[Contribution from the Chemical Laboratory of The Ohio State University]

THE MECHANISM OF CARBOHYDRATE OXIDATION. IV. THE ACTION OF POTASSIUM HYDROXIDE ON *d*-GLUCOSE[1] AND *d*-GALACTOSE[2]

By Wm. Lloyd Evans, Rachel Hartman Edgar and George Preston Hoff

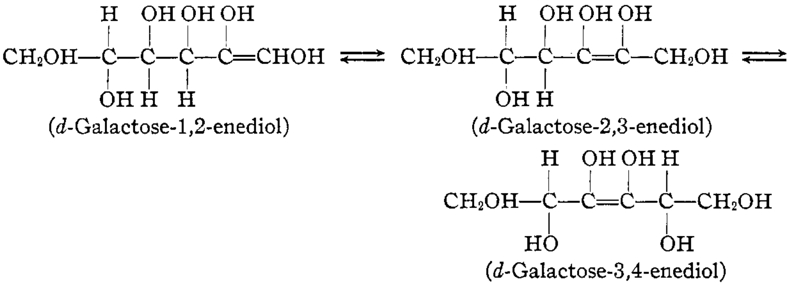
Received June 28, 1926 Published October 6, 1926

In some recent reports[3] concerning the work being done in this Laboratory on the oxidation of organic compounds it was pointed out that the results obtained by the action of neutral and alkaline potassium permanganate solutions on certain carbohydrates and polyatomic alcohols are best understood when they are considered in part both from the standpoint of the views of Lobry de Bruyn and Alberda van Ekenstein, and also from that of Nef and his students concerning the behavior of alkalies on these classes of organic compounds. The former investigators showed that both *d*-galactose and *d*-glucose undergo a series of changes in alkaline solutions which may be represented by Reactions 1 and 2.

d-galactose $\rightleftharpoons$ *d*-tagatose $\rightleftharpoons$ *d*-talose $\rightleftharpoons$ *l*-sorbose $\rightleftharpoons$ *d*-α-galtose $\rightleftharpoons$ *d*-β-galtose (1)

d-glucose $\rightleftharpoons$ *d*-fructose $\rightleftharpoons$ *d*-mannose $\rightleftharpoons$ *d*-pseudofructose $\rightleftharpoons$ *d*-α-glutose $\rightleftharpoons$ *d*-β-glutose (2)

As is well known, Nef and his collaborators postulated the existence in alkaline solutions of these hexoses of a series of enediols, consisting of 1,2, 2,3 and 3,4 forms which may be represented in the case of *d*-galactose by Reaction 3.



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

(*d*-Galactose-1,2-enediol) (*d*-Galactose-2,3-enediol)

$$\mathrm{CH_2OH{-}}\underset{\mathrm{HO}}{\overset{\mathrm{H}}{\mathrm{C}}}{-}\overset{\mathrm{OH}}{\mathrm{C}}{=}\overset{\mathrm{OH}}{\mathrm{C}}{-}\underset{\mathrm{OH}}{\overset{\mathrm{H}}{\mathrm{C}}}\mathrm{{-}CH_2OH} \qquad (3)$$

(*d*-Galactose-3,4-enediol)

It was furthermore pointed out by the authors in these reports that should there be a general equilibrium,[4] reversible or irreversible, in alkaline solutions of *d*-galactose, *d*-fructose, *d*-mannose and *d*-glucose between the

---

[1] Presented at the Baltimore meeting of the American Chemical Society, April, 1925.

[2] Presented at the First National Organic Symposium, Organic Section, American Chemical Society, Rochester, N. Y., December, 1925.

[3] Evans and co-workers, This Journal, **47** (a) 3085 (b) 3098 (c) 3102 (1925).

[4] Ref. 3 a. Compare also Spoehr and Wilbur, "Annual Report of the Director of the Laboratory for Plant Physiology," *Carnegie Inst. Pub.*, **1925**, p. 158.

substances indicated in Reactions 1, 2, 3, including also the various dissociation products arising from the splitting at the double bonds of the enediol forms of Reaction 3, then it was conceivable that there might be certain experimental factors, such as temperature and concentration of the alkali used, which would cause a shifting in the equilibrium point of such a system. In view of the results obtained in the oxidation experiments, it was now deemed of the greatest importance to study the behavior of *d*-glucose and *d*-galactose towards various concentrations of alkalies and at different temperatures in the absence of potassium permanganate for the purpose of ascertaining whether the properties of alkaline solutions of these carbohydrates would be in accord with the general views expressed above.

Experimental Part

Treatment of Aldohexoses with Potassium Hydroxide.—The general method of procedure employed with *d*-glucose and *d*-galactose varied slightly in some few details.

We began our work with *d*-glucose. Forty-five g. of this carbohydrate was placed in a 500cc. volumetric flask and dissolved in sufficient distilled water to bring the total volume to 500 cc. after the calculated volume of the standard potassium hydroxide solution necessary to give the desired normality had been added. This reaction mixture was placed in a 1000cc. round-bottomed Pyrex flask. Profiting by our experiences with *d*-glucose we found that our ends could be attained by the use of smaller quantities of material. Hence, with *d*-galactose one-sixteenth of a gram mole of the carbohydrate, also of known moisture content, was dissolved in 125 cc. of potassium hydroxide solution of the desired normality contained in a 500cc. flask. In both cases the flasks were immediately closed with a mercury-sealed stirrer and the contents were then agitated at a constant temperature for 48 hours. At the end of this time the reaction mixtures were treated with the calculated amounts of concd. hydrochloric acid. The resulting solutions were then distilled at 12–35 mm. from flasks of appropriate size fitted with stoppers containing a Kjeldahl bulb and a capillary tube. The suction flask used as a receiver was surrounded with ice water. When this operation was carried out with care no chlorides were found in the distillates. The chloride-free product was then made up to a known volume.

Volatile Acids. (a) Formic Acid.—From theoretical considerations, formic acid should be one of the products of the reaction between potassium hydroxide and these two aldohexoses, and hence it should be present in the distillate obtained as just described. Since the amount of this acid was determined by its reducing action, it was essential to show, if possible, that other reducing substances were absent and that the acid itself was present. (1) When the distillates from both *d*-glucose and *d*-galactose were examined for formic acid by the well-known Fincke's[5] test, a white precipitate of mercurous chloride was obtained in each case. (2) A portion of these distillates when treated with mercuric oxide[6] yielded metallic mercury. (3) Cold potassium

---

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

[6] Mulliken, "Methods for the Identification of Pure Organic Compounds," John Wiley and Sons, New York, **1914**, p. 83.

permanganate solutions[7] were also reduced. The formic acid was determined by the following modification of the well-known method of Jones.[8]

One-tenth of the distillate, diluted with 200 cc. of recently-boiled distilled water, was made alkaline with about 1 g. of sodium carbonate, then treated with 25 cc. of standard potassium permanganate solution and heated to 70–80° for half an hour. This solution, acidified with 4 cc. of sulfuric acid solution (1:1) after the addition of 25 cc. of 0.1 *N* oxalic acid, was then titrated with standard potassium permanganate solution. Each reported determination represents the average of at least three closely agreeing titrations.

(b) **Hydroxy Acids.**—The method of Denigès[9] gave only the faintest test for the presence of hydroxy acids. It should be noted that any undecomposed formic acid or any acetic acid would also give the characteristic color of this reaction. It is quite improbable that hydroxy acids are present in the distillates even in traces.

(c) **Acetol and Pyruvic Acid.**—When the distillates were examined for the presence of acetol by means of *o*-aminobenzaldehyde according to the method of Baudisch and Deuel,[10] no bluish fluorescence was obtained. No positive reaction was obtained when the distillates were examined for the presence of pyruvic acid by means of Hurtley's test.[11]

(d) **Acetic Acid.**—The presence of acetic acid in the distillate was proved by the following tests. (1) When the distillates were examined with *p*-amino-acetophenone by Arnold's[12] method, a purple-violet color was obtained. (2) Four parts of the salt residue obtained by concentrating the distillates previously neutralized with potassium hydroxide gave the characteristic odor of cacodyl oxide when treated with arsenious oxide. (3) The odor of ethyl acetate was detected by the well-known test with ethyl alcohol. (4) Our most convincing evidence for the presence of acetic acid was that obtained through the conversion of a portion of the neutralized distillates into *p*-acetotoluide (m. p., 146.5°).[13] The difference between the total acidity of an aliquot part of the distillate from the salt residue and the acidity due to its formic acid content was calculated as acetic acid.

Salt Residue. Lactic Acid.—After the distillation described above, the salt residue was dried at 65° in a 10cm. crystallization dish and then placed in a vacuum oven at the same temperature and 250–350 mm. for two days. The material was then ground to a uniform texture. The residues obtained from alkalinities below 1.0 *N* set to a gummy mass which resisted handling. In such cases the sample was mixed with a small amount of sand before evaporation, a procedure which gave a product easily handled.

A portion of the salt residue was weighed into fat-free Whatman paper thimbles and placed in a Soxhlet extractor, connected by a mercury seal to a Sy flask containing *dry* ether. This specimen was extracted for 120 hours at a rate which would be equivalent to the use of 40 liters of ether. At the end of this time the aqueous solution of the residue obtained from the ether extract was treated with zinc carbonate in the usual way, but no trace of zinc lactate was obtained. In our earlier experiments with *d*-glucose, the lactic acid was extracted from the moistened residues.

---

[7] Lieben, *Monatsh.*, **14**, 746 (1893).

[8] Jones, *Am. Chem. J.*, **17**, 539 (1895). Compare Griffin, "Technical Methods of Analysis," McGraw-Hill Book Company, New York, **1921**, pp. 81–82.

[9] Denigès, *Bull. soc. chim.*, [4] **5**, 647 (1909).

[10] Baudisch and Deuel, THIS JOURNAL, **44**, 1585 (1922).

[11] Plimmer, "Practical Organic and Biochemistry," Longmans, Green and Co., New York, **1915**, p. 113.

[12] Arnold, *Chem. Zentr.*, [II] **70**, 146 (1899).

[13] Ref. 6, p. 81.

We found that water was necessary for the extraction of lactic acid; hence, the salt residues were dissolved in the least amount of water and any insoluble charred material was removed by filtration. An aliquot part of this solution was placed in a specially constructed Soxhlet apparatus as shown in Fig. 1. During the progress of this work a similar instrument was described by Palkin, Murray and Watkins.[14] The extraction was continued for 48 hours, at the end of which time it was found that all of the lactic acid had been removed. The residue obtained from the ether extract was converted into zinc lactate, whose crystals could be washed free from any gummy material with minute amounts of water cooled below 5°, at which temperature the salt was only sparingly soluble. The zinc lactate crystals were then filtered off, washed with 95% alcohol, dried at 50°, cooled and weighed. Throughout this work the zinc lactate, $Zn(C_5H_5O_3)_2.3H_2O$, was constantly examined quantitatively for its water of hydration and its zinc oxide equivalent.

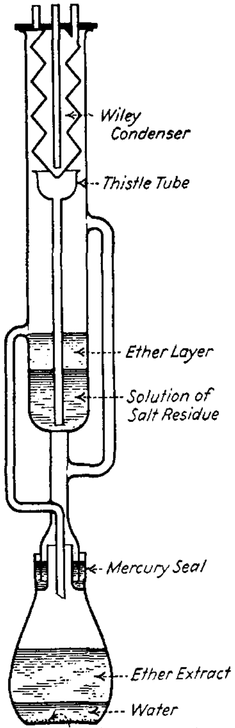


Fig. 1.—Diagram of extractor.

d-Galacto-α-metasaccharinic Acid Lactone.—The extracts (from d-galactose) with *dry* ether referred to above were retained because, after four to six hours' extraction of the salt residues from reactions carried out in 4 N alkali and in solutions of greater alkalinity, it was found that the ether became turbid and finally nodules of white crystals began to appear. These increased in amount until the third day, after which no further increase in the quantity could be noted. Owing to the very low solubility of these crystals in ether their total weight was determined by dissolving them in alcohol and then evaporating this solvent under diminished pressure. Successive crystallizations of the white solid from ten times its weight of hot alcohol gave clear, hard, tablet-like crystals; m. p., 143.5°. This substance had scarcely any taste, reacted neutral towards litmus, and was readily soluble in water and alcohol. It corresponded in every respect with d-galacto-α-metasaccharinic acid lactone described by Nef[15] who gave the melting point at 144°, while Upson[16] gave it as 142–144°, and Kiliani[17] as 142°. A molecular-weight determination of the lactone was made in the following way. A small portion of the substance was dissolved in water and heated to boiling. Standard alkali was added until the faint pink color of phenolphthalein was no longer discharged on warming. The following data confirmed the molecular weight as being 162; that is, $C_6H_{10}O_5$.

TABLE I

MOLECULAR WEIGHT OF LACTONE

Lactone used, g.	0.0521 N KOH needed, cc. Calcd.	Found
0.0200	2.39	2.38
.0190	2.27	2.25

As a further confirmation of the identity of the lactone, its phenylhydrazide was made

---

[14] Palkin, Murray and Watkins, *Ind. Eng. Chem.*, **17**, 612 (1925).

[15] Nef, *Ann.*, **357**, 303 (1907).

[16] Upson, *Am. Chem. J.*, **45**, 466 (1911).

[17] Kiliani, *Ber.*, **16**, 2628 (1883).

as follows. One-half g. of the purified compound was dissolved in 0.5 cc. of water and 1 cc. of 95% alcohol. To this solution was added 0.4 cc. of phenylhydrazine. At the end of three days the reaction mixture had solidified to a mass of yellow crystals which, when purified by the method of Kiliani and Eisenlohr,[18] melted at 110–113°. Upson[16] found 110–112°, while Nef[15] gave 113–115°.

Pyruvic Aldehyde Osazone.—To a solution of one-sixteenth of a gram mole of the aldohexose in 125 cc. of a standard potassium hydroxide solution contained in a 350cc. round-bottomed flask was added a solution of 25 cc. of phenylhydrazine in 87.5 cc. of 95% alcohol. Both solutions were at the desired temperature before being mixed. The alkali normality of the solution was based on the total volume (237.5 cc.). The reaction mixture was agitated on the thermostat for 48 hours, after which time the osazone was filtered off, washed with 30% alcohol, dried at 65° and weighed. As the normality of the alkali and the temperature were increased, greater quantities of tar were formed. This necessitated a purification of the product. The tar was found to be soluble in 95% alcohol, which in turn dissolved only a relatively small amount of the osazone. This substance and also its hydrochloride obtained from both *d*-glucose and *d*-galactose by this general procedure were found to be identical in every respect. The osazone melted at 148° while its hydrochloride melted at 197°.

Experimental Results.—The following compounds were quantitatively determined in the alkaline reaction mixtures: acetic, formic and lactic acids from *d*-glucose and *d*-galactose, *d*-galacto-α-metasaccharinic acid lactone, and pyruvic aldehyde osazone in aqueous-alcoholic alkaline solutions of the carbohydrates. The data are shown in the accompanying figures.

Theoretical Part

Since our main purpose was to ascertain whether changes in temperature and in the concentration of the potassium hydroxide would produce corresponding changes in a system such as is represented by Equation 3, it became necessary to examine the reaction mixture of *d*-glucose and *d*-galactose for compounds that could be regarded as being derived from these 1,2-, 2,3- and 3,4-hexose enediols. When one considers the sources of the compounds that we actually isolated and determined quantitatively it will become clear that this is possible. The evidence for the presence of the three enediols in the alkaline solutions of these two carbohydrates is presented in the following paragraphs.

3,4-Enediol. (a) Lactic Acid.—When the hexose-3,4-enediols undergo a cleavage at the double bond, two molecules of glyceric aldehyde methylenenol are obtained. These in turn may rearrange into the corresponding aldehyde and thus give rise to the formation of lactic acid through the intermediate formation of pyruvic aldehyde which Denis[19] has shown is easily converted by means of alkalies into lactic acid in the same manner as benzoylformaldehyde is changed into mandelic acid.[20] Pinkus[21] and

[18] Kiliani and Eisenlohr, *Ber.*, **42**, 2608 (1909).

[19] Denis, *Am. Chem. J.*, **38**, 561 (1907).

[20] Evans, *ibid.*, **35**, 122 (1905).

[21] Pinkus, *Ber.*, **31**, 31 (1898).

Nef[22] obtained pyruvic aldehyde phenylosazone from alkaline solutions of glucose. It is an extremely important fact in this connection that Wohl[23] also obtained the same osazone from alkaline solutions of glyceric aldehyde. It is also conceivable that this osazone may be coming from either lactic aldehyde or from acetol. Since lactic aldehyde is incapable of existence in aqueous solutions by reason of the fact that it rearranges into acetol,[24] and since alkaline solutions of acetol show no trace of lactic acid, it is clear that pyruvic aldehyde osazone obtained in alkaline solutions of *d*-glucose and *d*-galactose must come from pyruvic aldehyde and not from either acetol or lactic aldehyde. This point of view is further supported by the fact that *d*-glucose in the presence of zinc hydroxide is converted by ammonia into methylimidazole, a derivative of pyruvic aldehyde.[25] Furthermore, it has been pointed out in the experimental part that the distillates obtained from the acidified reaction mixtures gave no reaction for acetol when examined by the method of Baudisch and Deuel.[10] Hence, it is clear that the lactic acid obtained from alkaline solutions of *d*-glucose and *d*-galactose must be derived from the 3,4-enediol of these carbohydrates. That the concentration of the 3,4-enediol in these alkaline solutions is dependent upon both the temperature and the concentration of the alkali is clearly shown in Fig. 2. It is furthermore evident from Fig. 2, that the influence of these two factors is markedly

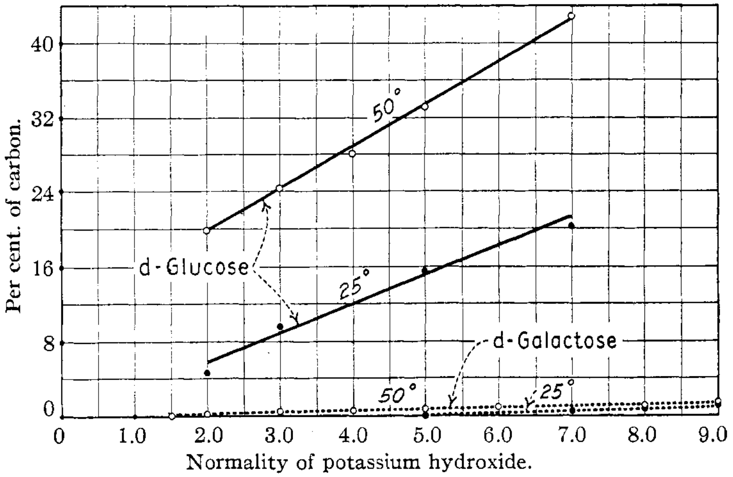


Fig. 2.—Per cent. of carbon obtained as lactic acid per molecule of *d*-glucose and *d*-galactose. Sugar concentration = 0.5 molecular; time, 48 hours.

---

[22] Nef, *Ann.*, **335**, 254 (1904).

[23] Wohl, *Biochem. Z.*, **5**, 56 (1907).

[24] Ref. 22. Compare also Evans and Parkinson, THIS JOURNAL, **35**, 1770 (1913).

[25] Windaus and Knoop, *Ber.*, **38**, 1166 (1905).

different in the case of *d*-glucose and *d*-galactose, the amount of the *d*-galactose existing in the 3,4-enediolic form being much less than that of *d*-glucose under the same conditions.

(b) **Pyruvic Aldehyde.**—It now became of much interest to know whether the concentrations of the pyruvic aldehyde, the intermediate to lactic acid formation, would bear the same general relationship to the temperature and the concentration of the alkali as did the lactic acid. This was ascertained by determining the osazone as described in the above experimental part; our results are shown in Fig. 3. The difference observed between the amounts of osazone obtained at 25° from *d*-glucose and *d*-galactose is in complete harmony with the difference observed in the lactic acid (Fig. 2) yields obtained from these two carbohydrates.

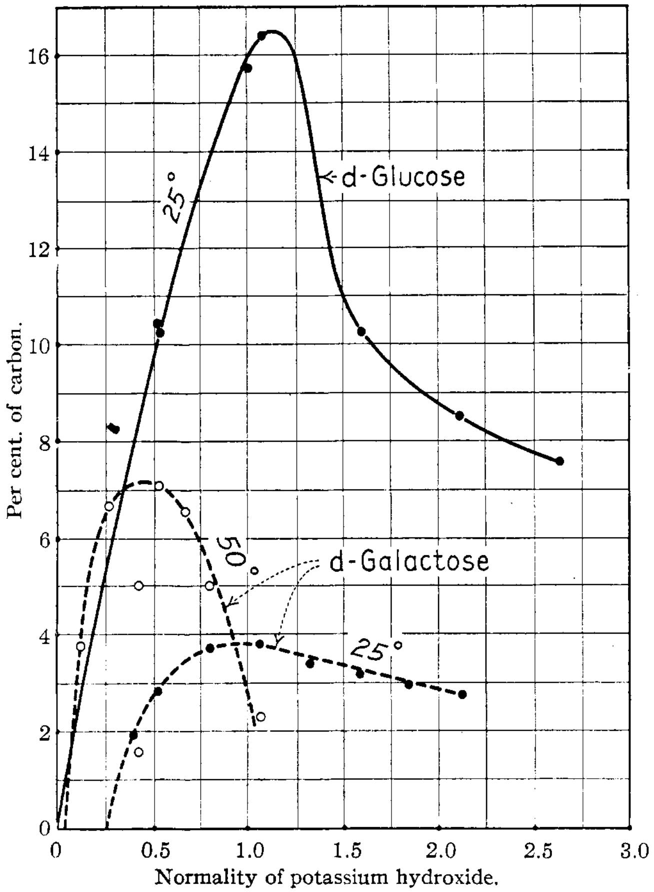


Fig. 3.—Per cent. of carbon obtained as pyruvic aldehyde per molecule of carbohydrate from *d*-glucose (96 hours) and *d*-galactose (48 hours). Sugar concentration = 0.5 molecular.

Furthermore, the amount of the osazone obtained from *d*-galactose was expected to be different from that from *d*-glucose because, as noted above, the experimental factors of temperature and alkali concentration influence the equilibrium in each alkaline system to a markedly different degree. By Fig. 3 it is observed that the amount of pyruvic aldehyde formed reaches a maximum in each case and then diminishes. This is undoubtedly due to the fact that the speed with which the pyruvic aldehyde is converted into lactic acid is also a function of the alkali concentration and hence becomes increasingly greater than the speed of osazone formation. Obviously this would result in diminishing yields of the osazone.

(c) **Acetic and Formic Acids.**—It is conceivable that pyruvic aldehyde obtained from glyceric aldehyde undergoes the following reactions.

$$CH_3{\cdot}CHO + CO \longleftarrow CH_3{\cdot}CO{\cdot}CHO \xrightarrow{+\,H_2O} CH_3{\cdot}CHOH{\cdot}COOH \qquad (4)$$

Under the conditions of our experiments it is clear that equimolecular amounts of acetic and formic acid should be obtained through the decomposition of pyruvic aldehyde. When the rate of lactic acid formation begins to exceed that of the decomposition into acetaldehyde and carbon monoxide, then the yield of acetic and formic acid will begin to decrease with an increasing alkali concentration and with increasing temperature. Then the maximum yield of acetic and formic acids for any given temperature must be at that alkalinity where the rate of formation of these two acids from pyruvic aldehyde is just equal to the rate of lactic acid formation from the same source. Fig. 4 shows that the formic acid at 50° and 75° has approximately the same maximum, while Fig. 5 shows that there is less acetic acid at 75° at the maximum than there is at 50°. These apparent irregularities may be due to two causes: (a) the well-known decomposition of alkali metal acetates and formates in hot alkaline solu-

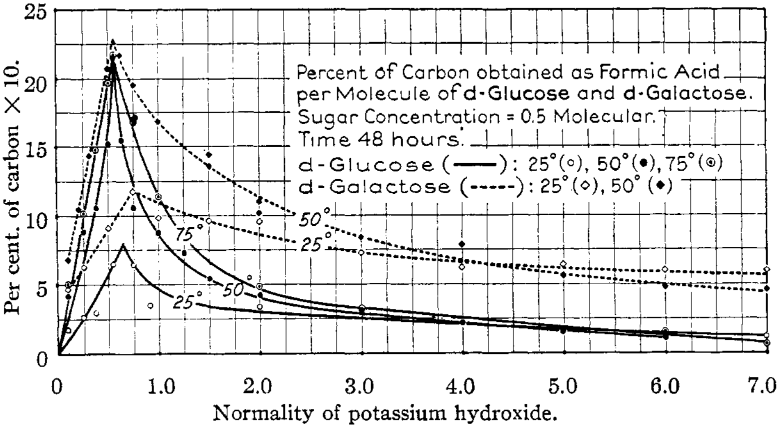


Fig. 4.

tions; (b) the greater temperature coefficient for lactic acid formation. In Fig. 6 the amount of acetic acid derived from *d*-galactose at 25° and 50° is shown to bear a logarithmic relationship to the alkali concentration.

1,2-Enediol. Formic Acid.—If the 1,2-enediols of the aldohexoses split at the double bond, formaldehyde methylenenol would be one of the products together with the appropriate pentose. Under the conditions of these experiments, the formaldehyde thus produced would yield formic acid. If the formic acid came only from this source, then this volatile reaction product could be considered as an index of the 1,2-hexose enediol.

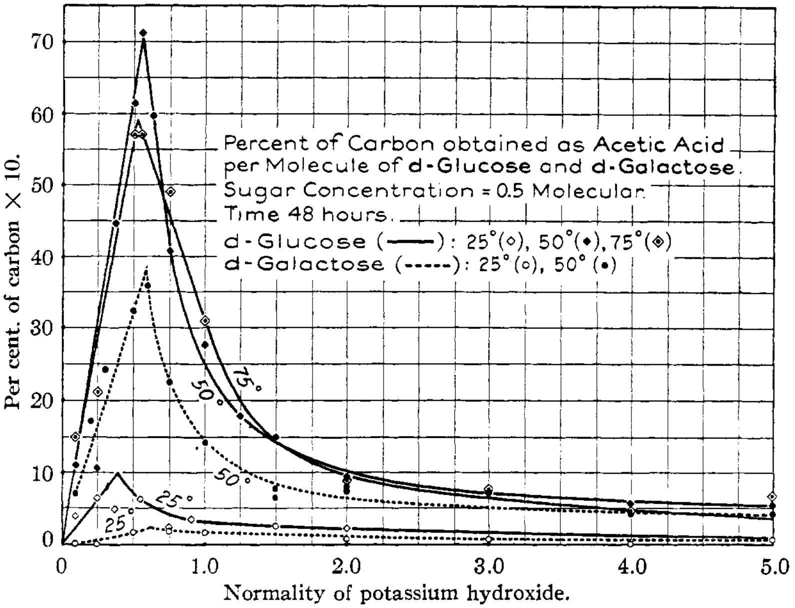


Fig. 5.

Computations based on our quantitative data show the following general facts. (a) At lower temperatures and in the regions of lower alkalinity the yields of formic acid are greater than the amounts that would be expected if the carbon monoxide of Reaction 4 were the only source of this acid; that is, the formic acid that is equivalent to the total acetic acid yields. This important fact points to another source of formic acid which we believe is the formaldehyde methylenenol formed from the hexose-1,2-enediol. (b) The difference in the amount of formic acid from these two sources becomes greater with increasing alkalinity until a maximum is reached, after which this difference tends to approach the limit zero; that is, the point at which equimolecular amounts of formic and acetic acids would be formed as indicated by Reaction 4. None of our results, however, reached this lower limit. By Figs. 4 and 5 it is also clear that the amounts of these two acids are rapidly diminishing due to the increasing rate of lactic formation from the pyruvic aldehyde with the increasing concentration of alkali. (c) The differences in the formic acid from the two sources pointed out in (a) and (b) are greater at lower temperature, a fact that shows that increasing temperature tends to shift the equilibrium toward the hexose-3,4-enediol of the system shown in Reaction 3.

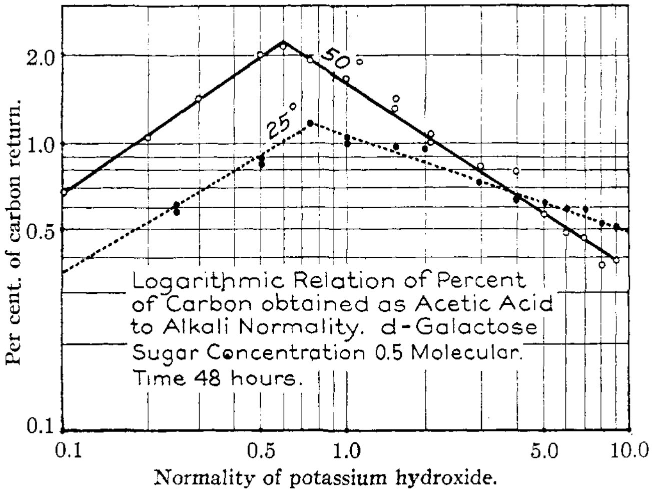


Fig. 6.

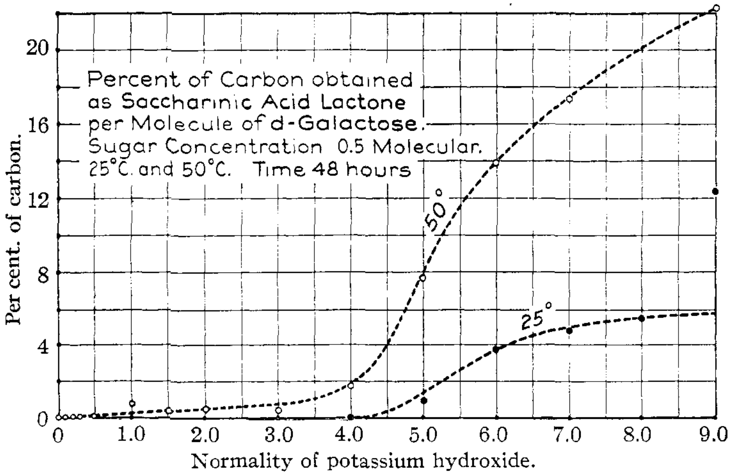
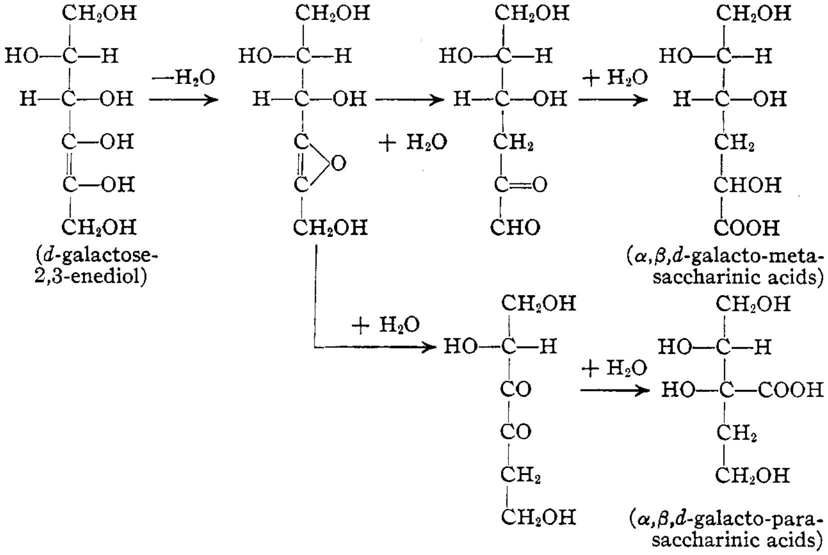


Fig. 7.

2,3-Enediol. ***d*-Galacto-α-metasaccharinic Acid Lactone (Metasaccharine).**—The percentage carbon equivalent of *d*-galactose converted into *d*-galacto-α-metasaccharinic acid lactone is shown in Fig. 7 as a function of the alkalinity. The effect of temperature and concentration of alkali on the yields of this acid are significant when considered from the equilibrium standpoint.

Nef[26] offered a theory for the formation of the saccharinic acids from the hexoses that involved the intermediate formation of *o*-diketo derivatives which were converted into the saccharinic acids through the benzilic acid rearrangement. In an investigation in this Laboratory on the action of alkalies on maltose, Miss Marjorie Pickard Benoy and the senior author of this paper have suggested a mechanism for the formation of these *o*-diketo derivatives that relates them directly to the 1,2-, 2,3- and 3,4-enediols. The importance of this suggestion lies in the fact that since the enediols themselves are regarded as the active components[27] of these alkaline solutions of the carbohydrates, the saccharinic acids as well as the products derived from the cleavage products of the hexose enediols are all considered as having a common source, rather than a special mechanism for the formation of saccharinic acids. The various saccharinic acids, therefore, become indices for the presence of the three enediols. On the basis of the proposed mechanism, the α-metasaccharinic acid obtained from *d*-galactose may be formed according to the following equation.

(a)



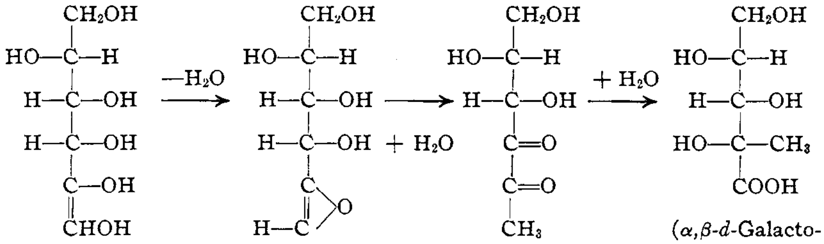
Thus, it is clear that these two different saccharinic acids may be derived from the 2,3-hexose enediol. Applying the same reaction to the 1,2- and the 3,4-hexose enediol, it is seen by the following equations that only one saccharinic acid is possible in each case, saccharinic and isosaccharinic acid, respectively.

---

[26] Nef, Ref. 12, p. 301; *Ann.*, **376**, 1 (1910).

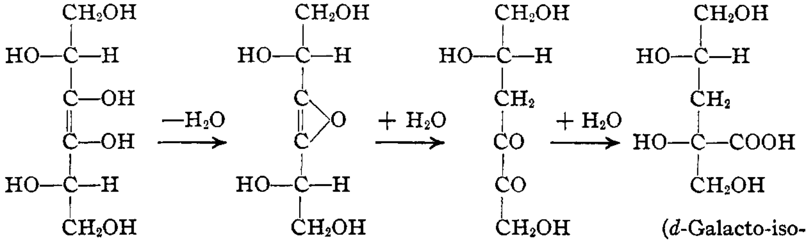
[27] Evans and Pickard, THIS JOURNAL, **47**, 3086 (1925).

(b)



(*d*-Galactose-1,2-enediol) (*α*,*β*-*d*-Galacto-saccharinic acids)

(c)



(*d*-Galactose enediol) (*d*-Galacto-iso-saccharinic acids)

Nef thought that the metasaccharinic acids were derived from the aldohexoses, the isosaccharinic acids from the 2-ketohexoses, and the saccharinic and the parasaccharinic acids from the 3-ketohexoses.

In conclusion we wish to thank Dr. Clyde S. Adams, of the Department of Chemistry, Antioch College, for kindly assistance in the preparation of *o*-aminobenzaldehyde used in these experiments.

Summary

1. The action of various concentrations of potassium hydroxide at different temperatures on aqueous solutions of *d*-glucose and *d*-galactose was studied for the purpose of ascertaining whether these two experimental factors would produce a change in the equilibrated system of enediols that are formed in alkaline solutions of these two carbohydrates.

2. The lactic acid obtained from alkaline solutions of these carbohydrates is formed by a cleavage of the 3,4-enediol into the methylenenol of glyceric aldehyde which in turn is converted to pyruvic aldehyde, a compound that yields lactic acid. The amount of lactic acid obtained from these carbohydrates is a function of both the alkali concentration and of the temperature, and is therefore regarded as an index of the extent to which the carbohydrates are converted into the 3,4-enediol.

3. The shifting of the equilibrium by means of alkali concentration and temperature in the enediol systems is much greater in glucose than in galactose solutions as is evidenced by the fact that lactic acid is obtained in much greater quantities from glucose than from galactose.

4. Acetic and formic acids are probably formed from the decomposition of pyruvic aldehyde into acetaldehyde and carbon monoxide. The pro-

duction of these two acids reaches a maximum with increasing concentration of alkali, after which there is a diminution in the yield of these acids. The maximum point is thought to be due to the speed of conversion of pyruvic aldehyde into lactic acid being just equal to that for the formation of acetic and formic acids at that alkalinity. The diminishing yields of these two acids is due to the increasing rate of lactic acid formation with increasing alkaline concentration.

5. The total yield of formic acid is greater than that obtained from pyruvic aldehyde. This is thought to be an index of the extent to which the carbohydrate is converted into the 1,2-hexose enediol, by reason of the cleavage of this enediol into formaldehyde methylenenol and a pentose. The total yield of formic acid tends to approach that equivalent to the total acetic acid yields from the 3,4-enediol as the alkali concentration and the temperature are increased.

6. The yield of pyruvic aldehyde osazone is a function of both the alkali concentration and the temperature, until a point of alkalinity is reached at which the rate of its conversion into lactic acid is greater than the osazone formation. Until this point is reached, in the absence of phenylhydrazine, the aldehyde yields acetic and formic acids.

7. *d*-Galacto-α-metasaccharinic acid lactone is thought to be an index of the extent to which the carbohydrate exists as 2,3-enediol, at any given alkalinity. The yields of this lactone are also found to be functions of the temperature and the alkali concentration.

8. A mechanism is offered by Miss Marjorie Pickard Benoy and the senior author for the formation of hexose *o*-diketo derivatives which are supposed to be the intermediates in the production of the various saccharinic acid lactones (saccharines). This mechanism directly relates these lactones to the three hexose enediols which are regarded as the active components of these alkaline solutions.

COLUMBUS, OHIO