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Simultaneous Formation of Acetol and Pyruvic Acid from Glucose

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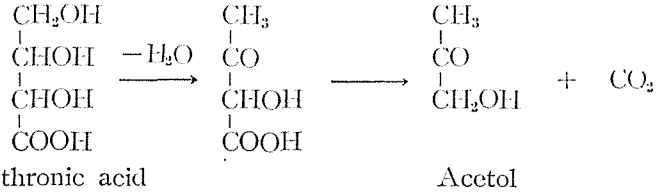
Formation of acetol from glucose by the influence of some alkaline reagents has often been reported by several investigators[1]. It seems, however, still quite obscure how the acetol is formed. Nef[2] postulated the acetol to be a reduction product of methylglyoxal which splits primarily from the sugar. As it is well recognized that glucose splits into two molecules of methylglyoxal by the action of alkalis, it seems quite possible the latter may in turn undergo dismutation, giving acetol and pyruvic acid[3]. On the other hand, Baudisch and Deuel[4] argued acetol to be a primary fission product of the sugar, since in contrast to glucose, methylglyoxal gave on distillation with sodium hydrogen carbonate only a very small quantity of acetol. But they said nothing about the product which should be formed from a glucose molecule simultaneously with acetol. Löb[5] assumed as an intermediate of acetol erythronic acid which may be formed from glucose and decomposed to acetol according to the following scheme:



Erythronic acid — Acetol

This assumption implies that the acetol is formed by successive cleav-

1. A. Emmerling and G. Loges, Ber., **16** (1883), 837; G. Pinkus, Ber., **31** (1898), 31; O. Baudisch, Biochem. Z., **89** (1918), 279; O. Baudisch and H. J. Deuel, J. Am. Chem. Soc., **44** (1922), 1585; R. Nodzu and R. Goto, Bull. Chem. Soc. Japan, **11** (1936), 381.
2. J. U. Nef, Ann., **335** (1904), 255.
3. C. Neuberg, Biochem. Z., **49** (1913), 502.
4. O. Baudisch and H. J. Deuel, Loc. cit.
5. W. Löb, Biochem. Z., **12** (1908), 78.

ages of C_1 or C_2- (but no C^3-) fragment, from a molecule of glucose or rather of its intramolecular transformation product.

In our previous work[1], it was observed that even when glucose is distilled with an acid (pH, 4–7) concentrated (about 40%) solution of potassium phosphate, acetol is found (in a yield about 5% of the sugar) in the distillate. Now, in the distillation residue, we were able to identify pyruvic acid as its 2 : 4-dinitrophenylhydrazone. But the amount of the acid was not large, i. e., at the largest 0.5% on the basis of glucose used. It should not obviously be hastily concluded that the pyruvic acid is a primary fission product of glucose, since such a small amount of the acid might be formed in the course of the distillation by autoxidation of some other primary C_3 fission products. Actually acetol and methylglyoxal were converted to the acid to a small extent under experimental conditions, but no lactic acid.

When, however, glucose was digested at 105–110°[2] with the phosphate solution under painfully air-free conditions—a vacuum higher than the order of 10^{-5}mm.—pyruvic acid was again found in the digestion product, though its yield was somewhat inferior to that in the distillation experiment. From the same reaction product, only traces of acetol could be obtained as its 2 : 4-dinitrophenylosazone. The yields of acetol and pyruvic acid do not necessarily imply that both were formed only in such scanty amounts. Acetol is well known to be an unstable substance. Pyruvic acid was only recoverable some to 50% after being subjected with the phosphate solution to the same distillation process as in the case of glucose, and to our interest, almost entirely disappeared when similarly treated in the presence of glucose. Moreover, methylsuccinic acid[3]—a condensation product of pyruvic acid—was found along with pyruvic acid in the distillation residue of glucose. Thus both acetol and pyruvic acid seem quite unstable and may suffer various transformations under experimental conditions. In view of the preceding results, there seems to be no doubt that by the action of the phosphate solution, glucose gives pyruvic acid simultaneously with acetol without any intervention of autoxidation.

On the other hand, methylglyoxal or d,l-glyceric aldehyde—a supposed precursor of the former—afforded on distillation with the

1. R. Nodzu and K. Matsui, Bull. Chem. Soc. Japan, **10** (1935), 122, 467.
2. The figures correspond approximately to the boiling temperature of the concentrated potassium phosphate solution.
3. L. Wolff, Ann., **317** (1901), 1.

phosphate solution mainly methylglyoxal and only a small quantity of acetol. This result is quite inconsistent with that from glucose, and indicates clearly that the simultaneous formation of acetol and pyruvic acid may not occur by a predictable route: glucose → 2 glyceric aldehyde → 2 methylglyoxal → acetol + pyruvic acid. Admitted, however, that under certain conditions a molecule of glucose decomposes into two molecules of methylglyoxal:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{-2H_2O} \underset{\text{Methylglyoxal}}{CH_3COCHO} + \underset{\text{Methylglyoxal}}{CH_3COCHO},$$

it is quite possible that under other conditions it may split disproportionally without intervention of methylglyoxal or glyceric aldehyde into one molecule each of acetol and pyruvic acid:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{-H_2O} \underset{\text{Acetol}}{CH_3COCH_2OH} + \underset{\text{Pyruvic acid}}{CH_3COCOOH}$$

And it seems very probable that the simultaneous formation of acetol and pyruvic acid may be ascribed rather to this process.

In the distillation experiment on glucose with the acid phosphate solution, the production of methylglyoxal was very small as already shown in the previous paper and that of lactic acid also far inferior to that by the action of some alkaline reagents on glucose. Since under the experimental conditions lactic acid was quite stable and methylglyoxal also tolerably stable—perhaps it transforms partly into the acid—, the decomposition of glucose favorable in alkaline media: Glucose → 2 methylglyoxal → 2 lactic acid, seems markedly depressed here in an acid medium.

As to whether the mechanism described above is also applicable to the formation of acetol from glucose by the influence of alkalis, further study is in progress.

Experimental Part.

Formation of Pyruvic Acid. In the same way as described in the previous paper, glucose in a certain volume of about 40% solution of potassium phosphate (pH 4.5 or 6.3) was distilled, keeping the volume constant by adding fresh water in small portions, and the distillate was analysed for acetol, diacetyl and methylglyoxal as their semicarbazones. The distillation residue was made up to one and a half in volume by addition of six-normal hydrochloric acid and a

precipitate soon formed was filtered off. To the filtrate was added an adequate volume of 2 : 4-dinitrophenylhydrazine reagent (1% in 2N-hydrochloric acid) and the mixture was kept over night at room temperature. A reddish-brown precipitate thus formed, was a quite complex mixture. From this, pyruvic acid-2 : 4-dinitrophenylhydrazone was isolated by exploiting the description of Case[1]. The precipitate was dissolved in a mixture of ethylacetate and toluene, and the solution was extracted by 25% sodium carbonate solution. The extract was then acidified with concentrated hydrochloric acid so as to precipitate the hydrazone. Since the precipitate of hydrazone was still contaminated with other compounds, it was again treated in the same way. Then it was recrystallized from alcohol. The melting points of the samples prepared in this way lay between 209° and 212°. After another recrystallization from alcohol, it melted at 215° and showed no depression in the mixed melting point (Found : N, 20.6. Calc. for $C_9H_9O_6N_4$: N, 20.9%).

The processes of isolation and purification of the hydrazone are tedious and wasteful, obviously not quantitative. Since, however, the crude hydrazone is always contaminated with some substances which give by alkali a coloration similar to that of the hydrazone, the colorimetry of Case can not be used here.

Amounts of the hydrazone actually isolated were always very meagre and two results from our experimental protocols are quoted in Table I.

Table I.

Glucose 20 g., 40% Solution of K-phosphate 200 c.c.

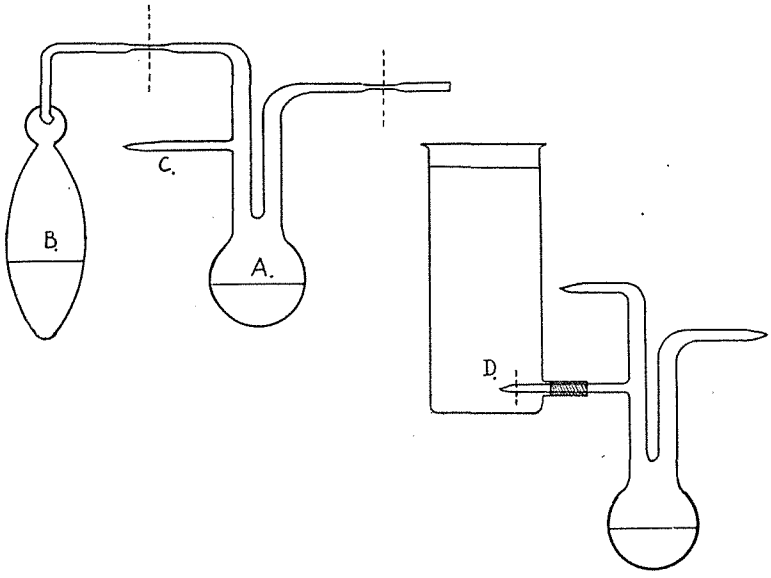
pH	4.5	6.4
Distill. duration (hour)	15	4.5
Distillate (c.c.)	2.340	820
Diacetyl-bis-semicarbazone (g.)	<0.01	—
Methylglyoxal-bis-semicarbazone (g.)	<0.01	—
Acetol-semicarbazone (g.)	0.32	0.17
Pyruvic acid-2 : 4-dinitrophenylhydrazone (m. p. 209–212°) (g.)	0.31	0.24

Formation of Pyruvic Acid under air-free conditions. 100 c.c. of the potassium phosphate solution of a certain pH, were evaporated

1. E. M. Case, Biochem. J., **26** (1932), 753.

to dryness. It was then powdered and mixed with 10 g. of glucose. This mixture was put in a glass flask A (Fig. 1.) of about 250 c.c. capacity. A glass bottle B (300 c.c.) contained about 150 c.c. of a mixture of stannous chloride and sodium hydroxide and water (0.5 :

Fig. 1



0.1 : 100). Cooling bottle B with liquid oxygen and warming flask A up to 80°, the whole system was highly evacuated up to a pressure of 10^{-6}mm. order.

Now the cooling and the warming devices were taken away, and anew flask A was cooled with liquid oxygen and bottle B was warmed very gradually up to the temperature of the human body. When the volume of water condensed in flask A reached 100 c.c., the flask was sealed in a blow-pipe at the two points marked with the dotted lines in the figure.

After digesting the flask content at 105–110° for a certain interval, it was brought to room temperature and scratched with a file near the end of side tube C. Side tube C was then connected with a thick rubber tubing to another side tube of a container D (about 500 c.c.) which held a sufficient quantity of the 2 : 4-dinitrophenylhydrazine reagent freed from air by boiling and cooling under a nitrogen current. Now side tube C was broken at the scratch by a pinch to let the hydrazine reagent spout into flask A. The precipitate thus

formed was analysed for pyruvic acid hydrazone as usual. In each experiment with the phosphate solution of pH. 6.4 (digestion interval 12 hours) and 4.5 (digestion interval 24 hours), about 0.01 g. of the hydrazone (m. p. 210–212°) was obtained. In these experiments, only traces of acetol osazone were obtained in an impure state (m. p. 290°–). This fact may be due partly to the difficulty of its separation from other products and partly to the disappearance of acetol once formed which might occur under the experimental conditions.

Formation of Lactic Acid. A mixture of 10 g. glucose and 100 c.c. of the phosphate solution of a certain pH was distilled as usual. When the distillate amounted to 2000 c.c. (distillation duration about 20 hours), lactic acid was isolated from the distillation residue as its zinc salt following Evans' description[1] and identified by Deniges' reaction. Also the same residue as above was quantitatively analysed for the acid by means of Tanaka and Endo's method[2]. Three experimental results with the phosphate solutions of different pH values are brought together in Table II.

Table II.

Glucose 10 g., 40% Solution of K-phosphate 100 c.c.

pH	4.5	6.4	8.4*
Zn-lactate	?	+	++
Lactic acid (g.)	0.06	0.10	0.56

*Na_2HPO_4 was used.

Formation of Methylsuccinic Acid. According to Wolff[3], pyruvic acid transforms spontaneously or especially by the influence of mineral acid, to methylsuccinic acid by way of α-ketovalerolactonecarboxylic acid. This transformation is to be expected under experimental conditions.

A distillation residue (distillation duration about 20 hours) from a mixture of 30 g. glucose and 300 c.c. of the potassium phosphate solution (pH 6.4) was neutralized with caustic potash and evaporated to syrup. After treatment with ether, the syrup was made strongly acidic by sulphuric acid, and then shaken with ether several times so as to extract acidic substances. The extract from which ether had been driven, was kept for about ten days in a desiccator over sulphuric

1. W. L. Evans and coworkers, J. Am. Chem. Soc., **48** (1926), 2665.
2. S. Tanaka and M. Endo, Biochem. Z., **210** (1929), 120.
3. L. Wolff, loc. cit.

acid. Some colourless fine crystals appeared in a viscous mass from which they were with difficulty separated by means of a porous plate. After a recrystallization from a mixture of ether and petroleum benzene, they weighed only 0.006 g. and melted at 109–110°, and showed no lowering of the melting point on admixing with an authentic specimen of methylsuccinic acid (Found: C, 45.4; H, 6.4. Calc. for $C_5H_8O_4$: C, 45.5; H, 6.1%) which was prepared from pyruvic acid according to Wolff.

α-Ketovalerolactonecarboxylic acid was in vain attempted to be identified as its phenylhydrazone in an identical distillation residue.

Instability of Pyruvic Acid. In the following experiments, a freshly distilled pyruvic acid (b. p. 48–50°/8 mm.) was used.

A solution of 0.105 g. of pyruvic acid (1 mol) in 50 c.c. of water was made about two-normal in regard to hydrochloric acid by addition of six-normal hydrochloric acid and mixed with 36 c.c. (1.5 mols) of the 2 : 4-dinitrophenylhydrazine reagent and left to stand at room temperature for completion of the precipitation. The precipitate of pyruvic acid-2 : 4-dinitrophenylhydrazone was filtered (filtrate A) and dried. It amounted to 0.301 g., corresponding to 94.2%[1] of pyruvic acid used and it melted at 215°. This sample was dissolved in 6 c.c. of a concentrated solution (about 25%) of sodium carbonate and the solution was filtered, and then the hydrazone was reprecipitated by addition of concentrated hydrochloric acid to the filtrate. It weighed 0.2964 g. which corresponds to 92.7% of the original pyruvic acid. In order to see if any material of the hydrazone remained in the filtrate A, Case's procedure was adopted. The filtrate was extracted with ethylacetate. After the ethylacetate liquor evaporated to a small volume, it was mixed with tenfold its volume of toluene and the mixture was filtered. The filtrate was well shaken with a few c.c. of the concentrated soda solution. However on acidifying the soda solution with hydrochloric acid none of the hydrazone was precipitated.

From a mixture of 0.25 g. of pyruvic acid and 50 c.c. of the concentrated phosphate solution (pH, 6.4), by the same procedure as above, was obtained 0.715 g. of the hydrazone (m. p. 214°, 94.0%) which decreased interestingly to 0.604 g. (m. p. 215°, 79.2%) after a purification by the soda treatment. However, after the same mixture

1. The datum differs noticeably from that of Simon and Neuberg, who recovered the acid up to 99% in a like experiment. (Biochem. Z., **232** (1931), 479.) The discrepancy might be due to a difference in purity of the materials.

of pyruvic acid and the phosphate solution was subjected to distillation for about 10 hours (distillate 1,000 c.c.), from the distillation residue was obtained 0.37 g. of the crude hydrazone (m. p. 210°–, 50%) which on further analysis resolved into 0.1 g. of the pure hydrazone (m. p. 215°), and some other acidic and neutral substances. It was not possible to identify pyruvic acid in the distillate.

The presence of glucose in the mixture of pyruvic acid and the phosphate solution made isolation of the hydrazone difficult—it was necessary to follow Case's process—and seemed to accelarate transformation of the acid. After it had stood at room temperature for an hour, a mixture of 3 g. of glucose and 0.02 g. of pyruvic acid and 30 c.c. of the phosphate solution (pH, 5.4) was analysed by Case's method for the acid, and 0.043 g. of the hydrazone (m. p. 214°, 72%) was obtained. When however, samples of the same mixture were distilled for an hour (distillate 55 c.c.) and for 16 hours (distillate 750 c.c.), 0.027 g. (m. p. 214°, 45%) and less than 0.001 g. (m. p. 190°–) of the hydrazone was isolated from the respective distillation residues.

Distillation of Glyceric Aldehyde and Methylglyoxal. d, l-Glyceric aldehyde was prepared by way of acrolein acetal from glycerine, following the description of Witzemann[1], and melted at 128°. Methylglyoxal* (b. p. 39–41°/15 mm.) was obtained by oxidation of acetone by means of selenium dioxide[2].

Each 2 g. of these two materials were respectively distilled with 50 c.c. of the phosphate solution of different pH values. The distillates and the distillation residues were analysed, and the results are summarized in Tables III and IV.

Table III.

Glyceric aldehyde 2 g. in 200 c.c. H_2O, 40% Solution of K-phosphate.

pH	4.5	6.3	9.3
Distillation duration (hour)†	22	7	7
Distillate (c.c.)	1.700	500	500
Diacetyl-bis-semicarbazone (g.)	0	0.01	0.01
Methylglyoxal-bis-semicarbazone (g.)	0.86	0.04	0
Acetol-semicarbazone (g.)	0	0.02	0.14
Pyruvic acid-2 : 4-dinitrophenylhydrazone (m. p. 210–212°) (g.)	0.07	0.02	0

1. E. I. Witzemann, J. Am. Chem. Soc. **36** (1914), 1908.
2. H. L. Riley, J. F. Morley and N. A. C. Friend, J. Chem. Soc., (1932), 1875.

* It was always contaminated with traces of pyruvic acid.

Table IV.

Methylglyoxal 2 g. in 200 c.c. H_2O, 40% Solution of K-phosphate.

pH	4.6	6.3	9.3
Distillation duration (hour)†	8	8	8
Distillate (c.c.)	600	650	620
Diacetyl-bis-semicarbazone (g.)	0	<0.01	0.02
Methylglyoxal-bis-semicarbazone (g.)	2.2	0.4	0
Acetol-semicarbazone (g.)	0	trace	0.02
Pyruvic acid-2 : 4-dinitrophenylhydrazone (m. p. 209—211°) (g.)	0.02	0.01	0

† The distillation was continued until no more iodoform reaction was observed in the distillate.

The actual formation of pyruvic acid in these two series of experiments led us to conclude that methylglyoxal suffers autoxydation. The acetol formed (especially in the alkaline region) might rather be a secondary product from some C_6-compound produced by condensation of glyceric aldehyde or methylglyoxal.

It is also worth noticing that methylglyoxal does not distill over from the alkaline solution.

Distillation of Acetol. Acetol (b. p. 53–54°/18 mm.) was prepared from monobromoacetone by the method of Levene and Walti[1] uncontaminated with pyruvic acid.

The distillation of 1 g. of acetol (in 500c.c. H_2O) with 50 c.c. of the phosphate solution (pH 6.4) was continued for 7.5 hours and 778 c.c. of the distillate were obtained. On analysis the distillate gave 1.20 g. of acetol-semicarbazone (0.68 g. as acetol), traces of methylglyoxal-bis-semicarbazone, and the distillation residue afforded 0.03 g. of pyruvic acid 2 : 4-dinitrophenylhydrazone (m. p. 214°).

This showed that acetol is also susceptible to autoxydation.

Distillation of Lactic Acid. 0.8 g. of lactide (m. p. 122°) was refluxed for 3 hours with 150 c.c. of N/10 soda solution. The product was neutralized by hydrochloric acid and made up to 500 c.c. by addition of water. Adding lactic acid solution thus prepared in small portions, 50 c.c. of the phosphate solution (pH 4.5, 50 c.c.) was distilled. The distillate gave no iodoform reaction. After continuing the distillation for 14 hours (distillate 1000 c.c.), an appreciable quantity of lactic acid in the form of zinc salt and a trace of a 2 : 4-dinitrophenylhydrazone (m. p. 204–208°) were obtained from the distillation residue. With

1 "Org. Syntheses." Vol. X. p. 1. (1930).

the phosphate solution of pH 9.3 the experimental results were quite alike.

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