

## XVI. THE OXIDATION OF THE ALKALI BUTYRATES BY HYDROGEN PEROXIDE WITH THE PRODUCTION OF SUCCINIC ACID.

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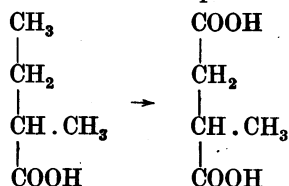
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The oxidation of butyric acid in the form of its ammonium salt has been studied by Dakin [1908]. He slowly distilled a solution of ammonium butyrate containing a slight excess of ammonia with two molecular proportions of 3 % hydrogen peroxide. To identify an unstable intermediate product—acetoacetic acid—he allowed ammonium butyrate and hydrogen peroxide to act upon each other at 37°. Dakin supposes that the attack on the fatty acid occurs both at the  $\alpha$ - and  $\beta$ -carbon atoms, the main attack being at the latter. The attack at the  $\alpha$ -carbon atom will lead to the production of propionic aldehyde, propionic acid, acetaldehyde, acetic and formic acids: the attack at the  $\beta$ -carbon atom will yield acetoacetic acid, which will readily break up into acetone and carbon dioxide. Dakin was able to prove the formation of all the substances mentioned, and he claims that the yield of acetone is as high as 50 % of that theoretically possible.

In a paper recently published by Hurtley [1916] the theory of the oxidation of the fatty acids by attack at the  $\beta$ -carbon atom has been subjected to some criticism on the ground of its inadequacy. It was there suggested that the oxidative attack occurred simultaneously at many points in the long chains of carbon atoms which are present in all the common higher fatty acids and in particular that the attack might occur at the terminal methyl group. This is the more likely because Dakin and Herter [1907] have shown that when hydrogen peroxide attacks the benzene ring it does not show the discrimination which is shown by the usual substituting agents: thus, when it attacks benzoic acid it yields a mixture of all three hydroxybenzoic acids. Direct evidence that oxidation of fatty acids occurs at the methyl group in the animal body is not available. Only one piece of evidence, as far as the authors know, exists showing that it occurs at the methyl group when a fatty acid is oxidised by hydrogen peroxide, and this is furnished by Raper

[1914], who found that, when  $\alpha$ -methylbutyric acid is oxidised by hydrogen peroxide, methylsuccinic acid is one of the products of the oxidation.



This surely indicates attack at the terminal methyl group. We have therefore taken up the question of the oxidation of the fatty acids by hydrogen peroxide and starting with butyric acid we have obtained results which lead us to suppose that the methyl group is a principal point of attack in this kind of oxidation and also encourage us to think that it will be worth while to study other fatty acids from this point of view. At first, following Dakin, we used the ammonium salt of butyric acid, but although the oxidation of this salt appears to take place more quickly than that of the sodium or potassium salts we have discarded it and now work with the sodium salt only, because:

- (1) The ammonium salt gave rather strongly coloured products.
- (2) It always left the product acid at the end of the reaction.
- (3) In the body it is more probable that the sodium or potassium salts and not the ammonium salts are those that are oxidised.
- (4) On submitting a liquid containing ammonium butyrate to steam distillation, both ammonia and butyric acid pass over with the steam.

To show this we distilled 200 cc. of a solution of ammonium butyrate containing 8.8 g. of butyric acid in a current of steam at such a rate that 50 cc. collected in about four minutes, collecting six fractions of 50 cc. each. In the table of results the first column gives the butyric acid in terms of normal sodium hydroxide: 50 cc. of the distillate were treated with a measured excess of normal sodium hydroxide, boiled till all ammonia was expelled and titrated with normal acid and phenolphthalein. These results are a little high. The second column gives the ammonia also in terms of normal sodium hydroxide: 50 cc. of the distillate were treated as in a Kjeldahl determination. This column gives the ammonia exactly.

Fraction	Butyric acid as cc. N NaOH	Ammonia as cc. N NaOH
1	32	36
2	23	19
3	14	12
4	10	8
5	8	6
6	8	5
Residue	19	14

It will be seen that the first distillate is alkaline and all the rest and the residue are acid in reaction. We wished to be able to distil our products without fear of obtaining ammonia in the distillate.

Our method of working is different from that of Dakin. After many preliminary experiments we adopted the following procedure: 1 g.-molecular proportion of sodium butyrate, equivalent to 4.4 g. of butyric acid, was dissolved in water and 3, 6, or 12 g.-molecular proportions of hydrogen peroxide (20 volume  $H_2O_2$  for the first two and 40 volume for the third) were added and the mixture made up to 190 cc. in all cases. In most of our experiments the mixture was heated at  $65^\circ$  in a flask with an efficient reflux condenser, until all, or all but a trace, of the hydrogen peroxide had disappeared. Temperature, as would be expected, has an exceedingly marked effect on the duration of the reaction: thus with 3 g.-molecules of peroxide at  $37^\circ$  the products are practically the same as at  $65^\circ$ , but the times required are 168 and 66 hours respectively. At  $100^\circ$  the reaction appears to be complete in four hours, but we observed that at this temperature much oxygen was given off and the product was much discoloured. At the end of an experiment with three atoms of oxygen the product is neutral: with six atoms of oxygen the reaction is acid: with twelve atoms of oxygen the reaction is again neutral, or slightly alkaline.

At the end of an oxidation the solution is neutralised, if it is not neutral. In one part aldehydes plus acetone are determined (*a*) directly by addition of standard iodine and caustic soda and titration of excess of iodine by standard thiosulphate in the usual way; (*b*) by distilling a part acidified with acetic acid exactly as in the Messinger-Huppert process. The other part is evaporated to dryness on the water-bath: acidified with a slight excess of normal sulphuric acid, and distilled in steam until all volatile acids are expelled: this usually requires 3000 cc. of distillate. This distillate is titrated and called volatile acids. The residue from this steam distillation is then extracted for 72 hours with ether in a continuous apparatus such as that described by one of us [Hurtley, 1916; see also Plimmer, 1915, p. 598]. After distilling off the ether the liquid is evaporated to dryness in a weighed platinum dish, the solid residue is dried at  $100^\circ$  and weighed. We call this weight the yield of crude acid. It is practically all succinic acid; the colour is usually slightly brown and it has a smell recalling that of pyruvic acid. A small portion of it is very sparingly soluble in water. To purify and identify the succinic acid it was redissolved in water, boiled with animal charcoal, filtered and crystallised. The crystals obtained were still slightly yellow

and were not improved by repeating this process of purification nor by recrystallising from alcohol. They melted at 185°, the melting point of succinic acid, and the melting point was not changed on mixing with succinic acid (Nos. 1, 2 and 3).

The acid was finally obtained pure by recrystallisation from pure concentrated nitric acid and then from water (No. 4).

*Analysis:*

			C	H	
1.	0.2513 g.	0.3875 g. CO <sub>2</sub>	0.1170 g. H <sub>2</sub> O	42.06	5.17
2.	0.2408 g.	0.3713 g. „	0.1092 g. „	42.06	5.05
3.	0.2264 g.	0.3484 g. „	0.1059 g. „	41.97	5.19
4.	0.2345 g.	0.3502 g. „	0.1070 g. „	40.72	5.07
	Calculated for succinic acid ...	...	...	40.67	5.09

The acid crystallises like succinic acid: in neutral solution it gives a buff coloured precipitate with ferric chloride. Of this acid 0.1672 g. required 14.4 cc. N/5 NaOH which corresponds to a molecular weight of 116: theory 118.

One g. of the acid was digested at 60° with 3–4 g. of acetyl chloride for three hours; on cooling it deposited beautiful crystals of succinic anhydride which melted at 119°–120°.

It is inconceivable that succinic acid is produced from butyric acid without the formation of intermediate products, and we have some evidence of the presence of such substances. The succinic acid before crystallisation from nitric acid gave high figures for the percentage of carbon and hydrogen; the acid also gave Schiff's test for aldehydes, and it reduced ammoniacal silver. All these observations would be explained by assuming that before crystallisation from nitric acid the acid contained a small amount of the half aldehyde of succinic acid. The following table will give some idea of the yield of succinic acid, of aldehydes and acetone taken together, and of carbon dioxide. In order to be able to calculate a yield for the succinic acid it is necessary to form an idea of the butyric acid left over and also of that part of it which has been converted into other products than succinic acid. At the end of one of our experiments the solution will contain (a) unchanged sodium butyrate, (b) sodium salts of lower fatty acids, (c) aldehydes (acetic and propionic as Dakin [1908] has shown) and acetone, (d) sodium succinate. If after neutralisation the liquid is evaporated to expel aldehydes and acetone, then acidified and distilled in steam, the distillate should give on titration, expressing the result as butyric acid, the sum of the unchanged butyric acid

and the other volatile fatty acids. Of the fatty acids in the distillate other than butyric, every molecule of propionic must correspond to one molecule of butyric acid oxidised. It is unlikely that acetic acid would arise from acetoacetic acid in our experiments, but it might arise from the oxidation of acetone. Some acetic acid must also be formed from the oxidation of propionic acid, but in each of these cases one molecule of acetic acid would correspond to one molecule of butyric acid oxidised. In the case of formic acid it is true that this acid might be formed in different ways so that each molecule of formic acid in our distillate would not represent one molecule of butyric acid oxidised, but perhaps more nearly one-third of a molecule. By far the greatest part of our volatile fatty acid is unchanged butyric acid and certainly it contains only a little formic acid, for in working up 250 g. of sodium salts from the steam distillate we obtained only a little carbon monoxide on treatment with pure concentrated sulphuric acid. We may therefore take the difference between the initial butyric acid and the titration of the volatile acid, expressed as butyric acid, as a measure of the butyric acid which has been converted into aldehydes, acetone and succinic acid, so that if we calculate the yield of succinic acid only on this difference we shall almost certainly be underestimating it. In the table the yield of succinic acid is so estimated.

From the table it is seen that the yield of succinic acid is variable even when the conditions appear to be the same; the only explanation we can offer of this phenomenon is that in some cases there may have been a greater loss of oxygen than in others. Nevertheless in spite of these differences certain results appear perfectly clearly:

1. A yield of 50 % and over of succinic acid can be obtained by oxidising sodium butyrate in neutral solution by hydrogen peroxide at 65°. It is therefore established that under these conditions a fatty acid such as butyric can be and is attacked to a very great extent at the methyl end of the chain.

2. The yield is much diminished in less concentrated solutions. In experiment number 2 when the volume is 1000 cc. instead of 720 cc. the yield of succinic acid is only 20 %.

3. When the concentration of the oxidising agent is doubled the yield of succinic acid is increased with respect to the butyric acid taken, but it is practically the same with respect to butyric acid oxidised. More acid is oxidised and the time taken is nearly doubled.

4. When the peroxide is quadrupled a more marked change occurs in the yield of the several products and in the time required to use up the

peroxide. The reaction is now complete in 23 hours; the amount of butyric acid oxidised is double the amount oxidised with three atoms of oxygen; the yield of succinic acid is greatly reduced while that of the carbon dioxide is enormously increased. Perhaps it is a fair inference to draw that under these conditions the succinic acid is formed, but a large part of it is oxidised as it is produced.

Number of experiment	Time in hours	Weight of butyric acid taken in g.	Total volume of solution in cc.	Hydrogen peroxide in atoms of available oxygen	Difference between column 3 and volatile acids calculated as butyric acid in g.	Weight of crude succinic acid in g.	Yield per cent. of theory of crude succinic acid	CO <sub>2</sub> evolved in g.	Aldehyde plus acetone calculated as butyric acid in g.	Remarks
1	48	17.6	720	3	3.08	2.13	51.5	—	—	At 65°
2	48	17.6	1000	3	5.63	1.54	20.3	—	—	"
3	66	4.4	190	3	1.14	0.48	31.6	0.36*	—	"
4	66	4.4	190	3	1.07	0.43	30.1	0.40*	—	"
5	66	4.4	190	3	1.41	0.47	25.1	—	0.65	"
6	66	4.4	190	3*	0.75	0.37	36.3	—	0.50	"
7	72	4.4	190	3	1.10	0.50	33.6	0.37*	—	"
8	72	4.4	190	3	1.02	0.46	33.6	—	0.64	"
9	72	4.4	190	3	0.75	0.51	50.7	0.54*	—	"
10	72	4.4	190	3	0.79	0.51	48.4	—	0.60	"
11	72	4.4	190	3	0.79	0.52	49.2	0.44	—	"
12	66	4.4	190	3	1.23	0.48	29.3	—	0.71	"
13	72	4.4	190	3	1.41	0.52	27.2	0.37	—	"
14	168	4.4	190	3	1.23	0.32	19.5	—	0.47	At 37°
15	72	17.6	720	6	6.69	3.18	35.5	—	—	At 65°
16	114	4.4	190	6	1.63	0.72	32.8	0.48*	—	"
17	114	4.4	190	6	1.54	0.69	33.2	—	1.10	"
18	114	4.4	190	6	1.50	0.77	38.0	—	—	"
19	114	4.4	190	6	1.36	0.71	38.8	—	—	"
20	114	4.4	190	6	1.32	0.67	37.8	0.88	—	"
21	114	4.4	190	6	1.89	0.72	28.5	—	1.16	"
22	114	4.4	190	6	1.72	0.75	32.5	1.12	—	"
23	672	4.4	190	6	0.79	0.71	67.3	—	0.73	At 37°
24	22	4.4	190	12	2.46	0.56	16.8	2.59	—	At 65°
25	23	4.4	190	12	2.46	0.51	15.4	—	0.80	"
26	23	4.4	190	12	2.46	0.50	15.2	2.72	—	"

\* Determined by passing the gas through baryta water and titrating.

A number of carbon dioxide determinations have been made, some by the use of baryta water and others by direct weighing; with care both methods give practically the same result. We do not find that our hydrogen peroxide contains more than a trace of carbon dioxide. Dakin found that his specimens contained a great deal. We have found however that the hydrogen peroxide acts upon rubber stoppers producing sulphur dioxide, which is absorbed by the potash used for absorbing the carbon dioxide—but this action is quite slow and only affects experiments which are continued for many hours.

In the last column but one are given some determinations of aldehyde plus acetone which are calculated to butyric acid. All the figures in this column were obtained by distilling an aliquot part of the oxidised product exactly as in a Messinger-Huppert acetone determination. We have also made some determinations by titrating after Messinger-Huppert without previous distillation. There was always a marked difference between the two results, a difference we attribute to the presence of less volatile aldehydes or aldehydo-acids.

We have also attempted to make separate determinations of aldehydes and acetone using Tollens' reagent for oxidation of the aldehydes. The following table gives some of our results. The amount of butyric acid used for the experiments and the volume of the solution are the same in all the experiments, namely 4.4 g. of the acid neutralised by sodium hydroxide, and 190 cc.

Hydrogen peroxide in atoms of available oxygen	Aldehydes + acetone expressed as g. of butyric acid		Acetone expressed as g. of butyric acid	Yield* % of theory
	By distillation	By direct titration		
3	0.76	1.02	0.206	20
3	0.73	1.06	0.407	39
6	0.94	1.46	0.457	32
6	0.73	1.43	0.290	20†
12	0.79	—	0.250	11
12	0.84	0.96	0.323	14
12	0.86	0.93	0.217	10

\* The yields are calculated on the *average* value of the butyric acid oxidised in each class of experiment because the volatile acids were not determined in all these experiments.

† Experiment at 37°.

From this table it is seen that by our method of oxidation we cannot approach Dakin's yield of 50 % of acetone. We believe our highest figure of 39 % is probably too high. Yet we believe that our method of working is a nearer approach to the conditions which prevail in the animal body than Dakin's method of actual distillation.

Butyric acid in the form of its sodium salt may be broken down by oxidation in three ways by hydrogen peroxide, namely by attack at each of its carbon atoms except that one present as carboxyl. Of these three modes of attack that at the terminal carbon atom results in the production of succinic acid; that at the  $\beta$ -carbon atom results in the production primarily no doubt of acetoacetic acid which is broken down to acetone and carbon dioxide; that at the  $\alpha$ -carbon atom probably yields propionic aldehyde, then the acid, and so on. Of these three we believe the

attack at the methyl group to be by far the most important. Butyric acid is easily oxidised in the body; of the intermediate products of its oxidation by hydrogen peroxide succinic acid is known to be easily oxidised in the body. Thus v. Longo [1878] took 13 g. of sodium succinate in two portions in 24 hours; no trace of the acid could be found in the urine. Marfori [1896] shows the same for animals. Batelli and Stern [1911] have shown that the acid is oxidised by nearly every tissue of the body. Acetoacetic acid on the contrary is difficult of oxidation in the body [see Hurltley, 1916, p. 380]. Propionic acid is known to be easily oxidised by the body. We believe therefore that in the body butyric acid is mainly attacked at the terminal carbon atom. If this is the case the same should be true of the higher fatty acids, and we propose to seek for evidence of this mode of attack in the case of some fatty acids higher in the series than butyric acid.

#### SUMMARY.

It is shown that a yield of succinic acid of over 50 % of the theoretical can be obtained by the oxidation of sodium butyrate by hydrogen peroxide.

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