XXXVIII. THE MODE OF OXIDATION OF CERTAIN FATTY ACIDS WITH BRANCHED CHAINS.

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Our knowledge of the mechanism by which the simpler fatty acids with branched chains are oxidised in the body is still very meagre. This is no doubt due to the difficulty in interpreting the experimental results so far obtained, in any way that will lead to a generalisation such as we possess for the normal fatty acids; namely, β -oxidation followed by removal of the two end carbon atoms of the acid. This difficulty may be illustrated by reference to the behaviour of α -methylbutyric acid and α -ethylbutyric acid. In the diabetic organism the former gives rise to increased "acetone body" production [Baer and Blum, 1906] whereas the latter yields, in part at any rate, methyl propyl ketone [Blum and Koppel, 1911]. Similarly α -methylpropionic acid (isobutyric acid), gives rise to extra glucose formation [Ringer, Frankel and Jonas, 1913] and traces of *d*-lactic acid [Baer and Blum, 1906], a result interpreted by Baer and Blum as indicating that lactic acid is a probable intermediate stage in the oxidation of isobutyric acid, the methyl group being replaced by a hydroxyl group, thus:

This view gains support from the fact that lactic acid gives rise to extra glucose formation in diabetics. Replacement of the methyl group by hydroxyl is not general however, since α -hydroxybutyric acid, which might be expected by the above hypothesis as an intermediate product in the oxidation of α -methylbutyric acid, does not give rise to "acetone body" formation whereas α -methylbutyric acid does.

Another explanation of the behaviour of isobutyric acid has been advanced by Ringer [Ringer, Frankel and Jonas, 1913]. This observer showed that propionic acid, like isobutyric acid, produces increased glucose formation

in phlorizinised dogs. He suggests therefore that demethylation takes place by hydrolysis, methyl alcohol and propionic acid being formed. This is a type of reaction however that is at present unknown in organic chemistry and had therefore better be reserved as an explanation until proof is supplied that it can take place.

A third possibility has been suggested, namely, that the α -methyl group in such acids as isobutyric and α -methylbutyric, is oxidised to a carboxyl group. This view was abandoned by Baer and Blum, who first put it forward, when they discovered that ethylmalonic acid, which under this scheme would be produced from α -methylbutyric acid, does not cause increased "acetone body" formation in diabetics.

The object of the present communication is to suggest a mechanism of oxidation which applies to the α -substituted fatty acids and will explain the facts so far known about the catabolism of these acids. It is merely an application of the rule of β -oxidation advanced by Knoop and postulates that the carbon atom of the methyl group, which in these acids is in the β -position to the carboxyl group, is selectively oxidised, rather than the β -carbon atom of the main chain. Instead of a β -ketonic acid being produced therefore, as with the normal fatty acids, a derivative of the half aldehyde of malonic acid would result:

Malonic semi-aldehyde has been prepared by Wohl [1900] who showed that it rapidly lost carbon dioxide, especially on warming, producing acetaldehyde. In this way it behaves like the β -ketonic acids, which also easily lose carbon dioxide but give a ketone instead. No derivatives of malonic semi-aldehyde have been described but it is very probable by analogy with the derivatives of the β -ketonic acids, that they would be equally unstable. The second reaction therefore in the scheme suggested would be the loss of carbon dioxide with production of the *normal* aldehyde of an acid containing one carbon atom less than the original methylated acid. This aldehyde on further oxidation, or by the Cannizzaro reaction, could then give rise to the corresponding normal fatty acid:

In this way, isobutyric acid would give rise to propionic acid, α -methyl butyric to *n*-butyric acid and α -methylvaleric to *n*-valeric acid. In support of this view is the fact that the fate of these methylated acids in the body is exactly that of the corresponding normal acids which would be produced from them by demethylation. In the case of α -ethylbutyric acid there is no methyl group in the β -position for selective oxidation, and this acid appears to undergo β -oxidation in the usual fashion producing α -ethyl-acetoacetic acid and then methyl propyl ketone. The scheme of oxidation suggested thus provides an explanation of the fact that α -methylbutyric acid on oxidation in the body gives rise to acetone formation and not methyl ethyl ketone, which might be expected by analogy with α -ethyl butyric acid [Blum and Koppel, 1911].

The hypothesis put forward above, to explain the mode of oxidation of the α -methylated acids, will also apply to those acids, such as isobutyl-acetic, which contain a methyl group in the γ -position, since by undergoing the usual β -oxidation they yield α -methylated acids. The mode of oxidation of β -methylated acids, such as isopropylacetic acid, cannot yet be explained by a simple scheme such as that suggested for the α -methylated acids. The reactions involved in their catabolism must be left for further experiment.

It has been shown by Dakin [1908] that on warming the ammonium salts of the normal fatty acids with dilute hydrogen peroxide, β -oxidation is regularly observed in all the cases investigated from butyric to stearic acids. A ketone can be isolated from the products of oxidation which is derived from a β -ketonic acid. Since in hydrogen peroxide we have an oxidising agent known to be capable of producing β -oxidation it has been employed in the first instance in this investigation to determine whether the type of oxidation represented above can actually take place with isobutyric acid and α -methylbutyric acid. If the scheme suggested for the oxidation of these acids be correct, then isobutyric acid should give propionaldehyde as an oxidation product and similarly α -methylbutyric acid should give butyraldehyde. Evidence has been obtained that the ammonium salts of these acids on oxidation with hydrogen peroxide do yield propionaldehyde and butyraldehyde respectively. Direct identification of the aldehydes was not possible, because of the simultaneous production of ketones resulting from oxidation at the α -carbon atom, acetone being obtained in the first case and methyl ethyl ketone in the second. The aldehydes were therefore oxidised to the corresponding acids and these isolated as silver salts. In addition to butyraldehyde, acetaldehyde was regularly observed as an

oxidation product of α -methylbutyric acid. The mechanism of its production appears to be somewhat obscure.

It is worthy of note that propionic acid is similarly constituted to the α -methylated fatty acids in that its β -carbon atom forms part of a methyl group, and it might be expected therefore to undergo the same kind of oxidation as the α -methylated acids. In that case it would yield malonic semi-aldehyde:

We already possess a certain amount of evidence in support of this view. It is certain for instance that β -phenylpropionic acid undergoes β -oxidation yielding benzoylacetic acid, and not α -oxidation which would yield phenylpyruvic or phenyl-lactic acid. Further, on oxidation with hydrogen peroxide, propionic acid yields a large amount of acetaldehyde [Dakin, 1908], although this might equally well be explained by α -oxidation, with the intermediate production of lactic acid, and not malonic semi-aldehyde. Ringer has observed that in phlorizinised dogs, propionic acid gives rise to extra glucose formation. No experiments have been performed with malonic semi-aldehyde but quite recently Ringer and Frankel [1914] have stated that acetaldehyde produces extra glucose formation in diabetic dogs. Since the administration of acetaldehyde causes a marked depression in the nitrogen excretion and the calculation of the extra glucose formed depends on the G/N ratio, the interpretation of these authors cannot be accepted unreservedly. It is noteworthy however that in spite of this variation an absolute rise in the glucose excretion was observed.

EXPERIMENTAL.

Oxidation of Ammonium Isobutyrate. Kahlbaum's isobutyric acid was used. Portions of ten grams were neutralised with ammonia, the solution diluted with about 100 cc. of water and twenty grams "perhydrol" added, the volume being then finally brought to about 200 cc. by further dilution with water. The oxidation was carried out by warming this solution in a flask connected with a well cooled condenser, the receiver being immersed in ice and salt. During the experiment a gentle current of air was aspirated through the apparatus in order to assist in the removal of the volatile products of oxidation from the flask in which the oxidation was carried out. The temperature was raised fairly rapidly to 70° and thereafter more slowly to boiling point. The heating was continued for half to one hour at this point

and the flame was maintained at such a height that distillation was very slow. The distillate, which was always alkaline, owing to the ammonia present, was redistilled to free the volatile oxidation products from possible traces of isobutyric acid. Direct isolation of a pure *p*-nitrophenylhydrazine derivative from the distillate was impossible, no substance with a sharp melting point being obtainable. The presence of aldehydes was easily detected by Tollen's reagent which was immediately reduced in the cold. Dakin [1908] using a slightly different method has shown that acetone is produced when ammonium isobutyrate is distilled with hydrogen peroxide. In order therefore to identify the aldehyde or aldehydes present, the distillate was oxidised with ammoniacal silver oxide containing a trace of sodium hydroxide. Oxidation was carried out at the ordinary temperature for several hours and then overnight in the incubator at 37°. The precipitated silver was filtered off, the filtrate nearly neutralised with phosphoric acid and distilled to remove the ketone. The distillate still contained a little aldehyde. This was removed by adding a few cc. of permanganate solution and redistilling. On treating the distillate with *p*-nitrophenylhydrazine acetate an immediate precipitate of hydrazone was obtained, which melted sharply at 148–9° after two recrystallisations from alcohol. This is the melting point of acetone-*p*-nitrophenylhydrazone. Dakin's observation of the formation of acetone was thus confirmed. The alkaline liquid from the oxidation with silver oxide, after removal of the acetone by distillation, was acidified with phosphoric acid and the volatile acids, corresponding to the aldehydes in the original distillate, distilled off. The distillate was neutralised with baryta and evaporated to dryness. The barium salts were washed with a little watery alcohol to remove barium acetate if present, then dissolved in a small amount of water and the solution left to crystallise. The crystalline barium salt was converted into the silver salt by precipitation with silver nitrate, washed with a little distilled water and dried in vacuo over sulphuric acid. The silver salt on analysis proved to be silver propionate.

0.1938 g. ; 0.1165g. Ag = 60.1 %.
Calculated for $C_3H_5O_2Ag$ 59.7 %.

Propionic aldehyde was therefore one of the oxidation products of isobutyric acid.

Non-volatile Oxidation Products. The residual liquid from the peroxide oxidation contained much unchanged isobutyric acid. It was tested specially for formic acid by the mercuric chloride reaction and this acid was

present in traces. After removal of the isobutyric acid by continuous extraction with petroleum ether, the liquid was extracted with ether but no methylmalonic acid could be isolated from this extract.

Oxidation of Ammonium α -Methylbutyrate.

The acid used was prepared from methylethylmalonic acid and partly from methylethylacetoacetic ester. The oxidation was carried out in the same way as that of the isobutyrate. The distillate containing the volatile oxidation products did not yield a pure *p*-nitrophenylhydrazine derivative so that a separation of the aldehydes and ketone by means of ammoniacal silver oxide was undertaken. In this way a ketone was obtained which gave a *p*-nitrophenylhydrazone crystallising in orange-coloured needles and melting sharply at 124–124.5°. This was found to be the melting point of the *p*-nitrophenylhydrazone of methyl ethyl ketone prepared from methylacetoacetic ester¹. A mixture of the two derivatives showed no change in the melting point. The acids obtained from the aldehydes by oxidation with silver oxide were isolated as barium salts which did not crystallise but were easily soluble in 95 % alcohol. The acids were therefore liberated by means of phosphoric acid, distilled off and converted into the calcium salts by neutralising the distillate with lime water. These crystallised readily on evaporating the solution and had the characteristic flocculent appearance of calcium acetate. The calcium salt was transformed into the silver salt and analysed.

$$0.1286 \text{ g.} : 0.0781 \text{ g. Ag} = 60.7 \%$$

$$\therefore \text{Mean molecular weight of acids} = 70.$$

The mean molecular weight of the acids lay therefore between that of propionic acid (74) and acetic acid (60). Propionic acid was not present in any considerable amount however as the barium salts of the mixed acids were easily soluble in 95 % alcohol, whereas barium propionate is almost insoluble. The acetic acid undoubtedly present was therefore mixed with a higher acid. That butyric acid was present was easily demonstrated by warming a small portion of the calcium salts with sulphuric acid and methyl alcohol when the characteristic odour of methyl butyrate was obtained. It was thought desirable to obtain more exact evidence of the presence of butyric acid. A trial with equal amounts of calcium acetate and butyrate

¹ Dakin [1908] gives 128–9° as the melting point of the *p*-nitrophenylhydrazone of methyl ketone, prepared by him, but does not state the source of the ketone.

dissolved in water and evaporated, showed that separation could not be obtained satisfactorily with 95 % alcohol, in which calcium acetate is practically insoluble and calcium butyrate easily soluble, due no doubt to the formation of a mixed salt with intermediate solubilities. Distillation of the free acids was therefore used as a means of separation. The acids obtained from several experiments were obtained as sodium salts, and the free acids then liberated by sulphuric acid and taken up in a small amount of petroleum ether. The petroleum ether and acetic acid were then removed by distillation up to a temperature of 155°. The residual acid was dissolved in water and converted into the calcium salt by boiling with calcium carbonate. The calcium salt, which was easily soluble in 95 % alcohol, was converted into the silver salt which was dried in vacuo over sulphuric acid and on analysis proved to be silver butyrate probably still containing traces of the acetate.

0.2245 g. ; 0.1255 g. Ag = 55.9 %.

Calculated for $C_4H_7O_2Ag$ 55.4 %.

Non-volatile Oxidation Products. As in the case of isobutyric acid traces of formic acid were detected by the mercuric chloride reaction. After removal of unchanged α -methylbutyric acid by distillation in steam, the residual liquid was neutralised, concentrated on the water bath, then acidified with phosphoric acid and extracted with ether for twelve hours. The ether extract contained a crystalline acid which, on recrystallisation from benzene containing a little acetone, and then from water, melted at 115°. This proved to be methylsuccinic acid. On heating with ammonia and zinc dust the pyrrole reaction was easily obtained.

0.1196 g. required 17.89 cc. 0.1 N. NaOH for neutralisation. Equivalent = 66.8.

Calculated for $C_3H_6(COOH)_2$ 66.

No evidence of the presence of ethylmalonic acid was obtained.

SUMMARY.

1. A scheme of oxidation is suggested which explains the results so far obtained in studying the catabolism of α -methylated fatty acids such as isobutyric, α -methylbutyric and α -methylvaleric acid, and also acids such as γ -methylvaleric acid which by β -oxidation would yield α -methylated acids.

2. The rule of β -oxidation is applied to these acids with the proviso that the carbon atom of that methyl group which is in the β -position undergoes oxidation first. The derivative of malonic semi-aldehyde produced,

would, by loss of carbon dioxide give rise to the *normal* aldehyde of the demethylated fatty acid and by subsequent oxidation, or the Cannizzaro reaction, to the normal acid itself.

3. In support of this view as a possible biochemical process it is shown that ammonium isobutyrate on oxidation with hydrogen peroxide yields amongst other products propionaldehyde, and that ammonium α -methylbutyrate yields similarly butyraldehyde.

4. The possibility of this scheme being applied to the catabolism of propionic acid is suggested, since this acid is similarly constituted as regards the position of its methyl group to the α -methylated acids.

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