

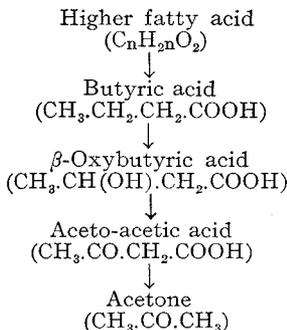
THE OXIDATION OF BUTYRIC ACID BY MEANS OF HYDROGEN PEROXIDE WITH FORMATION OF ACETONE, ALDEHYDES AND OTHER PRODUCTS.

BY H. D. DAKIN.

(From the Laboratory of Dr. C. A. Herter, New York.)

(Received for publication, December 7, 1907.)

Among the numerous investigations made with the object of throwing light upon the mode of oxidation of organic acids in the animal organism few have been more suggestive than those concerned with the origin of the "acetone bodies" from the saturated fatty acids. It will be recalled that the acetonuria resulting from starvation, from a carbohydrate-free dietary, or from experimental or human diabetes is associated with an excessive fat catabolism. It is generally conceded that the long carbon chain in the molecule of the higher fatty acids is resolved in such a way as to yield one or more molecules of butyric acid and that the acetone bodies (β -oxybutyric acid, aceto-acetic acid and acetone) result from the further oxidation of this substance:



There is a mass of indirect experimental evidence to support this view of the origin of β -oxybutyric acid, aceto-acetic acid and

acetone from butyric acid, and of the latter from the higher fatty acids. Thus increased excretion of acetone and of the "acetone bodies" results from the injection into diabetic animals of butyric acid and of a number of substances, such as caproic acid, isovaleric acid and leucin, which can give rise to butyric acid in the course of their oxidation in the animal body. Similar results are obtained when the same substances are administered to persons in whom the normal processes of oxidation have been influenced either through starvation or consumption of a carbohydrate-free diet, or by diabetic disease.

If butyric acid be the precursor of β -oxybutyric acid, acetoacetic acid and acetone, it is necessary to assume that in the organism, the first stages of the oxidation of butyric acid, if not exclusively at least in part, take place at the β -carbon atom. Up to the present, however, it has not been possible to bring about a similar oxidation of butyric acid outside the body. Thus on prolonged boiling with nitric acid, butyric acid yields succinic acid;¹ with manganese dioxide and dilute sulphuric acid as oxidizing agent, propyl butyrate and ethyl butyrate are formed.² Chromic acid oxidizes butyric acid to acetic acid and carbon dioxide,³ while alkaline potassium permanganate brings about complete oxidation to carbon dioxide and water.⁴ The object of the present paper is to show that by a suitable choice of oxidizing agents, it is possible to oxidize butyric acid outside the body in a way that bears the closest resemblance to that believed to occur in the organism, and thereby to secure a confirmation of the results already arrived at from physiological studies.

Evidence is slowly accumulating which tends to show that oxidation in the β -position is not an uncommon primary process in the breakdown of many organic acids in the organism. Reference may be made to the interesting work of Knoop upon the break down in the organism of the aromatic derivatives of fatty acids, *e. g.*, phenylpropionic, phenylbutyric and phenylvaleric acids, in which the occurrence of primary oxidation in the β -position is shown to be very probable. But on reviewing the

¹ Dessaignes: *Ann. d. Chem.*, lxxiv, p. 361.

² Veill: *Ibid.*, cxlviii, p. 164.

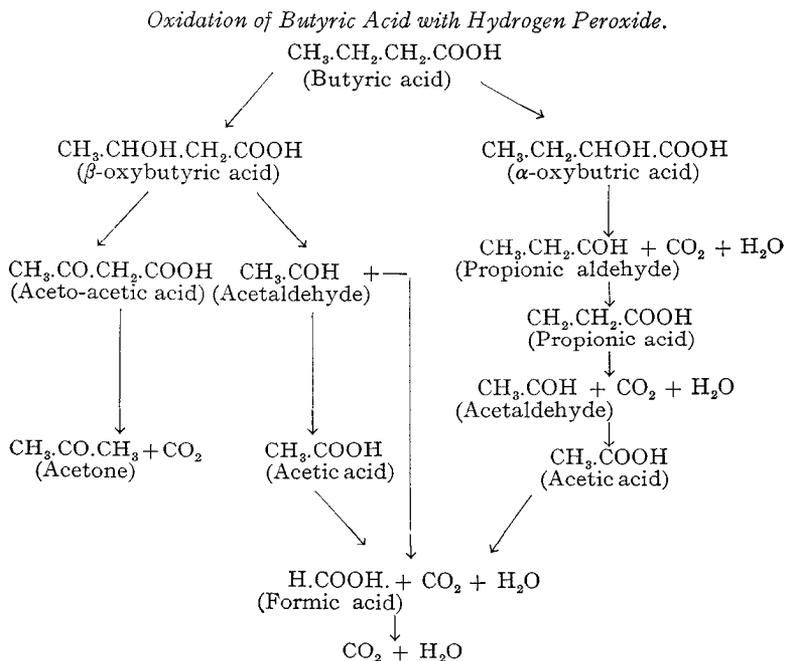
³ Grünzweig and Hecht: *Ber. d. deutsch. chem. Gesellsch.*, xi, p. 1053.

⁴ Meyer: *Ann. d. Chem.*, ccix, p. 241.

experimental results of studies upon the oxidation of fatty acids by purely chemical means it will be found that comparatively few cases are to be found that furnish clear evidence of the β -carbon atom being the first point of attack.

The probability that oxidation of saturated fatty acids at the β -carbon atom is a common biological reaction makes it desirable to know more of the conditions under which such changes may be brought about outside the body. A number of previous observations had shown a remarkably close similarity between oxidations carried out with hydrogen peroxide acting under certain conditions and those occurring in the organism. It was therefore decided to study the action of peroxide of hydrogen upon butyric acid in order to see whether convincing evidence might not be forthcoming of the possibility of imitating outside the body those changes that are believed to progress within. As in previous experiments of this kind, neutral peroxide was allowed to act upon the ammonium salt of the acid. It was found that the ammonium butyrate was attacked to some extent even at the temperature of the air, but more readily on gently warming. The following products were detected: Aceto-acetic acid, acetone, propionic aldehyde, acetaldehyde, acetic acid, formic acid and carbon dioxide. Indirect evidence was obtained of the presence of propionic acid. From the fact that both aceto-acetic acid and acetone were formed, the latter substance in large quantity (see experimental part), it is very probable that β -oxybutyric acid was an intermediate product of oxidation and this view was confirmed by the fact that β -oxybutyric acid itself on oxidation with peroxide under similar conditions also gave acetone, acetaldehyde, acetic acid, formic acid and carbon dioxide (see following paper). From a consideration of the products of oxidation together with a knowledge of the further action of peroxide of hydrogen upon some of the intermediate products it is possible to draw a picture of the successive steps in the breakdown of butyric acid.

Oxidation of Butyric Acid



It will be seen from the diagram that the initial breakdown of butyric acid appears to progress in two directions. It will be convenient to consider first that taking place with initial formation of β -oxybutyric acid. The β -oxybutyric acid undergoes further decomposition in two directions, yielding on the one hand aceto-acetic acid, which is then resolved into acetone and carbon dioxide, and on the other, acetaldehyde and probably other products which undergo further oxidation. The acetaldehyde in turn is oxidized to acetic acid and this eventually yields formic acid and carbon dioxide. The formic acid is lastly also oxidized to carbon dioxide and water. It is probable that several intermediate products are formed in the conversion of acetic acid into formic acid.¹ That acetic acid actually is oxidized to formic acid under the conditions prevailing in the oxidation of the butyric acid was proved by direct experiment. Hopkins and Cole² have investi-

¹ Cf. Dakin: *This Journal*, iii, p. 57.

² Hopkins and Cole: *Proc. Roy. Soc.*, lxxviii, p. 21.

gated the action of peroxide of hydrogen upon acetic acid under somewhat different conditions and obtained evidence of the formation of glyoxylic acid and formaldehyde in addition to formic acid. It is natural to assume that these substances were produced by the oxidation of the acetic acid formed during the oxidation of butyric acid, but to a large extent underwent further oxidation. No special attempt was made to isolate glycollic or glyoxylic acids, although qualitative reactions for the latter substance were obtained. No evidence was obtained of the presence of formaldehyde.

The presence of propionic aldehyde among the oxidation products of butyric acid indicates that part of the butyric acid underwent oxidation in a different way from that already indicated. The formation of this substance necessitates the assumption that oxidation at the α -carbon atom has taken place. It is natural to assume that α -oxybutyric acid is the primary product although this substance was not actually isolated. Identical products were obtained when α -oxybutyric acid was oxidized under similar conditions (see following paper). The propionic aldehyde on further oxidation yields propionic acid and the latter in turn yields acetic aldehyde and acetic acid. It is possible that lactic acid is an intermediate product in this reaction, but experiments to determine this point are at present lacking. Special experiments showed that acetaldehyde was actually formed by the action of hydrogen peroxide upon ammonium propionate. The acetic acid on further oxidation yields formic acid and eventually carbon dioxide and water as previously described for the products of oxidation of β -oxybutyric acid.

It will be seen that the earlier products of oxidation with the exception of acetone are all capable of further oxidation until they are eventually converted into carbon dioxide and water. Special experiments showed that acetone is very resistant to the oxidizing action of hydrogen peroxide and it is interesting to note that this is exactly comparable with the conditions believed to exist in the organism.¹ The difficulty of acetone combustion in the body has been demonstrated by Geelmuyden

¹ Wolfenstein has shown that acetone yields a peroxide on treatment with highly concentrated solutions of H_2O_2 .

while the easy tissue oxidation of the other products, such as propionic, acetic and formic acids, is well known.

The close similarity in many respects between tissue oxidation and oxidations affected by hydrogen peroxide has already been demonstrated¹ and in consequence it may be justifiable to try to draw some deductions from the results of the present investigation, as to the course of the breakdown of butyric acid in the body. It has long been a debatable question as to whether the breakdown of butyric acid with formation of the acetone bodies as intermediary products, as is known to take place under abnormal conditions, also represents the normal course of metabolism under physiological conditions. The difficulty of acetone combustion, even in the healthy organism, has been one of the main objections to the assumption that this substance is a product of normal intermediary metabolism. Notwithstanding this, the opinion just mentioned has been widely held, partly, no doubt, because hitherto it has been difficult to offer a satisfactory alternative theory of decomposition. In view of the already mentioned similarity between oxidation in the tissues and changes brought about by peroxide of hydrogen, is one not justified in assuming provisionally that butyric acid may break down in the body in more than one way, just as it does under the influence of peroxide of hydrogen? It may be that β -oxybutyric acid is the sole primary product and that this may break down so as to give either acetone, aceto-acetic acid being an intermediate product, or it may yield acetic and formic acids which then undergo further oxidation to carbon dioxide and water. The factors which determine which course of decomposition shall predominate have still to be determined. It must be conceded, too, that the possibility of α -oxidation of fatty acids in the organism is not entirely excluded and it may be that part of the butyric acid is broken down through the stage of propionic acid. The further complete breakdown of this substance in the organism presents no difficulty.

At any rate it appears from the results of the present investigation that there is at present no reason for assuming that acetone is necessarily a constant intermediate product of the tissue oxida-

¹ This *Journal*, i, pp. 171 and 271; iii, p. 419, and pending paper.

tion of butyric acid. It is probable that other modes of oxidation by way of acetic and formic acids (and possibly, although less likely, propionic acid) are at the disposal of the organism.

The demonstration of the easy formation of the acetone bodies by the direct oxidation of butyric acid renders superfluous the various hypotheses of the *synthetic* formation of these substances put forward at various times by von Jaksch, Geelmuyden, Magnus-Levy, Schwarz and others.

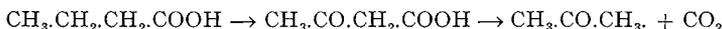
EXPERIMENTAL.

For the following experiments Kahlbaum's pure butyric acid was redistilled and the fraction boiling between 163.2° and 163.5° was employed. It was usually converted into the ammonium salt as required by addition of a slight excess of ammonia and then heated with neutral 3 per cent peroxide of hydrogen. In almost all the experiments it was so arranged that peroxide equivalent to two gram mols. was employed for the oxidation of every one gram mol. of the ammonium butyrate. Under these conditions a considerable amount of butyric acid remains unoxidized and there is opportunity for detecting some of the intermediate products of oxidation. The first point determined was the amount of carbon dioxide which was given off under these conditions.

Carbon dioxide. $\frac{1}{100}$ gm. mol. = 0.88 gm. butyric acid was neutralized with a slight excess of ammonia and the resulting solution boiled for a few minutes to expel any traces of carbon dioxide. The solution was then cooled and 3 per cent peroxide, equivalent to $\frac{3}{100}$ gm. mol. added. The mixture was contained in a flask placed in a water-bath and was connected with a series of absorption-bulbs all of which contained standard baryta solution with the exception of the first, which contained sulphuric acid to absorb any ammonia that might come over. A current of washed carbon-dioxide-free air was aspirated through the flask by means of a pump. The water-bath surrounding the flask was gradually heated, eventually to the boiling temperature. The current of air was maintained for almost three hours.

The excess of baryta in the absorption-bulbs was determined by titration with $\frac{N}{2}$ sulphuric acid using phenolphthalein as

indicator.¹ In two experiments carbon dioxide equivalent to 0.1155 gm. and 0.1100 gm. was obtained. If the whole of this carbon dioxide had resulted from the decomposition of butyric acid with formation of acetone according to the equation:



the amount would be equivalent to about 26 per cent decomposition. By adding more peroxide of hydrogen to the residue in the flask and again neutralizing and warming, a further evolution of carbon dioxide almost as large as the initial amount may be obtained. If desired, the process may be repeated until the whole of the butyric acid is decomposed.

Non-volatile products. That the reaction had not proceeded simply with formation of acetone and carbon dioxide was proved by determining the mean molecular weight of the unoxidized residual fatty acids. It was found to be lower than that of butyric acid, showing that fatty acids of lower molecular weight had been formed. The residue from the oxidation of butyric acid with two mols. peroxide was made exactly neutral with ammonia and precipitated with excess of silver nitrate. The silver salt was analyzed:

$$\begin{array}{l} 0.8577 \text{ gm. gave } 0.4883 \text{ gm. silver} = 56.93 \text{ per cent Ag} = \text{M.W. } 82.7 \\ \text{C}_4\text{H}_7\text{O}_2\text{Ag requires } 55.36 \quad \text{“} \quad \text{“} \quad \text{and} \quad \text{“} \quad 88 \end{array}$$

The non-volatile residues from a number of butyric acid oxidations were combined and evaporated to small bulk. An unsuccessful attempt was made to obtain evidence of the presence of β -oxybutyric acid by distilling the residue with strong sulphuric acid and examining the distillate for crotonic acid. The negative result does not, however, entirely preclude the possibility of its presence as its detection in small amount is rendered very difficult when accompanied by a large quantity of butyric acid.

Volatile products. The oxidation was carried out by very slowly distilling a mixture of ammonium butyrate (1 gm. mol.) and hydrogen peroxide (2 gm. mol.). The distillate was caught in a receiver placed in a freezing mixture. It smelt strongly of acetone and aldehydes and contained ammonia and some un-

¹ The liquid in the absorption-bulbs smelt strongly of acetone.

changed peroxide of hydrogen. The distillate was acidified with sulphuric acid and redistilled. The amount of acetone plus acetaldehyde was estimated by titration with standard iodine and calculated as acetone, and proved to be about 9 to 12 per cent of the amount of butyric acid taken for oxidation. But as less than 30 per cent of the butyric acid underwent oxidation the actual yield was about 40 per cent of the butyric acid oxidized, equivalent to about 50 per cent of the amount of acetone theoretically possible. Subsequent investigation showed that the proportion of acetone to aldehyde varied but was usually about 3 : 1.

The acetone was identified as follows. Distillates containing acetone and aldehydes were treated with ammoniacal silver solution together with some caustic soda. After gently warming and allowing to stand for some time the solution was filtered from precipitated silver and then almost but not quite neutralized with phosphoric acid. On distillation acetone passed over containing only traces of aldehydes. Part of the distillate was treated with paranitrophenylhydrazine acetate. An abundant precipitate of the hydrazone was at once obtained, which after recrystallization from dilute alcohol was obtained in the form of shining golden yellow needles melting at 149° . Bamberger and Sternitzki¹ give 148° to 148.5° as the melting point of acetone paranitrophenylhydrazone. Another portion of the distillate (15 cc.) containing approximately a decigram of acetone was shaken vigorously with 0.5 gram of benzaldehyde after addition of 3 cc. of a 10 per cent caustic soda solution. After 24 hours the somewhat pasty deposit of crystals was filtered off, washed with water and drained on a porous tile. It was then crystallized from alcohol. Well formed platelets of dibenzylidene-acetone melting sharply at 111.5° to 112.5° were readily obtained. The product dissolved with an orange-red color in strong sulphuric acid and with fuming nitric acid gave a deep red additive compound in the same manner as pure dibenzylidene-acetone prepared in other ways.

The preparation of the derivatives just described serves to demonstrate the presence of acetone convincingly, but several other reactions for acetone were carried out in addition. In every case a positive result was readily obtained. The tests used were

¹ *Ber. d. deutsch. chem. Gesellsch.*, xxvi, p. 1306.

(i) the orthonitrobenzaldehyde condensation with formation of indigo; (ii) the nitroprusside reaction; (iii) Stock's reaction with hydroxylamine, etc.; (iv) the iodoform reaction.

The satisfactory identification of the aldehydes found in the distillate offered considerable difficulty owing to the presence of large amounts of acetone. The presence of aldehydes was easily demonstrated with ammoniacal silver solution, Fehling's or Schiff's reagent and acetaldehyde was readily detected by Rimini's reaction with sodium nitroprusside and piperidin. Formaldehyde could not be detected with the usual qualitative tests. The question of the presence of propionic aldehyde could not be directly settled, but indirect evidence was obtained as follows. Distillates containing a mixture of acetone and aldehydes were digested with an excess of Tollen's ammoniacal solution, containing caustic soda. At first the oxidation was carried out at the ordinary temperature of the air, afterwards in an incubator at 37° . After some hours the precipitated silver was filtered off and the solution acidified and distilled. In this way a distillate was obtained which contained volatile fatty acids corresponding to the aldehydes originally present. The fatty acid was found to contain acetic acid corresponding to the acetaldehyde already detected but in addition there was present a higher fatty acid, which proved to be propionic acid. This was shown by a determination of the mean molecular weight of the acid by means of the silver and barium salts. Three separate experiments gave the following results:

- I. 0.1711 gm. silver salt gave 0.1049 gm. Ag = 61.3 per cent Ag =
M. W. 69.1
- II. 0.1099 gm. silver salt gave 0.0674 gm. Ag = 61.3 per cent Ag =
M. W. 69.1
- III. 0.1718 gm. barium salt gave 0.1226 gm. BaCO_3 = 49.6 per cent Ba
= M. W. 70.6

The molecular weight was found to be intermediate between that of acetic acid (60) and propionic acid (72).

It was thought desirable to attempt the isolation of the propionic acid in order to furnish convincing evidence of the formation of the propionic aldehyde from which it was derived. Accordingly distillates containing the aldehydes and acetone from the oxidation of butyric acid, were distilled after the addition of a

little ammonia, in order to remove any possible traces of butyric acid which might be present. The distillate was then oxidized with Tollen's silver solution with very gentle warming. The precipitated silver was then filtered off and after neutralizing the bulk of the alkali with phosphoric acid the acetone was distilled off. On acidifying, the volatile acids were obtained by further distillation. They were converted into the barium salts and after evaporation the residue was stirred up with dilute alcohol. The residue was washed with more alcohol and in this way the bulk of the more soluble acetate was removed. The residue was dissolved in a little water and precipitated with excess of silver nitrate. The silver salt was dried *in vacuo* and on analysis proved to be silver propionate:

0.1622 gm. silver salt gave 0.0972 gm. silver = 59.9 per cent Ag
 $\text{CH}_3\text{CH}_2\text{COOAg}$ requires 59.7 " "

Action of Hydrogen Peroxide upon Ammonium Butyrate at 40°.

I. *Detection of aceto-acetic acid.* Butyric acid (1 gm.) was neutralized with a very slight excess of ammonia and then 16 cc. of 3 per cent hydrogen peroxide was added. The whole was then incubated over night at 37°. The liquid was then shaken with pure charcoal to decompose the bulk of the excess of peroxide. The filtrate gave a strong reaction for aceto-acetic acid when tested for by the Arnold test with para-amidoacetophenone. The Bondy and Schwarz reaction with iodine was also obtained but the reaction with ferric chloride was largely obscured owing to the presence of large amounts of fatty acids. On warming the solution, the aceto-acetic acid readily decomposed with formation of acetone and carbon dioxide.

II. *Detection of acetone and acetaldehyde.* 8.8 grams of butyric acid ($\frac{1}{10}$ g. mol.) was neutralized with ammonia and $\frac{2}{10}$ g. mols. of 3 per cent hydrogen peroxide added. The mixture was placed in a tightly corked thick-walled flask and incubated over night at 37°. On shaking part of the fluid with either precipitated metallic silver or with charcoal so as to decompose the bulk of the excess of peroxide, it was found that the filtrate gave a strong iodoform reaction. This might, however, have been due to the aceto-acetic acid present in the liquid so that another method for the detection of acetone and aldehyde had to be devised. Accord-

ingly the liquid was distilled *in vacuo* at a temperature of not more than 40° . The distillate was caught in a receiver cooled with ice and salt. The distillate which contained ammonia and unchanged peroxide was acidified with phosphoric acid and redistilled. Aldehyde was readily detected in the distillate by the reactions with Tollen's silver solution, Schiff's reagent and by Rimini's reaction with sodium nitroprusside and piperidin. Part of the distillate was oxidized with Tollen's ammoniacal silver solution, then acidified and redistilled. Acetone was readily detected in the distillate with the iodoform reaction. In another series of experiments the distillation *in vacuo* was omitted and a current of air was bubbled through the liquid warmed to 40° . Aldehyde and acetone were readily volatilized and their presence detected by the tests previously employed.

SUMMARY.

Ammonium butyrate is readily oxidized by peroxide of hydrogen. The reaction progresses to a marked extent at low temperatures (37°) but is accelerated by warming.

The following products were detected: aceto-acetic acid, acetone, propionic aldehyde, acetaldehyde, acetic acid, formic acid and carbon dioxide. In addition propionic acid was probably present.

It is probable that α -oxybutyric acid and β -oxybutyric acid are the initial products of oxidation. With the exception of acetone, all the above products are capable of further oxidation with hydrogen peroxide until they are eventually converted into carbon dioxide and water. The probable course of the reaction is indicated diagrammatically on p. 80.

Acetone is very resistant to oxidation by hydrogen peroxide. A close similarity exists between the types of change involved in the oxidation of butyric acid with hydrogen peroxide and its oxidation in the body.

The view that there is no need to assume that the whole of the butyric acid formed in the body is oxidized with intermediate formation of acetone is supported. It is probable that β -oxybutyric acid may be decomposed so as to give acetic acid and its oxidation products. It is also possible, although

much less probable, that α -oxybutyric acid may be an alternative initial product of the tissue oxidation of butyric acid and this may be further oxidized through propionic acid and its subsequent oxidation products.