bromide, is increased thirty times and on addition of two moles, 300 times. On further addition of hydroxide the conductance is further increased, indicating appreciable dissociation of the complex compound in acetone.

On treating the dihydroxy bromide with silver oxide in an aqueous solution, the corresponding hydroxy derivative is obtained. The conductance of the solution clearly indicates that this complex hydroxide has considerable stability. It goes over gradually to trimethyl tin hydroxide, with which it is isomeric.

Trimethyl tin hydroxide forms a complex compound with methyl iodide in acetone. This compound is formed gradually and its solutions are relatively highly ionized. At a concentration of 0.1 *N*, the equivalent conductance has a value of the order of that of such salts as lithium nitrate and silver nitrate.

PROVIDENCE, RHODE ISLAND

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

THE MECHANISM OF CARBOHYDRATE OXIDATION. XIII. THE ACTION OF POTASSIUM HYDROXIDE ON CALCIUM HEXOSEDIPHOSPHATE. A COMPARISON WITH THAT OF GLUCOSE AND FRUCTOSE[1]

BY WILLIAM LLOYD EVANS AND ROBERT CASAD HOCKETT[2]

RECEIVED JULY 12, 1930 PUBLISHED OCTOBER 6, 1930

The rates of fermentation of glucose, mannose and fructose are almost the same and the products of these reactions are identical.[3] These facts suggested a fermentation mechanism involving the formation of common intermediates—a condition which seemed easily satisfied on the assumption that these substances were the enediols of the hexoses, especially since no other hexose can yield the same enediols as these, and no other is fermented at a comparable rate. This point of view seemed strengthened through Young's discovery that the same hexosediphosphoric acid[4] ($C_6H_{10}O_4(PO_2(OH)_2)_2$) is formed from each of these three sugars as an intermediate in fermentation as evidenced by the identity, regardless of its source, of all its properties, including optical rotation. Furthermore, this acid has little reducing power except on hydrolysis, and it does not form an osazone without hydrolysis of a phosphate group.

Schlubach and Rauchenberger[5] have recently methylated this common hexosediphosphoric acid and found that only seven methyl groups were

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[1] Read at the Columbus Meeting of the American Chemical Society May, 1929.

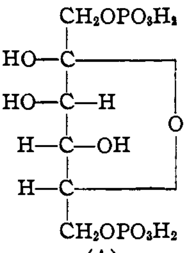
[2] E. I. du Pont de Nemours and Co. Fellow, 1928–1929.

[3] Harden, "Alcoholic Fermentation," Longmans, Green and Co., London, 1923, pp. 32, 48.

[4] Young, *Proc. Roy. Soc.* (London), 81, 528 (1909).

[5] Schlubach and Rauchenberger, *Ber.*, **60B**, 1178 (1927).

introduced into the molecule. If this acid were the derivative of an enediol, eight methyl groups should have been present in the resulting compound. The recently proposed structure (A) for this acid[6] easily satisfies the experimental data with reference to the formation of the tetramethyl ester of trimethylhexose-diphosphoric acid.



(A)

By reason of the results obtained in this Laboratory with reference to the action of glucose,[7a] fructose[7b] and mannose[7c] in aqueous solutions of potassium hydroxide, it became of much interest to know the behavior of calcium hexosediphosphate, commercially known as "candiolin," when it was subjected to the same experimental conditions as the three well-known hexose sugars. Especially were we anxious to obtain the experimental data with reference to lactic acid formation. These brief statements summarize our principal objectives in this work.

Experimental Part

Reagents.—The potassium hydroxide, zinc carbonate, phosphoric acid and ether used were of the same degree of purity as the reagents employed with glyceric aldehyde[8] and dihydroxy-acetone.[9] The glucose and fructose were Pfanstiehl's C. P. special anhydrous brand with specific rotations recorded, respectively, as +52.5° and −91.66°. The calcium hexosediphosphate was presented by the H. A. Metz Laboratories, and was purified before use by the method of Neuberg and Sabatay.[10]

Anal. Subs., 0.900: $Ca_3(PO_4)_2$ by ignition, 0.0411. Calcd. $Ca_3(PO_4)_2$: 45.73. Found: 45.66.

Procedure.—The experimental details were practically the same as in the case of glyceric aldehyde. Glucose and fructose samples of 2.25 g. were used and candiolin samples of 2.827 g. corresponding to 1.125 g. of hexose. The object was to conserve the difficultly prepared material. The three substances were treated as nearly alike as possible.

Products of the Reaction.—The reactions were all carried out at an alkali normality of five and at a temperature of 50°, with time as the variable. Qualitative examination was made for hydroxy acids, acetol, pyruvic acid, formic acid, acetic acid and lactic acid. Quantitative determinations were carried out for the last three named which were the only substances detected.

Data.—The quantitative data are shown in Fig. 1.

Theoretical Discussion and Summary

1. It was found that the yield of lactic acid from glucose in 5 N potassium hydroxide solutions at 50° rises rapidly to a maximum in about

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[6] Levene and Raymond, *J. Biol. Chem.*, **80**, 633 (1928); Morgan and Robison, *Biochem. J.*, **22**, 1270 (1928); "Annual Survey of American Chemistry," Vol. IV, 1930, p. 256.

[7] (a) Evans, Edgar and Hoff, THIS JOURNAL, **48**, 2665 (1926); (b) Evans and Hutchman, *ibid.*, **50**, 1496 (1928); (c) Evans and O'Donnell, *ibid.*, **50**, 2543 (1928).

[8] Evans and Hass, *ibid.*, **48**, 2763 (1926).

[9] Evans and Cornthwaite, *ibid.*, **50**, 486 (1928).

[10] Neuberg and Sabatay, *Biochem. Z.*, **151**, 240 (1925).

twenty-four hours and then remains constant up to at least five hundred and twenty-eight hours. The yields of formic and acetic acids are very low and remain constant over the same range of alkalinity. With fructose the yield of all three acids is greater in amount and constant, at least up to 374 hours. The maximum is reached more rapidly, however, so that in two hours a high yield of lactic acid was obtained. This behavior of fructose seems to be in harmony with the observation that this ketohexose is much more sensitive to some chemical reagents than either glucose or mannose.

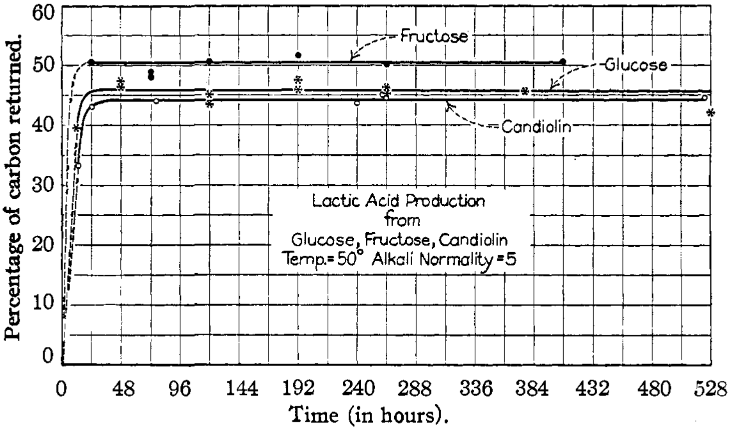

Fig. 1.

2. Candiolin shows a striking similarity in behavior to fructose and glucose. The maximum yield of lactic acid is a little more slowly attained and remains lower than with either of the other compounds, while acetic acid is obtained in larger quantity. It is clear that the rate of hydrolysis of the calcium salt in alkaline solution becomes an important factor in determining the concentration of the resulting hexose. It has been recently pointed out by Shaffer and Friedmann[11] that the yields of products obtained by the action of alkalies on the hexoses are dependent in part on the concentration of the sugar undergoing decomposition. The average yields after attaining a constant production are as follows

	Glucose, %	Fructose, %	Candiolin, %
Lactic	45.55	50.27	44.11
Acetic	0.85	1.52	2.31
Formic	0.47	0.63	0.20
Total	46.87	52.42	46.62

3. From these data and those compared in the diagram, it is clear that the mechanism involved in the formation of lactic, acetic and formic acids in alkaline systems of candiolin is best understood on the basis that

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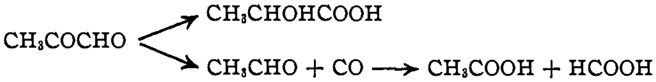
[11] Shaffer and Friedmann, *J. Biol. Chem.*, **86**, 361 (1930).

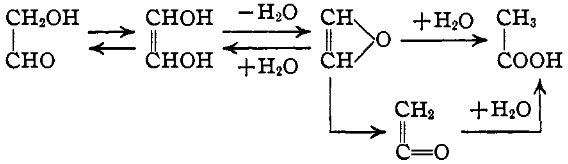
the calcium salt undergoes hydrolysis, thus liberating the keto-hexose, fructose. This sugar is then acted upon by the potassium hydroxide present, thus giving rise both to the sugars formed in the de Bruyn and van Ekenstein[12] interconversion and also to the enediols postulated by Nef.[13] It has been repeatedly pointed out in these studies that lactic acid arises from the hexose-3,4-enediols.

4. It has previously been considered[7a] that acetic and lactic acids, and also a portion of the formic acid obtained in the alkaline decomposition of the sugars are derived from pyruvic aldehyde by a series of reactions which are represented by the equations



$$CH_3COCHO \begin{cases} \nearrow CH_3CHOHCOOH \\ \searrow CH_3CHO + CO \longrightarrow CH_3COOH + HCOOH \end{cases}$$

Formic acid may also be derived from hydroxymethylene formed from the cleavage of hexose-1,2-enediol. From the figures given in paragraph 2, it is seen that the ratios of the yields of acetic and formic acids from glucose and fructose tend to approximate the stoichiometrical relations indicated in the above chemical equations. In the case of candiolin, however, the figures show that the acetic acid is proportionally too great for this equation. Furthermore, it should be pointed out that pyruvic aldehyde osazone has never been obtained in any notable quantity from either glucose, fructose or mannose in 5 N potassium hydroxide solutions.[7c] At this alkalinity the pyruvic aldehyde intermediate is completely converted into lactic acid. From these facts it follows that an independent source of acetic acid must be postulated. The junior author[14] proposes the following mechanism which is strictly analogous to that suggested by Evans and Benoy[7a] for the formation of lactic acid from glyceric aldehyde.



$$\begin{matrix}CH_2OH\\|\\CHO\end{matrix} \rightleftarrows \begin{matrix}CHOH\\\|\\CHOH\end{matrix} \underset{+H_2O}{\overset{-H_2O}{\rightleftarrows}} \begin{matrix}CH\\\|\\CH\end{matrix}\!\!>O \xrightarrow{+H_2O} \begin{matrix}CH_3\\|\\COOH\end{matrix}$$

$$\begin{matrix}CH\\\|\\CH\end{matrix}\!\!>O \longrightarrow \begin{matrix}CH_2\\\|\\C=O\end{matrix} \xrightarrow{+H_2O} \begin{matrix}CH_3\\|\\COOH\end{matrix}$$

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[12] De Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 156 (1895); **14**, 203 (1895); **15**, 92 (1896); **16**, 257 (1897); **16**, 274 (1897); **16**, 262 (1897); **19**, 1 (1900); **27**, 1 (1908).

[13] Nef., *Ann.*, **376**, 1 (1910); **403**, 204 (1914); **357**, 294 (1907).

[14] (a) R. C. Hockett, Master's Thesis, The Ohio State University, 1928. Note the analogy between the rearrangement of acetylene oxide into ketene and the well-known rearrangement of ethylene oxide into acetaldehyde. (b) Mr. P. G. Hoff, Doctor's Dissertation, The Ohio State University, 1926, made a similar proposal for the presence of acetic acid in the alkaline decomposition of galactose in which he assumed that glycol aldehyde, through the loss of water, would give rise to ketene, $CH_2=C=O$ as an intermediate, a compound which is known to add water with the formation of acetic acid. This proposal is included as a possibility in the above equations.

The presence of glycol aldehyde is due to the cleavage of the hexose-2,3-enediol or that of a triose-1,2-enediol. It is also conceivable that the formic acid in these reactions may arise from the cleavage of the ethene diol molecule into two molecules of hydroxymethylene.

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[CONTRIBUTION NO. 59 FROM THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY, RESEARCH LABORATORY OF ORGANIC CHEMISTRY]

THE DISSOCIATION PRESSURES OF CERTAIN METAL PYRIDINE THIOCYANATES

BY TENNEY L. DAVIS AND HOWARD R. BATCHELDER

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The determination of the dissociation pressures of the compounds of pyridine with metal thiocyanates is part of an investigation of the affinities by which pyridine and similar nitrogen bases are linked in their complex compounds. Of two compounds, such as nickelous tetrapyridine thiocyanate and cobaltous tetrapyridine thiocyanate, for example, which have the same molecular constitution and differ only in the metal which they contain, the one which has the lower dissociation pressure of pyridine is evidently the one in which the pyridine is attached more firmly to the metal. Since the pyridine is supposed to be attached to the coördination valence of the metal, it is to be expected that the order of the metals with reference to their affinity for pyridine will be the same in the series of the salts of one acid as in the series of the salts of another. By comparison, also, of the dissociation pressures of the corresponding pyridine derivatives of different salts of the same metal, it is possible to estimate the effect of the acid radical—for one acid will occupy more or less of the affinity of the metal than another acid and will leave less or more of the affinity available for combination through the coördination valences.

We have worked with cobaltous, cupric, nickelous and zinc pyridine thiocyanates. The compounds were prepared and purified by the methods which had been used earlier for the chloroform-soluble metal pyridine cyanates.[1] Dissociation pressures were measured by starting with a mixture of pyridine with the most highly pyridinated compound of the metal thiocyanate, evacuating the space above it and allowing to come to equilibrium, noting the pressure, then pumping off a small amount of pyridine, again allowing to come to equilibrium and again noting the pressure, and so on, until the pyridine was completely removed from the substance. Thus we were able to be certain that none of the intermediate pyridine complexes escaped us, and that the dissociation pressures of all of the compounds, from that which existed in the presence of an excess

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[1] Davis and Logan, THIS JOURNAL, 50, 2493 (1928).