*-glucopyranose pentaacetate seems to be more sensitive toward alkalies than at either 25, 50 or 62.5°.

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