XI.—*The Fermentation of Calcium Glycerate by the “ Bacillus Ethaceticus.”*

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IN a communication to the Royal Society by one of us, “On a Pure Fermentation of Mannite and Glycerin,” by P. F. Frankland and J. Fox (*Proc. Roy. Soc.*, **46**, 345), a micro-organism was described, having the power of fermenting suitable solutions of mannite and glycerin, and, in consequence of ethyl alcohol and acetic acid being the principal products of these fermentations, the name of *B. ethaceticus* was proposed for the organism in question.

In this paper, it was pointed out that the bacillus was also capable of setting up fermentative decompositions of glucose, cane-sugar, milk-sugar, starch, and calcium glycerate, but, most curiously, not of dulcete, although isomeric with and so closely akin to mannite.

The present paper deals with the fermentation of calcium glycerate by this organism.

Character of the Fermentation of Calcium Glycerate.—If sterile solutions (3 per cent.) of calcium glycerate to which the necessary nutritive material (see below), in the shape of a small quantity of peptone and minerals salts, has been added, are inoculated with a pure growth of the bacillus and kept at 35—38°, the liquid becomes turbid in the course of two or three days, and a day or two later a gentle but visible evolution of gas commences and continues for a period of about 7—14 days, after which the liquid again gradually becomes clear, although of considerably darker colour than at first. Occasion-

ally, however, a feeble secondary fermentation may follow the cessation of the first and continue for a few days.

The fermentation of calcium glycerate, as measured by the visible evolution of gas, is very much less vigorous than the fermentations previously described, and the organism not unfrequently entirely loses its capacity of setting up a palpable fermentation in this medium; we have, however, generally been able to restore its fermentative power by inoculating into the calcium glycerate solution from a vigorously fermenting solution of mannite or glucose. The restoration of its fermenting power towards calcium glycerate is, however, sometimes exceedingly troublesome.

All the precautions mentioned in the previous paper were taken both as to the use of perfectly pure cultures of the bacillus and as to the prevention of contamination during the process of fermentation, whilst the purity of the solutions was similarly tested by cultivation before proceeding to the chemical examination of the products formed.

The purity of the calcium glycerate employed was tested by determination of the calcium in the air-dried substance:—

0.2923 gram of the substance yielded 0.1365 gram CaSO_4 , corresponding to 13.73 per cent. Ca; calcium calculated upon $(\text{C}_3\text{H}_5\text{O}_4)_2\text{Ca} + 2\text{H}_2\text{O}$ gives 13.98 per cent.

The water of crystallisation is only partially lost at a temperature of $105\text{--}110^\circ$; thus the salt, on drying at that temperature, lost only 5.61 per cent., whilst the loss corresponding to 1 mol. H_2O is 6.29 per cent.

Fermentation No. 1.—The solution for fermentation had the following composition:—

Calcium glycerate.....	60 grams	} the whole diluted to 2000 c.c. with distilled water.
Peptone	2 "	
Calcium carbonate.....	10 "	
Salt solution*.....	200 c.c.	

The liquid was contained in a large flask plugged with cotton wool, and, after due sterilisation, was inoculated with a needle-loop of a pure culture of the *Bacillus ethaceticus*, and then kept in an incubator at 38° . Fermentation began on the 3rd, and was practically finished on the 7th day; the flask was not, however, removed from the incubator and examined until the 26th day.

The liquid was then submitted to distillation until a small quantity

* Prepared by dissolving 1 gram of potassium phosphate, 0.2 gram of magnesium sulphate (cryst.), and 0.1 gram of calcium chloride (fused) in 1000 c.c. of distilled water.

of the distillate yielded only the faintest reaction with Lieben's test (iodoform). The distillate, in which alcohols were to be expected, was found to be free from aldehyde, as indicated by the absence of reaction with rosaniline.

The alcohols in the liquid were determined by oxidation with potassium dichromate and sulphuric acid (A. Dupré, *J. Chem. Soc.*, **20**, 495). For this purpose 10 grams of potassium dichromate and 20 grams of strong sulphuric acid were made up to 100 c.c. with water, and 10 c.c. of this solution were added to every 20 c.c. of the alcoholic solution; the chromic acid solution, along with the alcoholic liquid, was placed in bottles, the mouths of which were closed with india-rubber stoppers tightly wired down. The bottles were then heated for seven hours in a steamer at 100°. The chromic acid was then reduced by the addition of a calculated quantity of zinc, and after the latter had passed into solution, the liquid was repeatedly distilled down to a small bulk, fresh quantities of water being added each time. In this manner, any volatile acids produced in the previous oxidation were obtained in the distillate. These acids were converted into barium salts by treatment with barium carbonate. The barium salts were dried at 130°, and the barium determined by conversion into barium sulphate. The following quantities of barium salts were obtained:—

- I. 1.3915 gram barium salt, yielding 91.16 per cent. BaSO_4
(barium acetate yields 91.37 per cent. BaSO_4).
- II. 1.9470 gram barium salt, yielding 91.16 per cent. BaSO_4 .
- III. 1.4010 gram barium salt, yielding 91.40 per cent. BaSO_4 .

The barium salts were thus practically pure barium acetate, indicating that the liquid before oxidation contained ethyl alcohol, and inasmuch as 4.7395 grams of barium acetate were in all obtained, ethyl alcohol to the extent of 1.711 gram must have been present.

In order to test this method of determining alcohol by oxidation, we have made experiments with known quantities of pure alcohol; the results of these determinations are recorded in the Appendix (see p. 93).

The residue from the first distillation of the fermented liquid was now treated with the requisite quantity of standard hydrochloric acid to neutralise the total calcium (both as glycerate and carbonate) present. 22.61 grams of HCl (or 619.4 c.c. of normal hydrochloric acid) were required for this purpose, but only 350 c.c. were in the first instance added, and then the liquid was repeatedly distilled to a small bulk in order to get over the volatile acids; the latter were converted into barium salts and analysed as above:—

I. 10.5940 grams barium salts, yielding BaSO_4 $\left\{ \begin{array}{l} 91.69 \text{ per cent.} \\ 91.88 \text{ „} \end{array} \right.$

100 c.c. more of the normal hydrochloric acid were added to the flask and distillation continued, from which the following salts were obtained :—

II. 4.1505 grams barium salts, yielding BaSO_4 $\left\{ \begin{array}{l} 96.50 \text{ per cent.} \\ 96.34 \text{ „} \end{array} \right.$

After a further addition of 100 c.c. normal HCl , there were obtained :—

III. 0.5685 gram barium salts, yielding BaSO_4 90.00 per cent. (the salt was pasty in appearance).

The remainder (69.4 c.c.) of the normal hydrochloric acid was then added, after which there were obtained :—

IV. 0.209 gram barium salts, yielding 84.68 per cent. BaSO_4 (the salt was again pasty in appearance).

10 c.c. of normal hydrochloric acid over and above the calculated amount were then added, but no further volatile acids could be obtained in the distillate.

In this and in similar experiments, we have always found that the last quantities of volatile acid obtained by repeated distillation in this manner are pasty and yield a low percentage of barium; we are, therefore, of opinion that the low percentages of barium in the fractions III and IV do not indicate the presence of volatile fatty acids of higher molecular weight, especially as such acids if they had been present should have come over in the first fraction (see Appendix, p. 95).

The salts thus consist of a mixture of barium acetate and formate, and taking into account that

Barium acetate yields 91.37 per cent. BaSO_4 ,
 „ formate „ 102.64 „ „

it can be calculated that the above salts consisted of 12.8873 grams barium acetate (or acetic acid, 6.065 grams) and 2.6347 grams barium formate (or formic acid, 1.067 gram), equivalent to 1.391 gram acetic acid.

In the liquid from which the volatile acids had been distilled off as above, there was left a brown, flocculent deposit; this was filtered off and washed with dilute hydrochloric acid. It was found to be entirely soluble in a boiling solution of caustic potash, from which it was again precipitated by hydrochloric acid. After thoroughly washing and drying until constant at 100° , it was found to weigh

0.0845 gram. This substance is of constant occurrence in all the fermentations with which we are acquainted, and appears to be derived from the cells of the bacteria themselves. It is the only portion of the fermentation products insoluble in dilute hydrochloric acid. Owing to the small quantity in which it is produced, however, we are not in a position to offer any further information as to its nature.

The filtrate from the above insoluble residue would obviously contain any unaltered calcium glycerate, together with any fixed acid that might have been produced in the fermentation; such acids would, however, be in the free state, inasmuch as an excess of hydrochloric acid had been added to the liquid. The liquid was repeatedly shaken out with ether. The ethereal extract, on drying at 100° , only amounted to 0.4785 gram, and a considerable part of this appeared to be succinic acid mixed with some gummy material.

The liquid, after extraction with ether as above, was boiled up with excess of calcium carbonate and filtered; the filtrate was evaporated to dryness on a water-bath, and then extracted with a large quantity of strong methylated spirit (93 per cent.), in order to separate any unaltered calcium glycerate. (We had previously determined that calcium glycerate can be most perfectly precipitated from solution by addition of strong spirit.) The calcium salt thus separated was unfortunately not weighed itself, but was converted into a lead salt by precipitation with lead acetate and alcohol. The lead salt thus obtained, after recrystallisation from water, yielded 68.89 per cent. of lead, corresponding closely to one of the basic lead glycerates referred to below (see pp. 87, 90).

After removing the calcium glycerate precipitated by means of alcohol as above, the alcoholic filtrate was evaporated down until free from alcohol, the residue was taken up with water, and the calcium precipitated with very slight excess of sodium carbonate, after which the calcium carbonate was filtered off. The filtrate, containing the sodium salts, was evaporated to dryness and repeatedly extracted with a mixture of 3 parts ether and 1 part alcohol, as it was thought that possibly some glycerin might have been formed in the fermentation; none was however found. The residue was then dried at 100 – 105° and weighed; it amounted to 71.408 grams. In addition to sodium chloride, this residue evidently contained the sodium salt of an organic acid, as it charred on heating. It was found to contain 82.02 per cent. of ash, and 68.28 per cent. of sodium chloride, leaving thus a considerable margin for the sodium salt of the organic acid. From this mixture, the sodium was as far as possible removed as sodium chloride by saturating the solution with hydrochloric acid gas. After filtering off the sodium chloride and wash-

ing with strong hydrochloric acid, the filtrate was evaporated to dryness and dried at 100° . The organic acid thus obtained (containing of course a small quantity of sodium chloride also) amounted to 15.790 grams; of this 13.400 grams were soluble in alcohol, 1.733 gram insoluble in alcohol but soluble in water, and 0.657 gram insoluble both in alcohol and water. The organic acid itself may thus be taken to have amounted to about 13.4 grams, whilst the remainder would be principally sodium chloride.

Some of the acid thus obtained was converted into a barium salt by boiling up with barium carbonate and filtering; the filtrate was evaporated to dryness and the residue dissolved in a small quantity of water, and treated with a large excess of alcohol, which produced a gummy precipitation. This precipitate was washed with alcohol several times, and then divided into several different portions, which were dried at different temperatures, and a barium determination made in each, thus:—

No. 1 dried at 105° yielded BaSO_4	{ 62.76 per cent.
	{ 62.82 „
No. 2 dried at 110 — 120° yielded BaSO_4 64.08	„
No. 3 dried at 130° yielded BaSO_4	64.95 „
No. 4 dried at 140° „	65.53 „

In a specimen of pure barium glycerate, after drying at 140° , we found the yield of barium sulphate to be 66.59 per cent. Even when dried at this temperature, the salt is not anhydrous, as anhydrous barium glycerate should yield 67.14 per cent. of barium sulphate. The effect of drying at different temperatures on pure barium glycerate was specially determined with the following results:—

Barium glycerate dried at 100 — 105° yielded 65.51 p.c. BaSO_4 .
„   „ 130° yielded 66.55 p.c. BaSO_4 .
„   „ 140° „   66.59   „   „

The coincidence between the barium salt in question and barium glycerate is thus very marked, for the percentage of barium itself in the latter is 39.16, and in the former 38.53.

We next endeavoured to obtain a lead salt of the acid by boiling its aqueous solution with lead carbonate and filtering; on cooling, a deposition took place in the filtrate; this precipitate was washed, dried, and submitted to analysis with the following results:—

A lead determination made on the salt dried over sulphuric acid, yielded 66.97 per cent. lead.

Another made on the salt dried at 100 — 105° yielded 66.99 per cent. lead.

In spite of repeated recrystallisation, we failed to obtain the lead salt free from chlorine, and it became necessary, therefore, to determine the latter; by precipitation with silver nitrate, 1.95 per cent. of chlorine was obtained.

The two following percentage compositions can be calculated from these results, according as we assume that the chlorine is present as PbCl_2 or as Pb_2OCl_2 :—

The percentage composition (*b*) is in very fair accord with that of a basic salt of the formula—

Fermentation No. 2.—The conditions of this experiment were precisely similar to those in the Fermentation No. 1.

The volatile acids were dealt with as before, and the following barium salts prepared and analysed :—

Corresponding to 11.364 grams barium acetate and
0.320 " " formate.

II. 3.4465 grams barium salt, yielding BaSO_4 $\left\{ \begin{array}{l} 97.40 \text{ per cent.} \\ 97.56 \text{ } \end{array} \right.$ „

Corresponding to 1.578 gram barium acetate and
1.8685 „ „ formate.

III. 0.4995 gram barium salt, of pasty appearance, which, after extraction with strong alcohol, left 0.3215 gram insoluble; this yielded 97.35 per cent. BaSO_4 ,

Corresponding to 0.147 gram barium acetate and
0.174 „ „ formate.

IV. 0.1255 gram barium salt, again of very pasty appearance, but as the quantity was too small to purify as above, it was converted into BaSO_4 , of which it yielded only 82.47 per cent.; assuming, however, that it had the same proportional composition as fraction III, it would correspond to 0.0575 gram barium acetate, and 0.068 gram barium formate.

Thus the total yield of volatile acids consisted of:—

13.1465 grams barium acetate, corresponding to 6.187 grams acetic acid,
2.4305 grams barium formate, corresponding to 0.985 gram formic acid.

After distilling off the volatile acids, there remained, as in the case of the first fermentation, a brown, insoluble residue which, after thoroughly washing free from hydrochloric acid and drying at 105° , amounted to 0.1665 gram

From the filtrate, there was obtained by repeatedly shaking out with ether an extract which, after drying at 100° , amounted to 0.501 gram, and appeared to consist to a great extent of succinic acid mixed with some gummy material, just as in the previous fermentation.

The acid liquid, after extracting with ether as above, was boiled up with an excess of calcium carbonate, filtered, and the filtrate evaporated as nearly to dryness as possible on the water-bath. The residue of calcium salts was then extracted with a large quantity of strong alcohol (93 per cent.), and further quantities of alcohol were added to the liquid until no more precipitation was occasioned. The insoluble part, consisting presumably of unaltered calcium glycerate, was filtered off; it was quite soluble in water, and amounted, after drying at 105° , to 2.037 grams (corresponding to 1.61 grams of glyceric acid, taking into account the loss of a part of the water of crystallisation at 105° , see p. 82). This calcium salt was purified by dissolving in water and precipitating with alcohol four times, after which it

was dried at 105—110°, and a calcium determination made, which yielded 14·29 per cent. of calcium. Pure calcium glycerate, when similarly dried, was found to yield 14·81 per cent. of calcium.

After removal of the above calcium glycerate, the further treatment was similar to that described for the first fermentation; in the first instance, the calcium was precipitated by means of a very slight excess of sodium carbonate, the sodium was then removed as far as possible by saturating with hydrochloric acid gas, and filtering off the precipitated sodium chloride. On evaporating the filtrate to dryness on the water-bath and drying at 100°, there remained 18·11 grams of residue (consisting of an organic acid, with a little sodium chloride which had escaped precipitation); of this, 13·90 grams were soluble in alcohol (90 per cent.), 2·81 grams were insoluble in alcohol, but soluble in water, therefore doubtless sodium chloride, whilst 1·40 gram was insoluble in water also. The amount of organic acid, 13·90 grams, was thus almost identical with that obtained in the previous fermentation, 13·40 grams.

Owing to the unsatisfactory and gummy appearance of the barium salt prepared from this organic acid in the first fermentation, we determined on this occasion to prepare only the lead salt. For this purpose, the portion of the above residue soluble in alcohol was dissolved in water, boiled up with lead carbonate, and filtered hot; on cooling, however, no deposition of lead salt took place. We therefore decomposed the solution with sulphuretted hydrogen, filtered off the lead sulphide, heated the filtrate until free from sulphuretted hydrogen, and then again boiled up with lead carbonate and filtered. On cooling, a quantity of lead salt separated from the filtrate; this we washed, dried at 105°, and submitted to analysis:—

The salt yielded Pb ..... $\left\{ \begin{array}{l} 68\cdot66 \text{ per cent.} \\ 68\cdot69 \text{ „} \end{array} \right.$

As this lead salt was also found to contain chlorine, as in the case of the one obtained in the first fermentation, a determination of the halogen was made by precipitation with silver nitrate, with the following result:—

Chlorine ..... $\left\{ \begin{array}{l} 1\cdot13 \text{ per cent.} \\ 1\cdot23 \text{ „} \end{array} \right.$

On combustion in oxygen with CuO and silver foil in front, the following results were obtained:—

- I. 0·5525 gram yielded 0·060 gram OH₂, and 0·203 gram CO₂; corresponding to H = 1·21 per cent., C = 10·02 per cent.
- II. 0·59525 gram yielded 0·065 gram OH₂, and 0·21675 gram CO₂; corresponding to H = 1·21 per cent., C = 9·93 per cent.

Assuming, however, that the chlorine found in the salt is present as PbCl_2 or as Pb_2OCl_2 , the following figures may be calculated respectively:—

	Cl as PbCl_2 .	Cl as Pb_2OCl_2 .
Pb	68·39	67·47
C	10·48	10·79
H	1·26	1·32
O	19·87	20·42
	100·00	100·00

On comparing these with the results obtained in the case of the similar salt in the first fermentation, it will be seen that this one contains more lead in proportion to the carbon and hydrogen, which is doubtless due to its being a more basic salt, or possibly a mixture of the former salt with a more basic one. This becomes the more probable when it is remembered that this salt was only precipitated after *repeatedly* boiling with lead carbonate, and that after each successive treatment with lead carbonate, a fresh quantity of precipitate formed on cooling. In fact, this second lead salt corresponds approximately in composition with a basic salt of the following constitution:—

$\text{Pb} \left\langle \begin{array}{c} \text{CH}_2\cdot\text{OH} \\ \text{OOC}\cdot\text{CHO} \\ \text{OOC}\cdot\text{CHO} \\ \text{CH}_2\cdot\text{OH} \end{array} \right\rangle \text{Pb}$	Pb	66·56
	C	11·57
	H	1·28
	O	20·59
		100·00

A similar basic salt has been prepared in the case of tartaric acid, and has the formula

Unfortunately, the quantity of material at our disposal was insufficient to effect any further purification of the salt, and we have had to leave the further investigation of this acid until a larger quantity of the products of fermentation have been obtained. It is, however, perfectly obvious that this non-volatile acid left after fermentation has the very strongest resemblance, both in composition and properties, to glyceric acid, and we hope very shortly to be in a position to communicate some further points of interest and importance concerning it.

Fermentations Nos. 3 and 4.—Two further fermentations were conducted in order to re-determine the quantities of alcohol and volatile

acids produced, as well as the relationship between the two. The mode of procedure was just the same as before, and the following quantities of products were obtained on aliquot parts of the whole fermented liquid:—

Volatile Acids.

	Fermentation III.	Fermentation IV.
Barium salt dried at 130°.	2·015 grams, yielding BaSO ₄ 91·96 per cent.	3·813 grams, yielding BaSO ₄ 92·10 per cent.
Total barium salt produced	(2·015 × 4 = 8·06 grams). See note below	3·813 × 4 = 15·262 grams
Corresponding to—		
Acetic acid.....	(3·694 grams)	7·00 grams
Formic „	(0·0896 „)	0·132 „
Total volatile acid, calculated as acetic acid	(3·799 „)	7·198 „

Alcohols on Oxidation yielded:—

Barium salt dried at 130°.	1·3125 gram, yielding BaSO ₄ 90·95 per cent.	1·8015 gram, yielding BaSO ₄ 90·95 per cent.
Total barium salt produced	(1·3125 × $\frac{3}{2}$ = 1·968 gram)	1·8015 × 2 = 3·603 grams
Corresponding to ethyl alcohol	(0·71 gram)	1·299 gram

Note.—Only in the case of No. IV are the quantities of alcohol and acetic acid obtained absolute, for in No. III about one-half of the fermented liquid was accidentally lost just in commencing the first distillation. Thus, whilst the proportion of alcohol to acetic acid remained undisturbed by the mishap, the amounts of alcohol and acid must be multiplied by about 2, in order to obtain the quantities actually produced in the fermentation.

From these results, the alcohol and acetic acid are seen to stand in the following relationship:—

Fermentation III. Alcohol : acetic acid :: 1 : 5·35
 „ IV. „ „ :: 1 : 5·54

This relationship corresponds approximately to 1 mol. of alcohol to 4 mols. of acetic acid:—

Of Fermentations Nos. 1 and 2, only No. 1 can be used for comparison, as in No. 2 the portion containing the alcohol was lost. In No. 1 the relationship was,

Alcohol : acetic acid :: 1 : 4·35.

We believe that this deficiency of acetic acid in the case of No. 1 is probably to be accounted for by the very considerable proportion of formic acid produced in that fermentation, for there is evidence, as already pointed out by one of us in the case of mannite and glycerin, that whenever formic acid is found amongst the products of fermentation of the *Bacillus ethaceticus*, the proportion of acetic acid is diminished, and we are of opinion that the formic acid is produced at the expense of the acetic acid already formed, in that feeble secondary fermentation, mentioned on p. 82, which occasionally takes place. This belief is supported by the fact that in the case of Nos. 1 and 2, in which the amount of formic acid was considerable, such a secondary fermentation was actually observed, whilst in the case of Nos. 3 and 4, in which the proportion of formic acid was quite insignificant, it was not.

We may provisionally suggest the following as the equation according to which the glyceric acid undergoes fermentative decomposition by this organism:—

This equation not only indicates the observed relationship between the alcohol and acetic acid formed, but the proportion of these substances to the glyceric acid decomposed is in fair accord with the quantities actually obtained. Thus, in Fermentations 1 and 2, there were in each case 60 grams of calcium glycerate, corresponding to 44.47 grams of glyceric acid, employed, and of this, almost exactly one-half was still present as glyceric acid after the fermentation was completed; assuming, then, that 22 grams of glyceric acid had suffered fermentative decomposition, there should have been formed, according to the above equation, 1.9 gram of alcohol and 9.9 grams of acetic acid; as a matter of fact, there were actually found by the methods of analysis employed, 1.7 gram alcohol in Fermentation 1 (in Fermentation 2 the alcohol was lost), and 1.3 gram alcohol in Fermentation 4, also 7.5 grams volatile acid calculated as acetic acid in Fermentation 1, 7.47 grams in Fermentation 2, and 7.2 grams in Fermentation 4. Now, it will be shown in the Appendix to this paper (see pp. 94, 95) that, in the process of repeated distillation with hydrochloric acid, only about 95 per cent. of the volatile acid present is actually recovered, so that, by introducing this correction, the above figures approximate more closely to those calculated from the equation, and the difference may doubtless be accounted for by loss in the secondary change from acetic to formic acid, as well as in the production of the small quantity of succinic acid, which has not been taken into consideration in the equation.

We may summarise the results of our investigation as follows:—

- (1.) Suitable solutions of calcium glycerate undergo a very well-defined fermentation on inoculation with pure cultures of the *Bacillus ethaceticus*.
- (2.) The fermentation is less vigorous than those induced by the same organism in similar solutions of glucose, mannite, and glycerin; indeed, in old cultures the organism is frequently found to have lost its power of fermenting the glycerate, although it still grows in the solution, rendering it highly turbid.
- (3.) The products of the fermentation are essentially alcohol and acetic acid, with a small and variable proportion of formic acid, together with a trace of succinic acid.
- (4.) The alcohol and acetic acid are produced approximately in the proportion of 1 mol. of alcohol to 4 mols. of acetic acid.
- (5.) The production of formic acid appears to be at the expense of acetic acid, as previously observed by one of us in the case of other fermentations induced by this organism.
- (6.) There remains, after the completion of the fermentation, in addition to the above products, also the calcium salt of a fixed acid, the amount of which corresponds almost exactly to one-half of the glyceric acid originally present. We have reason to believe that this acid is glyceric acid, but we are still engaged upon its further investigation.

APPENDIX.

We wish to record in this appendix some of the experiments undertaken with a view to testing the accuracy of the methods employed in the separation of the various products of the fermentation.

I. *Determination of Alcohol by Oxidation with Potassium Dichromate and Sulphuric Acid.*

10·7475 grams of absolute alcohol (calculated from sp. gr. of strong spirit of wine) were diluted to 1000 c.c. with distilled water; 100 grams of potassium dichromate and 200 grams of strong sulphuric acid dissolved in 1000 c.c. of water were then added, and the whole was heated in a closed bottle at 100° for seven hours. The liquid was then reduced with zinc, the total volume now amounting to 2070 c.c. Of this, 255 c.c. (containing the products of oxidation of 1·323 gram alcohol corresponding to 1·727 gram acetic acid) were taken and submitted to repeated distillation as long as the distillate came over acid; on titrating the three distillates so obtained with normal alkali, the following quantities of acid, calculated as acetic acid, were recovered:—

Distillate.	Acidity, calculated as acetic acid.
I	0.762 gram
II	0.929 „
III	0.004 „
Total.....	1.695 „

1.695 gram acetic acid corresponds to 1.299 gram ethyl alcohol, so that 98.17 per cent. of the alcohol employed was actually found.

II. *Determination of the Volatile Acids by Distillation with Hydrochloric Acid.*

In the first experiment :—

5 grams of pure barium formate were dissolved in about 300 c.c. of water and a quantity of (20 c.c. of normal hydrochloric acid) hydrochloric acid insufficient to decompose the whole of the formate was added; the liquid was then repeatedly distilled to one quarter of its bulk until the distillate was no longer acid. Each distillate was then titrated with normal alkali, and the following quantities of formic acid were thus obtained in the several distillates :—

Distillate.	Acidity, calculated as formic acid.
I	0.4784 gram
II	0.2300 „
III	0.0920 „
IV	0.0460 „
V	0.0090 „
VI	0.0140 „
Total	0.8694 „

This 0.8694 gram of formic acid actually found corresponds to 94.5 per cent. of the formic acid theoretically liberated by the quantity of normal hydrochloric acid originally added.

In a second experiment the conditions were varied :—

4.593 grams of barium formate were dissolved in about 750 c.c. of water, and then the calculated amount of normal hydrochloric acid was added so as to liberate the whole of the formic acid; the liquid was then repeatedly evaporated to about one-fourth of its bulk until the distillate was no longer acid, the various distillates were then titrated with decinormal alkali in quantities of 1000 c.c. at a time, when the following proportions of formic acid were found :—

Distillate.	Acidity, calculated as formic acid.
I	0·6900 gram
II	0·6440 „
III	0·2806 „
IV	0·0920 „
V	0·0506 „
VI	0·0115 „
Total....	1·7687 „

This total of 1·7687 gram corresponds to 95·00 per cent. of the formic acid which should have been theoretically found (namely, 1·862 gram).

III. *Determination of the Order of Distillation of the Fatty Acids.*

These experiments were made in order to ascertain the succession in which the several fatty acids distil over from a mixture of their salts to which hydrochloric acid has been added.

Thus a mixture of barium propionate, acetate, and formate was made in the proportion of:—

Barium propionate	3·000 grams
„ acetate	2·703 „
„ formate	2·406 „

Each of these quantities of salt requires 21·2 c.c. of normal hydrochloric acid for the liberation of its respective acid.

1. 21·2 c.c. of normal acid were added along with a considerable volume of water, and distillation was proceeded with as long as an acid distillate came over, fresh water being repeatedly added to the distilling flask. The acid in the distillate was then converted into barium salt in the usual way, and after drying at 130° until constant, the latter was weighed and a barium determination made:—

2·6825 grams of barium salt were obtained, yielding:—

- (a.) 85·43 per cent. BaSO₄,
- (b.) 85·63 „ „

Barium propionate yields 82·13 per cent. BaSO₄.

2. The whole of the remaining acid (42·4 c.c. of normal hydrochloric acid) was then added, and distillation continued down to one-fourth the volume of liquid; from this first distillate, there were obtained:—

2.6515 grams barium salt, yielding $\begin{cases} 92.42 \text{ per cent. BaSO}_4, \\ 92.00 \text{ „ „ „} \end{cases}$

Barium acetate yields 91.37 per cent. BaSO₄,
 „ formate „ 102.64 „ „

Water was again added, and the liquid distilled down to one-fourth of its volume; from this second distillate, there was obtained:—

1.549 gram of barium salt, yielding $\begin{cases} 96.81 \text{ per cent. BaSO}_4. \\ 96.65 \text{ „ „} \end{cases}$

This salt, therefore, contained a larger proportion of formate than the last.

The liquid was again three times distilled down to one-fourth of its bulk, and on uniting these distillates (3, 4, and 5), there was obtained:—

0.364 gram of barium salt, yielding 100.00 per cent. BaSO₄, and thus consisting chiefly of formate with a smaller proportion of acetate.

Two more distillates (the 6th and 7th) were similarly obtained and, on uniting them, a further quantity of—

0.2275 gram of barium salt was prepared, yielding 101.98 per cent. BaSO₄.

This was, therefore, almost pure formate.

These results clearly show, what has already been amply demonstrated in the course of our examinations of the fermented liquids, that from such a mixture of the salts of the fatty acids the acids distil over in the order of their molecular weights, the heaviest first and the lightest last.