39. *Quantitative Determination of Methylglyoxal, and the Mechanism of its Reaction with Hypoiodite Solution.*

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It has been shown that the reaction established by Fischler and Boettner, *i.e.*,

$$CH_3{\cdot}CO{\cdot}CHO + 4I_2 + 7NaOH = CHI_3 + (CO_2Na)_2 + 5NaI + 5H_2O \quad . \; . \; . \; . \quad (1)$$

cannot be used as a quantitative method for determining methylglyoxal. The degree of completeness of this reaction varies greatly with the experimental conditions, never exceeding 94%. Excess of iodine and sodium hydroxide have been shown to convert methylglyoxal into pyruvate and oxalate, and an accurate quantitative method has been based on this reaction, the iodine consumed and the oxalate produced being determined.

The mechanism of the total reaction is discussed.

We have examined several methods for the determination of methylglyoxal. Friedemann's method (*J. Biol. Chem.*, 1927, **73**, 331) gave consistent results, but that of Fischler and Boettner (*Z. anal. Chem.*, 1928, **74**, 28), though sometimes consistent in itself, gave lower results. Since in neither case was there an independent check by a trustworthy process, the absolute accuracy of these two methods is unknown. Takahashi (*J. Biochem. Japan*, 1933, **17**, 299) found that the latter method gave results 6% lower than the former and nearer to those obtained by Neuberg and Kobel's method (*Biochem. Z.*, 1928, **203**, 463), but nevertheless concluded that these three methods were the best available.

Since anhydrous methylglyoxal is difficult to prepare and very hygroscopic, the methods could not be checked against accurately weighed quantities, so another procedure had to be devised. Fortunately, by the methods of Riley, Morley, and Friend (J., 1932, 1875) and Riley and Gray (*Org. Synth.*, 1935, **15**, 67), glyoxals may be prepared conveniently in pure condition, and by testing the methods on weighed quantities of pure phenylglyoxal monohydrate, one of us (S.-C. W.), together with (Miss) Wang Chêng-I, proved the accuracy of Friedemann's method to within an error of 1%.* Hence, the concentration of a methylglyoxal solution so determined could be used as a standard for examining the Fischler–Boettner method. This involves the use of an alkaline hypoiodite, and although this reaction has been much used for the determination of organic compounds, its dependence upon experimental conditions and its mechanism have only recently been thoroughly studied. Hatcher and Mueller (*Trans. Roy. Soc. Canada*, 1929, III, **23**, 35) and Hatcher and Horwood (*Canadian J. Res.*, 1934, **11**, 378) found that even for acetone the extent to which reaction proceeds is greatly influenced by the quantity of alkali used and by the order of mixing of the reagents. It is now generally accepted (see, *e.g.*, Fuson and Bull, *Chem. Rev.*, 1934, **15**, 275) that the rate of this reaction is chiefly controlled by the rate of enolisation of acetone, which, in turn, depends on the concentration of the alkali. It was thought that the reactions involved in the Fischler–Boettner method might show similar characteristics. Consequently, a systematic study of this method under various conditions was undertaken in order to elucidate its mechanism and that of hypoiodite in general, and to find an accurate method for the determination of methylglyoxal.

Experimental.

The methylglyoxal used was prepared by the method of Riley, Morley, and Friend (*loc. cit.*), the higher-boiling portion being further purified by vacuum distillation. The sodium hydroxide, iodine, iodate-free potassium iodide, sodium thiosulphate, potassium permanganate, and hydrochloric acid were of the highest grade of purity available, and their solutions were standardised as usual, results agreeing to within 0·2%. These were checked about every 10 days.

When methylglyoxal is mixed with an excess of iodine and sodium hydroxide solutions, iodoform and oxalate are produced [see (1), above], as proved by Fischler and Boettner. Their procedure was as follows: A solution of methylglyoxal was mixed with a measured excess (1—1·5 times the theoretical quantity) of N/10-iodine solution, the mixture made alkaline with 6—10 times the theoretical volume of N-sodium hydroxide, and, after ½ hour, acidified with hydrochloric acid, the liberated iodine being titrated with N/10-sodium thiosulphate. For the determination of iodoform and oxalate, a duplicate portion was treated with iodine and alkali exactly as above, and after ½ hour the iodoform was filtered off, washed, dried, and weighed, the oxalate in the filtrate being determined as calcium salt (monohydrated) by acidification with acetic acid and addition of calcium chloride.

Our preliminary experiments were done (*a*) by the above procedure; (*b*) by using hydrochloric acid, followed by calcium chloride and ammonia, as usual, to precipitate the oxalate; (*c*) by simplifying the procedure to make the three determinations in one solution. All three methods gave essentially the same results, so our final procedure was as follows: After the mixture of methylglyoxal, iodine, and sodium hydroxide had been kept for ½ hour, it was acidified with hydrochloric acid, the unconsumed iodine titrated with thiosulphate, and the iodoform filtered off on a sintered glass filter, dried in a vacuum desiccator, and weighed; to the filtrate, excess of calcium chloride solution was added, and calcium oxalate precipitated at 70—80° as usual, filtered off, dissolved in sulphuric acid and titrated with standard potassium permanganate.

* These results will be reported later, together with other methods for determination of phenylglyoxal.

From these results it was found that the concentration determined from the iodine consumed was lower than that determined by Friedemann's method, the yield of oxalate was somewhat lower than that calculated from the consumed iodine, and that of iodoform still lower. Moreover, the iodine consumed and the oxalate produced varied considerably with the experimental conditions. Consequently, a systematic investigation was made by wide variations in (1) the excess of sodium hydroxide, (2) the excess of iodine, (3) the concentration of alkali, and (4) the order and (5) the manner of mixing of reagents. Experiments were also carried out to determine the rate of reaction after the addition of alkali to the mixture of methylglyoxal and iodine.

RESULTS AND DISCUSSION.

The results are summarised in the tables. In the main table, all the experiments were made by adding the alkali last. The three necessary determinations were then made.

10 *C.c. of* 0·1057 (*or* 0·0639)M-$CH_3\cdot CO\cdot CHO$ + 40(*or* 80) *c.c.* of 0·3N-*iodine* + NaOH.

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
							% of $CH_3\cdot CO\cdot CHO$ converted into			
No.	$CH_3\cdot CO\cdot CHO$, millimols.	I_2 added, milliequivs.	NaOH, milli-equivs.	I_2, cons., milli-equivs.	$(CO_2Na)_2$, millimols.	CHI_3, millimols.	$(CO_2Na)_2$.	$CH_3\cdot CO\cdot CO_2Na$.	Total of 8 and 9.	$CH_3\cdot CO\cdot CHO$, millimols., calc.
1	1·057	12	7 (1N)	3·050	0·1542	—	14·59	85·9	100·5	1·063
Series A: NaOH added gently down the sides of the flask, with shaking after each addition, and final dilution to 174 c.c.*										
2*a*	1·057	12	14 (1N)	5·537	0·5582	0·523	52·81	50·7	103·5	1·094
2*b*	1·057	12	14 (1N)	5·532	0·5683	0·526	53·77	46·6	100·4	1·061
2*c*	1·057	12	14 (1N)	5·262	—	—	—	—	—	—
3*a*	1·057	12	35 (1N)	7·301	0·8560	—	80·98	21·4	102·4	1·083
3*b*	1·057	12	35 (1N)	7·973	0·9761	—	92·34	7·8	100·1	1·058
3*c*	1·057	12	35 (1N)	7·331	0·8538	—	80·79	23·7	104·5	1·104
4*a*	1·057	12	84 (1N)	7·965	0·9726	0·888	92·02	8·7	100·7	1·065
4*b*	1·057	12	84 (1N)	7·959	0·9748	0·881	92·22	7·6	99·8	1·055
5*a*	1·057	24	14 (1N)	3·711	0·2863	0·233	27·09	67·2	94·3	0·997
5*b*	1·057	24	14 (1N)	3·797	0·2855	0·253	27·01	71·6	98·6	1·042
Series B: NaOH added from a burette at normal rate.										
1	1·057	12	3 (1N)	1·251	—	—	—	—	—	—
2	1·057	12	5 (1N)	2·100	—	—	—	—	—	—
3	1·057	12	14 (1N)	4·458	(0·3907)	—	(37·0)	—	—	—
4	1·057	12	35 (1N)	6·744	(0·7716)	—	(73·0)	—	—	—
5	1·057	12	84 (1N)	6·588	(0·7457)	—	(70·6)	—	—	—
6	1·057	12	35 (2N)	7·837	(0·9539)	—	(90·3)	—	—	—
7	1·057	12	35 (4N)	7·927	(0·9689)	—	(91·7)	—	—	—
8*a*	0·639	12	14 (1N)	3·928	0·4433	—	69·37	29·89	99·26	0·634
8*b*	0·639	12	14 (1N)	3·916	0·4409	—	69·00	30·44	99·44	0·635
Series C: The quantity of N-sodium hydroxide, diluted to 84 c.c., was added dropwise with constant stirring.										
1	1·057	12	7 (N/12)	3·249	(0·189)	—	(17·9)	—	—	—
2	1·057	12	14 (N/6)	4·917	0·467	—	44·18	55·86	100·04	1·057
3	1·057	12	35 (5N/12)	4·950	(0·473)	—	(44·8)	—	—	—
4	1·057	12	56 (2N/3)	4·920	(0·468)	—	(44·3)	—	—	—
5	1·057	12	84 (1N)	5·244	(0·522)	—	(49·4)	—	—	—

* Under these conditions (see p. 165) the reaction stopped very quickly after the addition of alkali, and hence the dilution here would not show any effect.

From equation (1), 1 mol. of methylglyoxal should consume 8 equivs. of iodine and yield 1 mol. each of iodoform and sodium oxalate, but from the tabulated results the following facts may be noticed. First, the amounts of all these three species involved varied greatly with the quantity and the manner of addition of the alkali, and to a lesser extent, with the quantity of iodine and the concentration of alkali. Under all conditions, too little iodine was consumed; the highest value (expt. 3*b*) corresponds to 7·973 × 100/8 × 1·057 = 94% of the methylglyoxal present, agreeing with Takahashi's value for the ratio between the results of the Fischler–Boettner and the Friedemann method. Secondly, the oxalate produced was always less than one-eighth of the iodine consumed; hence, some of the iodine must have been consumed in side reactions. Thirdly, the iodoform produced was even lower than the oxalate; this may be explained partly by the greater experimental error in its determination and partly by its appreciable solubility, *i.e.*, 0·0106 g. per 100 g. of water at 25° (Seidell, " Solubilities, etc.," van Nostrand, 1919, p. 335).

The aldehyde group of methylglyoxal may be easily oxidised by hypoiodite, and Neuberg and Corr (*Biochem. Z.*, 1925, **166**, 442) have oxidised it with bromine to pyruvic acid, the reaction being accelerated by sodium acetate; hence some of the iodine may have been used for oxidation of methylglyoxal to pyruvate:

$$CH_3\cdot CO\cdot CHO + I_2 + 3NaOH = CH_3\cdot CO\cdot CO_2Na + 2NaI + 2H_2O \quad . \; . \; . \; . \quad (2)$$

This would require that

$$\text{Total } I_2 \text{ consumed} = 8[(CO_2Na)_2] + 2[CH_3\cdot CO\cdot CO_2Na] \quad . \; . \; . \; . \; . \; . \; . \; . \quad (3)$$

also $CH_3\cdot CO\cdot CHO$ converted into $(CO_2Na)_2 = [CO_2Na)_2] \times 100/1\cdot 057\%$

and ,, ,, ,, $CH_3\cdot CO\cdot CO_2Na = \dfrac{\text{Total } I_2 \text{ consumed} - 8[(CO_2Na)_2]}{2 \times 1\cdot 057} \times 100\%$. (4)

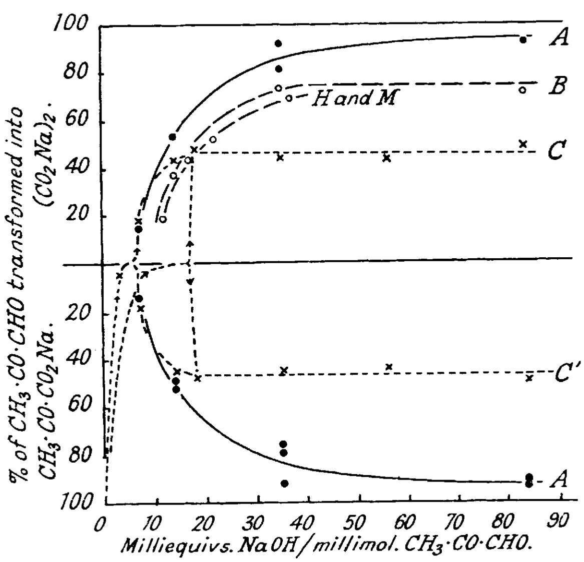
where iodine is expressed as milliequivs. and the salts in millimols. The percentages so calculated are listed in cols. 8 and 9 of the table (without parentheses). It will be seen from col. 10 that the total amount of methylglyoxal undergoing the reaction is $100 \pm 4\%$. This means that under any experimental conditions when iodine and alkali were in excess, all the methylglyoxal has been converted into pyruvate and/or oxalate. Although the 4% error seems large, it must be remembered that the experimental conditions varied over a very wide range; moreover, the pyruvate is obtained by difference in (3) and so is sensitive to experimental error.

Now if y is the number of millimols. of methylglyoxal and x is the fraction converted into oxalate, then $1 - x$ is the fraction converted into pyruvate, and (3) can be transformed into

$$\text{Total } I_2 \text{ consumed} = 8xy + 2y(1 - x) = 2y + 6xy = 2[CH_3{\cdot}CO{\cdot}CHO] + 6[(CO_2Na)_2] \quad . \quad . \quad (3a)$$

whence, if iodine and alkali are in excess, we can calculate the quantity of methylglyoxal from determinations of the iodine consumed and the oxalate produced. The results so obtained are given in col. 11, and although the error may be as high as 4%, many of the results (especially in series B and C) agree with the quantities, 1·057 or 0·639 millimols., determined by Friedemann's method. Furthermore, this method has the advantage of not being much affected by the experimental conditions. Hence, we suggest the following amplification of the details on p. 162.

The aqueous solution of methylglyoxal is mixed with about 12 equivs. of 0·1—0·3N-iodine, and then with 14—30 equivs. of N-sodium hydroxide, added from a burette and stirred. After a few minutes, the mixture is acidified with dilute hydrochloric acid, the iodine is titrated with sodium thiosulphate, and the iodoform filtered off. In the filtrate, oxalate is determined gravimetrically or volumetrically by standard procedure, and the weight of methylglyoxal calculated from equation (3*a*).



In order to determine whether pyruvate is formed at a low pH, and therefore before the oxalate, we made the following experiments.

(1) To find whether methylglyoxal and iodine react in absence of alkalis, 10 c.c. of 0·1057M-methylglyoxal were mixed with 40 c.c. of 0·3N-iodine and 124 c.c. of water; after ½ hour, the unconsumed iodine was titrated, but only 0·030 and 0·036 milliequiv. were used in two different experiments.

(2) To find whether there is any consumption of iodine before formation of iodoform in weakly alkaline solution, 30·5 c.c. of 0·1N-sodium hydroxide (barely sufficient for the formation of pyruvate only) were added dropwise with constant stirring to 10 c.c. of 0·1057M-methylglyoxal, 40 c.c. of 0·3N-iodine, and 94 c.c. of water. No iodoform was formed, but 1·99 and 2·03 milliequivs. of iodine were found to have been consumed on titration, respectively, immediately and after ½ hour. These correspond to 94 and 96% of conversion into pyruvate.

(3) To determine whether the first appearance of iodoform could be used as an indication of the completeness of the formation of pyruvate, 0·01N-sodium hydroxide was added dropwise with constant stirring to a mixture of methylglyoxal and iodine as in (2) with 40 c.c. of water till iodoform appeared; after ½ hour, the unconsumed iodine was titrated, and 5·20 milliequivs. found to have been consumed, corresponding to 48·7% conversion into oxalate.

From these experiments, it is clear that at low pH transformation into pyruvate predominates, but that higher pH favours formation of oxalate. We have the following consecutive and simultaneous reactions:

(i) $I_2 + 2NaOH = NaI + NaIO + H_2O$
(ii) $CH_3{\cdot}CO{\cdot}CHO + NaIO + NaOH = CH_3{\cdot}CO{\cdot}CO_2Na + NaI + H_2O$
(iii) $CH_3{\cdot}CO{\cdot}CO_2Na + 3I_2 + 4NaOH = CHI_3 + (CO_2Na)_2 + 3NaI + 3H_2O$

Reaction (i) is probably the most rapid, and although this would consume sodium hydroxide almost instantly if it were slowly added, there might be sufficient hydroxyl-ion concentration to facilitate reaction (ii). Only when this has been completed will reaction (iii) begin to take place with further addition of alkali, but the onset of this depends on the concentration and the manner of addition of the alkali as depicted by curves *C* and *C′* (see Fig.). However, in rapid addition of alkali, the local hydroxyl-ion concentration may suffice to start reactions (i), (ii), and (iii), simultaneously. This explains why the iodine consumed and the oxalate produced were so much influenced by the manner of addition of the alkali.

The formation of pyruvate as an intermediate step was also postulated by Fischler and Boettner, but its further oxidation requires such a high hydroxyl-ion concentration that, as shown by Hatcher and Mueller, it can never be complete when alkali is added to a mixture of pyruvic acid and iodine. From the iodine consumed, we have calculated by equation (4) the proportion of methylglyoxal transformed into oxalate; these values are listed in the main table in parentheses and plotted in the figure. It will be seen that they vary with the manner of addition of alkali, approaching a different maximum which does not increase with further addition of alkali. These maximum values, though fluctuating somewhat, are different in each case. If the pyruvate is indeed the intermediate in the oxidation of methylglyoxal, these values are actually the proportion of pyruvate converted into oxalate. Consequently, the values of our series B experiments (see table) should agree roughly with those obtained by Hatcher and Mueller for pyruvic acid :

$$CH_3\cdot CO\cdot CO_2H + 3I_2 + 5NaOH = CHI_3 + (CO_2Na)_2 + 3NaI + 4H_2O \quad . \quad . \quad . \quad . \quad (1a)$$

The results of those authors, for 1 millimol. of methylglyoxal and 14 milliequivs. of iodine, were as follows (with a slight modification to allow for iodine and alkali used for oxidation of the aldehyde to the acid) :

NaOH, milliequivs.	12	17	22	27	32
% Conversion into $(CO_2Na)_2$	18·5	43·5	52·0	62·4	68·8

These values are also plotted in the figure. It will be seen that they agree well with our curve B, except for small differences which might arise from differences in manner of addition and in the quantities of iodine used for these two cases.

It might still be doubted whether the difference in oxalate production could not be partly due to an error arising from not stopping the reaction immediately after the addition of alkali. Preliminary experiments for the determination of reaction rate were thus made for the different manners of addition and different quantities of sodium hydroxide. It was found that, under our experimental conditions, the reaction practically stopped after 10 mins., no difference being detected after a further 50 mins.

Hatcher and his collaborators also found that for acetone and pyruvic acid the rate of the reaction producing iodoform is controlled by the enolisation of these compounds, which again depends on the pH value. Consequently, the completeness of the reaction depends upon the order of mixing of the reagents. Orders of addition being denoted by (I) $CH_3\cdot CO\cdot CO_2H$, NaOH, I_2; (II) $CH_3\cdot CO\cdot CO_2H$, I_2, NaOH; (III) I_2, NaOH, $CH_3\cdot CO\cdot CO_2H$, Hatcher and Mueller found that in (I), reaction approached almost to completion when the alkali exceeded 6 times the theoretical quantity; in (III) the reaction proceeded the least. Our results for methylglyoxal agree fairly well with those of series (II) in their work, but we have also made experiments upon this compound with the other two orders of mixing, the results of which are given below.

Order of mixing.	$CH_3\cdot CO\cdot CHO$, millimols.	H_2O, c.c.	N-NaOH, milliequivs.	I_2 added, milliequivs.	I_2 consumed, milliequivs.	$(CO_2Na)_2$, millimols.
$CH_3\cdot CO\cdot CHO$, NaOH, I_2	1·057	40	14	12	2·765	0·3368
I_2, NaOH, $CH_3\cdot CO\cdot CHO$	1·057	40	14	12	0·114	—

With the last order of mixing, the consumption of iodine was very small, the pH in the hypoiodite solution apparently being too low even for the formation of pyruvate. The other order of mixing would have resulted in a large production of oxalate were it not for the fact that methylglyoxal might have undergone a Cannizzaro reaction, affording lactate, before it could be oxidised to pyruvate; nevertheless, the methylglyoxal which had not undergone this reaction must have been almost completely converted into oxalate. (It is presumed that lactate is much more slowly oxidised than pyruvate.) Hence, although the iodine consumed and the oxalate produced were both too low, the latter corresponded to 97·4% of the total iodine consumed.

In another experiment, instead of sodium hydroxide, 14 milliequivs. of N-sodium acetate were added to a mixture of 10 c.c. of 0·1057M-methylglyoxal, 40 c.c. of 0·3N-iodine, and 40 c.c. of water; after ½ hour, only 0·099 milliequiv. of iodine had been consumed.

This work is being continued.

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