THE α-OXIDATION OF ACETALDEHYDE AND THE MECHANISM OF THE OXIDATION OF LACTIC ACID.

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(Received for publication, July 19, 1930.)

In previous papers (1, 2) it has been shown that in dilute aqueous solutions aldehydes may be oxidized in the α position by oxidizing agents such as ceric sulfate, potassium dichromate, and potassium ferricyanide. Normal butyraldehyde and isobutyraldehyde yielded the most conclusive results; the latter was converted by ceric sulfate at 80° into α-hydroxyisobutyraldehyde and acetone. The evolution of carbon dioxide by the oxidation of acetaldehyde with potassium permanganate in acid solution under special conditions indicated that the α-oxidation could proceed also with this aldehyde. A study of the action of ceric sulfate on acetaldehyde in dilute solution at 80° has now shown that with this reagent α-oxidation is the normal course of the reaction. A study of the action of the same reagent on lactic acid shows that the acetaldehyde first formed undergoes α-oxidation. The possible biochemical implications of this fact are considered in the concluding paragraphs of this paper.

Oxidation of Acetaldehyde.

The oxidation of acetaldehyde by a 0.2 N solution of ceric sulfate in 0.5 M sulfuric acid at 80° yields formic acid as the chief product. In addition, about 0.25 mol of carbon dioxide per mol of aldehyde is produced and small amounts of volatile and non-volatile aldehydes. The presence of glycolic aldehyde or glyoxal was established by the formation of the p-nitrophenylsazone which was identified by a mixed melting point. The fact that the solution (after removal of the cerous salts) reduced Fehling's solution rapidly at room temperature but reacted very slowly with phenylhydrazine showed that the source of the osazone was glycolic
aldehyde and not glyoxal, although the presence of some of this compound is not excluded. Glyoxylic acid is also formed in varying amounts depending on the conditions. A positive test for it was obtained in the distillate in several experiments. A small amount of formaldehyde is probably also formed but not in sufficient amounts to obtain a solid derivative.

In all the experiments the temperature was kept at 80-90°; in order to study the volatile aldehyde it was necessary to be sure that all the acetaldehyde had been oxidized. This was accomplished in a number of experiments by employing a 5:1 molecular proportion of oxidizing agent and prolonging the action for 2 hours. A typical experiment was as follows: 50 cc. of freshly prepared 0.2 M acetaldehyde solution (0.01 of a mol) were added to 200 cc. of a 0.24 M solution of ceric sulfate (0.048 of a mol) in 0.5 M sulfuric acid. The temperature was kept at 80-90° for 2 hours; a slight excess of ceric sulfate was still present at the end of this time, as shown by the yellow color. The solution was cooled, filtered (a large amount of cerous sulfate precipitated during the reaction), and about 200 cc. distilled. The distillate contained no acetaldehyde as judged by the absence of a positive iodoform test. Tests with fuchsin reagent, and Tollens' reagent, were positive, while the solution failed to reduce Fehling's solution. A slight murkiness was formed in treating the distillate with p-nitrophenylhydrazine. A Hopkins-Cole test for glyoxylic acid was positive. Another sample of the distillate was neutralized with sodium hydroxide (in some experiments a redistillation was performed before the neutralization). The sodium salt was obtained by evaporation to dryness (each run yielded a few tenths of a gm.). The salt was found to be chiefly sodium formate contaminated with a little sodium carbonate. (Possibly small amounts of sodium acetate were also present.) The typical reactions of sodium formate (including the evolution of 1 mol of carbon dioxide on oxidation), an analysis of the barium salt, and the actual isolation of formic acid from several gm. of material, completed the identification.

The nature of the non-volatile aldehyde was established in another similar experiment in which the reaction mixture stood 15 minutes and only 150 cc. were distilled in order to avoid decomposition. Such a residue on treatment with p-nitrophenyl-
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Hydrazine yielded a small amount of reddish brown precipitate (a few mg. in each experiment). This precipitate gave the test with sodium hydroxide and alcohol characteristic of the p-nitrophenylosazone of glyoxal. The yield of the osazone could be increased by evaporation of the initial solution to a small volume before the precipitation; the evaporation was carried out under diminished pressure at 80°. After almost one-third of the water had been distilled, the solution was neutralized with sodium carbonate, the precipitated cerous carbonate filtered off, and the evaporation continued, inorganic solids being removed from time to time. In this way the volume was diminished to almost 0.1 of the original. The concentrated solution reduced Fehling's solution in the cold; in the Hopkins-Cole test for glyoxylic acid a deep brown ring was formed which obscured the glyoxylic acid reaction if such were present. About 1 gm. of p-nitrophenylosazone per 0.05 mol of acetaldehyde was obtained from this concentrated solution; this is a 10 per cent yield if the precipitate is assumed to be pure glycolic aldehyde osazone. The orange color and the fact that a portion of it was soluble in alcohol indicated that the p-nitrophenylhydrazone of glyoxylic acid was also present. Pure p-nitrophenylosazone of glycolic aldehyde was obtained by extraction with alcohol and recrystallization from pyridine; identification was made complete by a mixed melting point with a known specimen. Experiments in which the molecular ratio of ceric sulfate to aldehyde was 2:1 and 4:1 yielded the same amount of product.

Oxidation of Lactic Acid.

If a solution of lactic acid and a solution of 0.2 M ceric sulfate are slowly dropped into boiling 0.5 M sulfuric acid, the distillate contains acetaldehyde (identified by the formation of the p-nitrophenyldrazine). However, if the acetaldehyde is not removed immediately but allowed to react further with the ceric sulfate, the final products of the oxidation of lactic acid by ceric sulfate are glycolic aldehyde, glyoxylic acid, formic acid, and formaldehyde. Strangely enough the yield of formaldehyde is greater than when acetaldehyde itself is oxidized.

A typical experiment was as follows: To 200 cc. of 0.24 M ceric sulfate (0.048 mol) in 0.5 M sulfuric acid at 80°, 35 cc. of 0.2 M
lactic acid (0.007 mol) were added either at once or slowly. (In some experiments a solution of recrystallized zinc lactate was employed without altering the results.) After 2 hours, the solution was distilled (a slight excess of ceric sulfate was present) and the distillate examined in exactly the same manner as described in the experiments with acetaldehyde. The fuchsin and Tollens's tests were very much stronger than in the experiments with acetaldehyde while the glyoxylic acid test was of about the same intensity; the iodoform test was negative. The p-nitrophenylhydrazone of formaldehyde was obtained as a yellow solid by the addition of the reagent to the distillate. It was identified by a mixed melting point determination. The chief component of the distillate was identified as formic acid.

The essentially non-volatile products of the oxidation of lactic acid by ceric sulfate are a mixture of glyoxylic acid and glycolic aldehyde as in the case of acetaldehyde. As a rule the yield of these products seems to be less; glyoxylic acid appears to be the sole product when a large excess of ceric sulfate is employed. Thus, if lactic acid is slowly added to boiling ceric sulfate in a 6:1 molecular ratio, only half of the ceric sulfate is reduced since the major portion of the product is acetaldehyde which at once escapes. After concentration of the residual liquid, a yellow precipitate was formed with p-nitrophenylhydrazine which had the same appearance and solubility as the p-nitrophenylhydrazone of glyoxylic acid (a complete identification was not possible because of the unsatisfactory melting point of this compound); the Hopkins-Cole test was strongly positive. The absence of glycolic aldehyde was shown by the fact that the solution did not reduce Fehling's solution at room temperature and by the yellow color and solubility of the precipitate with p-nitrophenylhydrazine. It being assumed that the yellow precipitate was composed solely of the hydrazone of glyoxylic acid, the yield was about 3 per cent.

The best yield of glycolic aldehyde from lactic acid was obtained by having only a 4:1 ratio of ceric sulfate to acid and allowing the reaction mixture to stand 15 to 30 minutes at 80° before distillation. The concentrated solution obtained under these conditions reduced Fehling's solution in the cold, gave a brown ring with no purple color in the Hopkins-Cole test, and yielded an orange precipitate with p-nitrophenylhydrazine. The pure p-nitrophenyl-
osazone was readily prepared from this precipitate by the pro-
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procedure described above. It was identified by a mixed melting point determination. The yield was about 8 per cent. With a ratio of 6:1 of reagent to acid, the yield was only about 3 per cent and though the precipitate was orange the preponderance of glyoxylic acid was shown by a strong Hopkins-Cole test and a negative Fehling’s test in the cold.

**DISCUSSION.**

That the first step in the oxidation of acetaldehyde by ceric sulfate consists in the introduction of oxygen in the α position can hardly be doubted in view of the facts given above. The possibility of the process proceeding through acetic acid seems excluded by the fact that acetic acid, like formic acid, is not attacked by hot ceric sulfate. We cannot be certain that the intermediate product between acetaldehyde and formic acid is glycolic aldehyde. Some unstable intermediates such as \( \text{CH}_2\text{C=CH}_2 \) may first be formed, but the formation of the \( p \) -nitrophenylosazone of glyoxal from the reaction mixture proves that some glycolic aldehyde is present at the end. The formation of formic acid may proceed through glycolic acid but this substance on oxidation with ceric sulfate yields 1 mol of carbon dioxide for each mol of formic acid, whereas the ratio of formic acid to carbon dioxide with acetaldehyde is about 3:1. The most conservative method of formulating our results would seem to be as follows:

\[
\text{CH}_3\text{CHO} + [\text{O}] \rightarrow [\text{C}_2\text{H}_4\text{O}_2] \quad \rightarrow \quad 2 \text{H COOH} + \text{H}_2\text{O}
\]

Further oxidation

Intermediate unknown structure

Rearrangement

\[
\text{CH}_2\text{OH} \quad \text{H COOH} + \text{CO}_2 + \text{H}_2\text{O}
\]

\[
\text{CHO COOH}
\]
Heimrod and Levene (3) found that the oxidation of acetaldehyde by hydrogen peroxide in alkaline solution yielded formic acid. They assumed that the intermediates were vinyl alcohol, glycolic aldehyde, and glyoxal, and showed that the last two substances yielded formic acid under the conditions of the experiment. Our identification of glycolic aldehyde and glyoxylic acid among the products of the oxidation of acetaldehyde might be taken as supporting evidence for their hypothesis although our reagent and conditions were very different from theirs. The enol form of acetaldehyde may be involved in oxidations which occur in alkaline solution (Heimrod and Levene's least alkaline conditions were with dilute ammonia), but it is much less probable that a reaction would take this course in 20.5 M sulfuric acid. Furthermore, the oxidation of vinyl alcohol directly to glycolic aldehyde by the oxidation of two hydroxyl groups to the ethylene linkage is very improbable since ceric sulfate does not attack the double carbon linkage. It differs markedly in this respect from the equally strong oxidizing agent, potassium permanganate, and from hydrogen peroxide. For this reason we prefer to write an intermediate C2H4O2 of unknown structure. Considering the fact that the yield of glycolic aldehyde is about the same whether a 2:1 or a 4:1 ratio of oxidizing agent is employed, it seems as if this aldehyde were produced by a side reaction.

We must conclude that acetaldehyde is an intermediate in the main reaction between ceric sulfate and lactic acid since it escapes from the reaction mixture if the opportunity is provided. The further oxidation of acetaldehyde then yields the products noted in the scheme outlined in the preceding paragraph. This simple explanation is not entirely satisfactory, however, since it does not account for the fact that very definite amounts of formaldehyde are formed from lactic acid while with acetaldehyde the amount of this aldehyde in the oxidation products was too small to make its identification certain. Probably in addition to the main reaction which follows the course lactic acid → acetaldehyde, there is another side reaction in which lactic acid is oxidized to formaldehyde. The other products of this side reaction may well be identical with those formed from acetaldehyde. Another possibility is that the first oxidation product of acetaldehyde may react with lactic acid, one of the products of this interaction being formaldehyde.
The results obtained with lactic acid and acetaldehyde suggest that perhaps the same mode of oxidation may occur in biological processes. One would expect that under certain circumstances the first product of the oxidation of acetaldehyde (i.e., glycolic aldehyde or the intermediate $C_2H_4O_2$) might undergo reactions other than those we have found. In particular, one would expect polymerization to carbohydrates. The mechanism of the oxidation of lactic acid in the muscle and the resynthesis of glycogen might be imagined to take some such course as the following.

$$3 \text{CH}_2\text{CHOH COOH} + 3 \text{O}_2 \rightarrow 3 [\text{C}_4\text{H}_8\text{O}_4] + 3 \text{CO}_2 + 3 \text{H}_2\text{O}$$

The actual process in the muscle is specific for lactic acid (and pyruvic) and impossible of duplication with acetaldehyde or glycolic aldehyde. This is not inconsistent with the suggested mechanism; such a situation appears to exist in regard to the production of formaldehyde by oxidation in vitro, as has been shown above. The ratio of oxygen to carbon dioxide given by the above equation is, of course, in accord with the facts. The portion of acid resynthesized is two-thirds (0.66) according to the scheme outlined above, while Meyerhof (4) believes that under favorable circumstances almost four-fifths (0.80) of the lactic acid may be resynthesized. If one were willing to postulate that a molecule of lactic acid or its isomer methyl glyoxal as well as the intermediate were involved in the polymerization, the following reaction involving the resynthesis of four-fifths of the acid might be written:

$$3 \text{C}_2\text{H}_4\text{O}_2 + 2 \text{C}_2\text{H}_4\text{O}_3 \rightarrow 2 \text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O}$$

While this mechanism has the advantage of clearly explaining why the process is specific for lactic acid, it would involve the cleavage of 1 molecule of the compound $C_2H_4O_2$ into formaldehyde in order for the six carbon chain in glycogen to be synthesized. These mechanisms provide a direct chemical path by which the energy of the oxidation process might effect the resynthesis of the carbohydrate. Since it has been shown that the oxidation in vitro of
lactic acid yields such reactive substances as formaldehyde and glycolic aldehyde, it is very tempting to imagine that similar products are formed in the muscle where they would be just the compounds needed for the resynthesis of carbohydrates without further expenditure of energy.

We are in indebted to Mr. W. F. Bruce for valuable assistance in performing some of the experiments reported in this paper.

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