# 734. *Submicro-methods for the Analysis of Organic Compounds. Part XXII.* The Malaprade Reaction*

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The oxidation of various carbohydrates with periodate was studied for samples of 30—80 μg. Methods were developed for determination of the formic acid produced, the periodate consumption, and the aldehyde produced, by successive titrations on the same solution. The method was entirely satisfactory for compounds producing no aldehyde other than formaldehyde. For other materials, determination of formaldehyde by a chromotropic acid method was preferable; but only the periodate consumption and formaldehyde could be determined successively. The accuracy obtained was similar to that of macro-methods and the reactions followed the same course.

THE selective oxidising action of periodic acid on polyhydroxy-compounds was first noted by Malaprade [1] and has since become very widely utilised in analysis and structural studies.[2-4] Normally, such analyses are done on the macro-scale but, with the great extension of paper and thin-layer chromatography for separation and purification, it would clearly be advantageous in many cases to carry out an analysis on a very small sample; the work described below was therefore undertaken. The methods were developed for sugar analysis, but they should be readily applicable to other materials which are oxidised by periodate. It was found possible to determine the periodate consumption, and the acid and the aldehyde formed in the reaction, without any separations, on a sample of only *ca.* 50 μg, with a degree of accuracy equivalent to that obtained on a macro-scale. For some compounds, better accuracy for formaldehyde was obtained by taking a separate sample, but even so, the total amount of sample needed was less than 100 μg.

*General Method for Submicro Periodate Oxidations.*—Sodium metaperiodate was chosen as the oxidant because the pH of its aqueous solutions (*ca.* 4) is convenient for the oxidation of most 1,2-glycols and 1,2-diketones, etc., and for a simple titration of the acid formed in the reaction. An aqueous medium was found satisfactory for all the compounds examined. With a 200—300% excess of periodate, the oxidation of simple sugars was complete in 15 min. at room temperature; where the oxidation required more time, the reaction vessel was placed in the dark in order to avoid decomposition of the periodate or the oxidation of the simple reaction products, which would be catalysed by light [5].

Numerous methods have been described for the determination of the excess of periodate,[2-4] the most straightforward methods and those most readily adaptable to the microgram scale are based on iodometric titrations. In preliminary tests, three methods were examined for submicro-titrations of 0·025M-sodium periodate alone: direct titration with thiosulphate in acidic medium;[1] direct titration with arsenite solution at pH 7·5—8; and addition of excess of arsenite followed by titration with iodine solution.[6] All three methods gave results in good agreement with each other, and none of the conditions was particularly critical, even when the titrations were carried out on mannitol oxidation mixtures; the end-points were readily reproducible to within 0·2 μl. of 0·0125M-titrant when detected with Thyodene indicator. For further work, the direct titration with arsenite was preferred, for arsenite solution is very stable when correctly prepared and stored. Moreover,

* Part XXI, *J.*, 1964, 5698.

[1] L. Malaprade, *Bull. Soc. chim. France*, 1926, (4) **39**, 325; 1934, (5) **1**, 833.
[2] J. H. Dyer, in "Methods of Biochemical Analysis," ed. D. Glick, vol. III, Interscience, New York, 1956, p. 111.
[3] J. M. Bobbit, *Adv. Carbohydrate Chem.*, 1956, **11**, 10.
[4] R. J. Dimler, *Adv. Carbohydrate Chem.*, 1952, **7**, 46.
[5] F. S. H. Head and G. J. Hughes, *J.*, 1952, 2046.
[6] P. F. Fleury and J. Lange, *J. Pharm. Chim.*, 1933, (8) **17**, 107.

only periodate is reduced by arsenite at pH 8 whereas, in a thiosulphate titration, iodate formed from periodate would also be determined, so that the final results would be less precise. When mannitol, as the standard test compound, was analysed, there was no significant difference in accuracy between the three titrations, but the direct arsenite method showed a better precision.

The effect of time on the oxidation of 30—60 μg. samples of mannitol to form formaldehyde and formic acid was examined for reaction periods of 15 min. to 16 hr. The oxidation with 150 μl. of 0·025M-periodate was essentially complete after 15 min. and values of 100·5 ± 0·7% of theory were obtained after up to 4 hr.; on oxidation for 16 hr., the results decreased very slightly to 98·7% of theory, because of some decomposition of periodate, which affected the simultaneous blank determination more than the sample determination. The oxidation of sorbitol was also found to be complete in 15 min. at *ca.* pH 4. As some compounds cannot be oxidised accurately under mildly acidic conditions, mannitol was oxidised in a sodium hydrogen carbonate medium in order to check the procedure; the results were the same as at pH 4.

*Determination of Acid Formed.*—Formic acid is the commonest acid formed in the Malaprade reaction, being produced from 1,2-hydroxyaldehydes, from the central carbon atom of a vicinal trihydroxy-structure and in some non-glycol cleavages. It is comparatively rare for glyoxylic or glycollic acid to appear. Attention was therefore concentrated on the determination of formic acid; obviously, acetic acid produced, for example, from acetoin, could be determined in the same way. On the macro-scale, it is customary to titrate the acid with sodium or barium hydroxide after removal of the excess of periodate with ethanediol, or after distillation of formic acid (if periodic acid is used as the oxidant or if the oxidation medium is buffered).

For submicro-application, iodometric titration of a pure 0·0217M-solution of formic acid was first tested.[7] However, results were always about 10% low, even under the optimum conditions, which involved addition of excess of thiosulphate to the reaction medium containing iodate and iodide, and back-titration after 1 hr. with standard iodine solution. Even on the micro-scale with smaller dilution factors, the results of the iodometric method were 4% lower than those obtained by an acid–base titration. When the submicro-method was applied to periodate oxidation mixtures, values for formic acid were 20—50% low, and the method was therefore abandoned.

Straightforward acid–base titrations were then examined. Reasonable end-points and good results were obtained with Methyl Red as indicator, provided that excess of hydroxide was added and back-titrated with hydrochloric acid solution; neither a nitrogen atmosphere, nor the use of Methylene Blue as a screen, was necessary. When phenolphthalein indicator was used with a nitrogen atmosphere, the end-points were better, but sodium metaperiodate interfered; it could be destroyed with ethanediol, but its subsequent determination would then have been impossible. With Methyl Red as indicator, it was possible to titrate formic acid, adjust the pH to 8, and then titrate the excess of periodate with arsenite solution. When five 40—60 μg. samples of mannitol were analysed under these conditions, the production of formic acid was found to be 98·4 ± 1% of theory, and the total consumption of periodate was 100·5 ± 1·1% of theory. The slight diminution in the precision of the periodate results was due to the Methyl Red indicator. Aqueous indicator solutions were used because alcohol would have interfered in subsequent attempts to determine aldehyde by iodometric techniques.

*Determination of Aldehyde.*—In the Malaprade reaction, aldehydes are formed from 1,2-glycols, 1,2-hydroxyaldehydes or ketones and their corresponding amino-derivatives. Formaldehyde is the commonest aldehyde formed by the oxidation of sugars, being produced from terminal carbon atoms bearing a primary hydroxyl group. Many methods have been recommended for the determination of simple aldehydes after a periodate oxidation

[7] I. M. Kolthoff and R. Belcher, "Volumetric Analysis," vol. III, Interscience, New York, 1957, p. 276.

on a macro- or semimicro-scale; of the titrimetric procedures, various modifications of the bisulphite-addition method appear to be favoured,[2-4,7] whereas the dominant colorimetric procedures involve chromotropic acid or phenylhydrazine–hexacyanoferrate(III).[2-4] It is usual to distil the aldehyde from the oxidation mixture or to remove the excess of periodate by reduction or precipitation.

Various attempts to distil or aerate formaldehyde from the reaction mixture on the submicro-scale were a complete failure; precipitation–filtration methods of removing excess of periodate were not tested because, on the submicro-scale, the technique involved is rather critical. Because taking microlitre aliquots of a solution is difficult and wasteful, the problem was to determine formaldehyde in a mixture containing iodate, iodide, bicarbonate, arsenate, formate, Methyl Red and Thyodene, *i.e.*, the solution after the titration of acid and periodate. The most elegant possibility appeared to be method of Romijn,[8] in which aldehyde is oxidised with hypoiodite in alkaline medium and the excess is titrated with thiosulphate after acidification; the method is reported[9] to be very accurate for dilute solutions.

Preliminary tests showed that the Romijn method was applicable to microgram quantities of formaldehyde; alteration in the reaction volume from 0·2 to 1·2 ml. had little effect on the results, but increase in the hydroxide concentration from 0·2N, as used previously,[8,9] to 0·5N lowered the results to 95% of theoretical. A large excess of iodine was essential for complete oxidation; under the conditions used, a 300—600% excess (50—100 μl. of 0·1N-iodine) gave excellent results (relative error ±3%), but a 1000% excess produced very high and variable results. The order of addition of hydroxide and iodine did not appear to be critical.[10]

For application of this method to a reaction mixture, it was essential to reduce the iodate formed from the periodate, otherwise far too much iodine would have been released on acidification. Thiosulphate could not be used, because tetrathionate reacts with hypoiodite; accordingly, after the accurate titration of the periodate, the mixture was acidified to release iodine from iodate and then the pH was readjusted with sodium hydrogen carbonate before further titration with arsenite; the modified Romijn method was then applied directly. For eight analyses of mannitol, the average amount of formaldehyde produced was found to be 101·3% of theory with a maximum relative error of ±9%. Hypoiodite did not oxidise formaldehyde in a bicarbonate medium, but so long as sodium hydroxide was added, there was no need to remove bicarbonate.

A disadvantage of the Romijn method is that it is not specific for formaldehyde, but provided that formaldehyde is the only aldehyde formed in the periodate oxidation, the method is excellent. If unknown aldehydes are formed, interpretation of the results may be difficult. Acetaldehyde, which is formed in the reaction of L-rhamnitol, for example, reacts with hypoiodite in two ways:

$$CH_3CHO + IO^- \longrightarrow CH_3CO_2H + I^- \quad (1)$$

$$CH_3CHO + 3IO^- \longrightarrow CHI_3 + HCO_2^- + 2OH^- \quad (2)$$

The formation of iodoform was noted by Romijn; Bruckhausen[11] calculated that *ca.* 42% of the acetaldehyde present reacted to produce iodoform, whereas Mach and Herrmann[12] found 36—38%. On the submicro-scale, it appeared that 55—60% of the acetaldehyde reacted according to equation (2).

*Analysis of Various Carbohydrates.*—The methods described above, *i.e.*, periodate oxidation followed by successive determinations of acidity, excess of periodate, and aldehyde, were applied to the selection of compounds shown in Table 1; samples of 30—80 μg.

[8] G. Romijn, *Z. analyt. Chem.*, 1897, **36**, 18.
[9] R. Signer, *Helv. Chim. Acta*, 1930, **13**, 43.
[10] Cf. L. P. Goodwin, *J. Amer. Chem. Soc.*, 1920, **42**, 39.
[11] F. Bruckhausen, *Apoth.-Ztg.*, 1939, **34**, 428.
[12] F. Mach and R. Herrmann, *Z. analyt. Chem.*, 1923, **63**, 417.

were used throughout. The straightforward hexitols caused no difficulty, nor did L-arabitol or erythritol; from these results, it appeared that the reactions proceeded to completion rapidly, and that the periodate consumption and the formation of formic acid and formaldehyde could be measured on the microgram scale with an accuracy equivalent to that generally attainable on the macro-scale.

The formic acid figures for the hexitols were 1—4% low; this has been found also in macro analysis and is probably due to the very slow hydrolysis of *O*-formyl esters at low pH values.[13] With myoinositol, the oxidation was obviously incomplete under the recommended conditions; when a larger excess of periodate (300 μl.) was used and the oxidation time was extended to 2 hr., the consumption of periodate levelled off to *ca.* 107% of theoretical, but the production of formic acid was always low. These results agree very well with macro-scale investigations of inositols.[14, 15] For example, Stephen [15] found for myoinositol, periodate consumptions of 5·55 mol. after 12 min., 6·50 mol. after 62 min., and 6·62 mol. after 160 min. In the present work, the periodate consumption was 5·71 mol. after 60 min. and 6·40 mol. after 120 min.; the lag in the oxidation was obviously due to the greater dilutions on the submicro-scale. The production of formic acid was 5·2 mol. in precise agreement with Stephen's results. As expected, no aldehyde was found.

With methyl α-D-glucopyranoside, 300 μl. of periodate solution and a reaction time of 22 hr. were needed for complete oxidation. After 24 hr. over-oxidation and over-production of formic acid occurred, which were apparently in agreement with previous results [16] on a macro-scale. No formaldehyde should be found in the oxidation of methyl α-D-glucopyranoside but when the hypoiodite procedure was applied, *ca.* 2 mol. of aldehyde were found. This was consistent with complete oxidation of the dialdehyde formed in the periodate reaction; cleavage of the dicarboxylic acid to form glyceric and glyoxylic acids and methanol would probably follow.

TABLE 1

Successive determination of acid, periodate consumption, and aldehyde.

| | | | Percentage recovery | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Reaction time (hr.) | No. of detn. | $HCO_2H$ | Average devn. | Compound recovery * | Average devn. | HCHO | Average devn. |
| D-Mannitol ..... | 0·25 | 4 | 98·73 | 0·6 | 101·0 | 1·02 | 96·4 | 2·9 |
| Sorbitol ..... | 0·25 | 4 | 95·9 | 0·6 | 98·8 | 0·38 | 97·6 | 2·0 |
| Dulcitol ..... | 0·25 | 4 | 97·9 | 0·6 | 98·0 | 0·75 | 95·5 | 3·7 |
| Erythritol ..... | 0·25 | 2 | 99·6 | 0·2 | 99·7 | 0·6 | 93·5 | 3·8 |
| L-Arabitol ..... | 0·25 | 2 | 99·1 | 0·1 | 99·9 | 0·5 | 93·9 | 1·5 |
| Myoinositol † ..... | 2 | 2 | 87·2 | 0·4 | 107·1 | 0·4 | 0·0 | — |
| Methyl α-D-glucopyranoside † ... | 24 | 2 | 103·9 | 0·1 | 119·0 | 10 | See text | |
| L-Rhamnitol ..... | 0·25 | 2 | 100·3 | 0·5 | 100·1 | 0·1 | See text | |

* Based on consumption of periodate. † 300 μl. sodium periodate added.

When L-rhamnitol was oxidised, excellent results were obtained for periodate consumption and acid production; however, this compound formed both formaldehyde and acetaldehyde in the oxidation. When the theoretical hypoiodite consumption of the formaldehyde formed was deducted from the total consumption and the quantity of acetaldehyde was calculated on the basis of a 1 : 1 ratio between reactions (1) and (2), the recoveries were 103 ± 4% of theory. Thus, the hypoiodite method can provide useful confirmatory evidence, if the behaviour of a compound is predictable.

Several isopropylidene derivatives were examined as model compounds containing acid-sensitive blocking groups; the oxidation had to be done in sodium hydrogen carbonate

[13] T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *J.*, 1947, 1427.
[14] J. C. P. Schwarz, *Chem. and Ind.*, 1955, 1388.
[15] A. M. Stephen, *J.*, 1952, 738.
[16] G. Neumuller and R. Vasseur, *Arkiv Kemi*, 1953, **5**, 235.

medium, and no values for the production of acid could be obtained. The periodate consumptions were found to lie within ±3% of the theoretical values, but the consumption of alkaline hypoiodite was always very high. For example, with 1,2:5,6-di-isopropylidene-D-mannitol an average of 2·2 mol. of hypoiodite was consumed; the consumption of periodate averaged 0·991 mol. No formaldehyde should be produced, and this was confirmed by the chromotropic acid method (see below). The consumption of hypoiodite can be explained as follows: the 1,2-isopropylidene glyceraldehyde formed in the periodate oxidation was partially hydrolysed to glyceraldehyde and acetone during the brief acidification before the hypoiodite procedure; the aldehydes then consumed 2 mol. of hypoiodite, the additional hypoiodite being consumed by acetone to produce iodoform. When the acidified mixture was left for 30 min. before the hypoiodite procedure, 7·7 mol. of hypoiodite were consumed instead of the theoretical 8, because of almost complete production and reaction of acetone. However, it was obvious that a method more specific for simple aldehydes was essential.

*Chromotropic Acid Method for Formaldehyde.*—Of the many modifications of the chromotropic acid procedure, that of Lambert and Neish [17] in which periodate is reduced with arsenite, and formaldehyde is determined *in situ*, seemed most appropriate for submicro-application. It was found that a very large excess of arsenite was necessary to prevent the reappearance of iodine during the chromotropic acid reaction. At least 100 μg. of acetaldehyde did not interfere. When the recommended procedure was applied, Beer's Law was obeyed over the range 6—40 μg. of formaldehyde produced from the original sample, the calibration graph being prepared with samples of mannitol oxidised at *ca.* pH 4; larger amounts of formaldehyde were not tested. However, determinations of formaldehyde produced from sorbitol and dulcitol were then 10—20% low, whereas results for erythritol were high. When another calibration graph was prepared from mannitol oxidised in hydrogen carbonate medium, a line of greater slope was obtained and recoveries of formaldehyde for the other samples approach more closely to the theoretical values (Table 2). The errors involved in the determination after oxidation at *ca.* pH 4 were presumably due again to *O*-formyl ester formation, which would not have occurred with erythritol, nor with the hexitols, in a hydrogen carbonate oxidation medium. Again, this is in agreement with earlier results on the macro-scale.[18]

TABLE 2

Spectrophotometric determination of formaldehyde (oxidation in hydrogen carbonate medium)

| | Wt. of sample (μg.) | HCHO found (μg.) | (%) |
|---|---|---|---|
| Sorbitol | 34·08 | 11·40 | 101·5 |
| | 54·94 | 17·80 | 98·3 |
| Dulcitol | 76·82 | 25·60 | 101·2 |
| | 58·82 | 19·60 | 101·1 |
| Erythritol | 59·74 | 29·20 | 99·5 |
| | 36·49 | 18·00 | 100·4 |
| L-Arabitol | 56·89 | 21·90 | 97·6 |
| | 54·20 | 21·00 | 98·2 |
| 1,2:5,6-Di-isopropylidene-D-mannitol | 48·15 | ~1·10 | — |
| | 39·09 | ~0·80 | — |
| 1,4-Anhydro-2,3-isopropylidene-D-talitol | 70·23 | 9·80 | 95·0 |
| | 44·76 | 6·35 | 97·0 |

When the isopropylidene derivatives (Table 2) were analysed by the chromotropic acid method, the production of formaldehyde was found to be more in accordance with expectation; for the mannitol derivative, essentially no formaldehyde was formed, and for the talitol derivative, 1 mol. was formed, so that the results of the hypoiodite method then became useful in indicating the more complex products of the periodate oxidation.

When attempts were made to determine the periodate consumption with subsequent

[17] M. Lambert and A. C. Neish, *Canad. J. Res.*, 1950, **28**, *B*, 83.
[18] L. Haugh, D. B. Powell, and B. M. Wood, *J.*, 1956, 4799.

determination of formaldehyde on the same solution, the blank determinations were very variable because of interference from the Thyodene indicator; when a 1% starch solution (*ca.* 20 μl.) was used, better results were obtained. When D-glucose was treated with periodate in hydrogen carbonate medium for 2 hr., the results for periodate consumption and formaldehyde production were excellent (100·2 and 102·8% of theory, respectively); at pH 4, complete oxidation required 24 hr.

*General Conclusions.*—If the periodate oxidation was done at *ca.* pH 4 (*i.e.*, the pH of the sodium metaperiodate solution itself), accurate determinations of periodate consumption and of simple carboxylic acids produced were possible with a single sample of 30—80 μg. Titrimetric determination of formaldehyde could be made on the same solution, but if other aldehydes were present, the results could be difficult to interpret and could only provide confirmatory evidence of structure. Analogous successive titrations with visual indicators would be impossible on larger scales of working. Periodate oxidations in hydrogen carbonate media were more suitable, if formaldehyde was to be determined specifically by the chromotropic acid method, or if high accuracy was needed. It was then possible to determine the periodate consumption and the formaldehyde production on a single sample, though better accuracy was obtained when a separate sample was taken, because of mild interference by the starch indicator. When the spectrophotometric method was used, a prior acid determination was impossible, because of interference by the acid–base indicator; doubtless, potentiometric titration of the acid would solve this difficulty

In general, the reactions of different compounds with periodate followed the same course on the submicro-scale as on the macro- or semimicro-scale, though they were slower because of the greater dilutions used.

## Experimental

*Apparatus.*—The QO1 balance, its accessories and the titration apparatus were those described previously.[19]

The reaction vessels were similar in shape to a submicro-"oxygen" flask,[19] but were 8 cm. high with a B10 stopper; the lower part of the vessel was 2·5 cm. high with a diameter of 2 cm. and the bulb was 2 cm. high with a diameter of 3·5 cm. The bulb was introduced to reduce the danger of sample particles remaining unnoticed on the upper parts of the wall.

Spectrophotometric measurements were made with a Unicam S.P. 600 instrument.

*Reagents.—Water.* Glass-distilled water was used throughout.

*Sodium periodate solution,* 0·025M. Sodium metaperiodate (5·35 g.; AnalaR) was dissolved in water and diluted to 1 l. For standardisation, 100·00 μl. aliquots were transferred to a reaction vessel and about 1 ml. of water was added. Sodium hydrogen carbonate (*ca.* 10 mg.) and 0·1 ml. of fresh aqueous 10% potassium iodide solution were added and the iodine was titrated with the standard arsenite solution in the presence of *ca.* 5 mg. of Thyodene as indicator.

*Standard sodium arsenite solution,* 0·0125M. Primary standard arsenic(III) oxide (0·61813 g.) was dissolved in *ca.* 10 ml. of 1N-sodium hydroxide solution; the solution was then made very slightly acidic by the dropwise addition of 1N-sulphuric acid, and diluted to 250 ml. in a volumetric flask.

*Standard sodium hydroxide solution,* 0·025M. This was prepared by suitable dilution of Sörensen's oily lye or of concentrate from a standard ampoule with carbonate-free distilled water. For standardisation, 100·00 μl. of the hydroxide solution, 0·05 ml. of aqueous 0·01% Methyl Red, and 0·2 ml. of water were mixed and titrated with standard 0·01M-potassium hydrogen iodate, added from an Agla burette, to the first permanent red end-point. The indicator blank was determined in the same way and was usually negligible (<0·5 μl.).

*Standard hydrochloric acid solution,* 0·025M. This was standardised against the sodium hydroxide solution as described above.

*Standard sodium thiosulphate solution,* 0·02N. An approximately 0·02N-solution was standardised against *ca.* 150 μg. of potassium hydrogen iodate dissolved in 0·5 ml. of water after addition of 0·1 ml. of 4N-sulphuric acid and 0·1 ml. of fresh 10% potassium iodide solution; Thyodene was used as indicator.

[19] R. Belcher, P. Gouverneur, and A. M. G. Macdonald, *J.*, 1962, 1938.

*Chromotropic acid solutions*, 0·2%. Chromotropic acid (1·00 g.) was dissolved in 100 ml. of distilled water and the solution was filtered. A cooled mixture of 300 ml. of concentrated sulphuric acid and 150 ml. of water was added and the solution was diluted to 500 ml. with sulphuric acid. It was stored in a brown bottle and prepared every 10 days.

*Procedures.—Oxidation method.* When the successive-titration method was used, a 30—80 μg. sample was transferred to the reaction vessel, and exactly 150·00 μl. (or more, depending on the compound) of 0·025M-sodium metaperiodate were added, the burette tip being rinsed with 0·05—0·1 ml. of water from a hypodermic syringe. The stoppered vessel was then placed in the dark for the required reaction period.

When the compound was oxidised in a hydrogen carbonate medium, 10 mg. of sodium hydrogen carbonate and 0·2—0·3 ml. of water for dissolution were added before the periodate solution. When the spectrophotometric method for formaldehyde was used, the sample was placed in a clean, dry 5-ml. graduated flask and sodium hydrogen carbonate, water, and periodate solution were added as above.

Blank determinations were done simultaneously with each batch of samples.

*Titration of the acid formed in the reaction.* The vessel was placed on a magnetic stirrer and 0·05 ml. of aqueous 0·01% Methyl Red indicator was added followed by exactly 100·00 μl. of standard 0·025M-sodium hydroxide, the tip of the Agla burette being rinsed with 0·05 ml. of water. The excess of alkali was then titrated with standard 0·025M-hydrochloric acid to the first appearance of a red colour. The end-point was less sharp than in the standardisation, because of the buffering effect of sodium formate, but was still easily detectable.

*Titration of excess of periodate.* The tip of the acid burette was rinsed with 0·05 ml. of water and 10 mg. of sodium hydrogen carbonate and 0·05 ml. of fresh aqueous 10% potassium iodide solution were added. The iodine liberated was then titrated with standard 0·0125M-sodium arsenite solution; Thyodene indicator was added after the iodine colour had faded only slightly and the end-point was marked by a change from brownish purple to the clear yellow of Methyl Red. If the spectrophotometric method for formaldehyde was to be used, 0·02 ml. of aqueous 1% starch solution was used instead of Thyodene.

*Titration of formaldehyde formed in the reaction.* Sufficient 4N-hydrochloric acid (usually 0·05 ml.) was added to remove hydrogen carbonate and iodate, and the solution was then carefully rebuffered with *ca.* 15 mg. of sodium hydrogen carbonate. The iodine liberated was then titrated with 0·0125M-arsenite solution until the colour became clear yellow. Sodium hydroxide (0·12—0·15 ml. of 4N) and exactly 50·0 μl. of 0·1N-iodine solution were added with rapid stirring, and the solution was placed in the dark for 30 min. Sufficient 4N-hydrochloric acid (0·25 ml.) was then added to make the solution slightly acidic and liberate the iodine, which was titrated with standard 0·02N-sodium thiosulphate solution to the end-point from brownish purple to clear red.

*Spectrophotometric determination of formaldehyde.* After the reaction period and titration of excess of periodate, the magnetic stirring bar was removed and washed with 1 ml. of water. Then 2·0 ml. of 0·0125M-sodium arsenite solution were added from a pipette and the mixture was shaken occasionally over a period of 10—15 min. before dilution to exactly 5 ml.

A 1·00-ml. aliquot portion was transferred by pipette to a 25-ml. vessel and 10·00 ml. of chromotropic acid solution added. The solution was mixed carefully, and the flask, loosely stoppered, was placed in a vigorously boiling water-bath away from direct light. The solution was then cooled and the optical density was measured at 570 mμ. in a 10-mm. cell. Blank determinations were carried out in exactly the same way; the blanks were usually negligible.

A calibration graph was prepared from samples of mannitol covering the range 5—40 μg. of formaldehyde, treated as described above, except that the exact titration of periodate was omitted; oxidation was done in a hydrogen carbonate medium.

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