269. *Oxidation of Carbohydrates by the Periodate Ion.*

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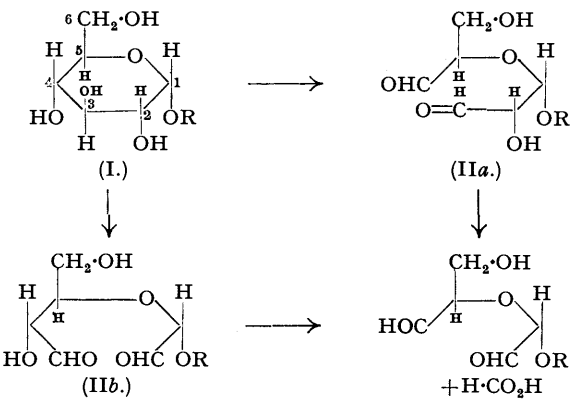
The oxidation, with salts of periodic acid, of the methylglycosides of the hexose and pentose sugars and of the disaccharides has been investigated. Sugar derivatives containing hydroxyl groups on each of three adjacent carbon atoms are oxidised with the formation of 1 mol. of formic acid, and conditions have been established for the quantitative estimation of the formic acid. Methyl hexo- and pento-pyranosides and the methyl glycosides of maltose and cellobiose give rise quantitatively to 1 mol. of formic acid, but reducing sugars, methylglycosides of the uronic acids, and methylhexofuranosides yield acidic material equivalent to more than 1 mol. of formic acid per mol. of sugar. The causes of this continued oxidation are discussed.

The observation by Hudson and Jackson (*J. Amer. Chem. Soc.*, **1936**, **58**, 378; **1937**, **59**, 994), that the α- and β-methylglucosides yield on oxidation with periodic acid a dialdehyde together with 1 mol. of formic acid, affords a possible method of estimating the number of end groups present in a polysaccharide which has terminal groups of the type (I), provided that the non-terminal groups are such that they do not yield formic acid. Such a method should therefore be applicable to a wide range of polysaccharides, including starch, cellulose, and glycogen, which possess chains of 1 : 4-linked hexopyranose residues, but hitherto attempts to make use of this procedure have encountered difficulty in that the oxidation is not arrested at



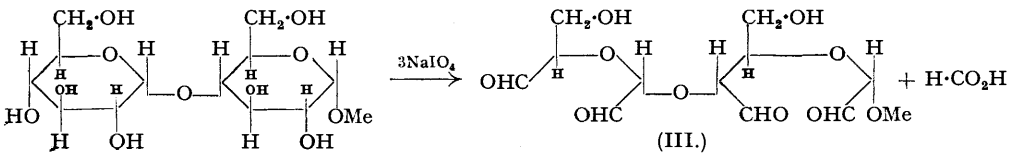
the stage when 1 mol. of formic acid has been libertated from the terminal group (Davidson, *J. Textile Inst.*, 1941, **32**, T, 109). Nevertheless the reaction has been used successfully in other

ways in the study of polysaccharides. For example, the stepwise degradation of laminarin, which consists of 1 : 3-linked residues, has been described by Barry (*Nature*, **1943**, **152**, 538) and by Dillon (*ibid.*, **1945**, **155**, 546), and attempts have been made to estimate the formaldehyde produced from the reducing end of the chain in starch dextrins (Caldwell and Hixon, *J. Biol. Chem.*, **1938**, **123**, 595).

In view of our interest in methods for estimating end groups in polysaccharides we decided to undertake a more detailed survey of the conditions requisite for the prevention of over-oxidation and thus facilitate the development of a method of estimation which would possess many advantages over the present available techniques. This was all the more necessary in that we had found in the course of experiments on plant gums, in which we had hoped to remove certain side chains with the aid of periodic acid, that difficulties due to over oxidation were encountered.

As a preliminary, therefore, to the application of the periodate method of oxidation to the polysaccharides, model experiments were carried out with simple sugars containing groups similar to those present in polysaccharides. These included α-methylglucopyranoside, α-methylgalactopyranoside, α-methylmannopyranoside, α-methyl-*d*-mannofuranoside, β-methylmaltoside, β-methylcellobioside, β-methyl-*l*-arabopyranoside, α-methyl-*d*-xylopyranoside, amygdalin, mannitol, lactose, lactal, and the methyl ester of α-methyl-*d*-galacturonoside. Control experiments with formic acid, ethylene glycol, and oxalic acid were also carried out.

It was our aim to estimate small amounts of formic acid, and this we found practicable only when the excess of periodate had been destroyed by the addition of ethylene glycol with the resultant formation of formaldehyde and sodium iodate, both of which were without action on methyl-red used as indicator for the titration of the formic acid. Sodium periodate slowly destroys formic acid, and the estimated amount of the latter may be low unless precautions are taken. It was found, however, that the oxidation of formic acid is considerably slowed down in the presence of sodium iodate, and, as this substance is always formed by reduction of the periodate, loss of formic acid during the period of oxidation is negligible. Sodium periodate gives satisfactory results for the oxidation of small amounts of methylhexopyranosides and pentopyranosides, all of which on oxidation give 1 mol. of formic acid. On the other hand when this reagent is applied to the methylglycosides of the reducing disaccharides oxidation proceeds beyond the stage represented by (III), and ultimately considerably more than 1 mol. of formic acid is produced per mol. of methylglycoside. The rate of production of formic acid is much slower, however, after the liberation of 1 mol., but with a large excess of sodium periodate as oxidising agent there is not an easy differentiation between the two stages of the reaction.

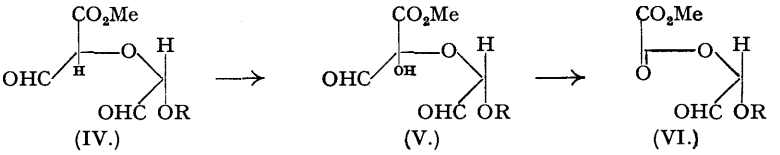


(III.)

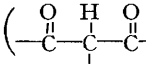
We therefore examined other salts of periodic acid which had lower solubility and would provide reaction solutions of lower acidity. Most of the salts examined were unsatisfactory since they possessed either basic or strongly acidic characteristics. Potassium metaperiodate, however, is only very slightly soluble in water and gives a solution having a pH of about 4. Using this salt and keeping the concentration of formic acid produced to a low value (*ca.* 10 mg. per 100 c.c.), we were able to obtain consistent and reliable figures for the amount of formic acid produced. This lowering of concentration of the periodate ion, however, considerably reduces the rate of reaction, and instead of being complete in 6 hours it now requires 150 hours at 15°. On oxidation with this reagent the methylglycosides of (*a*) the hexose, (*b*) the pentose sugars, and (*c*) the reducing disaccharides gave normal amounts of formic acid. The methylglycosides of uronic acids and of hexofuranosides underwent further oxidation with the formation of additional amounts of acidic material. Similar behaviour was shown by reducing sugars.

The oxidation of the glycosides of the uronic acids has been the subject of previous publications. Huebner, Lohmar, Moore, and Link (*J. Biol. Chem.*, 1945, **159**, 502) demonstrated that, in the oxidation of zinc borneol glucuronoside, more than 1 mol of acid was produced per mol. of glucuronoside and that one of the reaction products was bornyl formate. They suggested that the uronic acid residue, after undergoing oxidation with the formation of formic acid and the dialdehyde (IV), was further oxidised by the periodate. The first step in this oxidation, it was suggested, was the oxidation of the active hydrogen situated on the carbon atom between

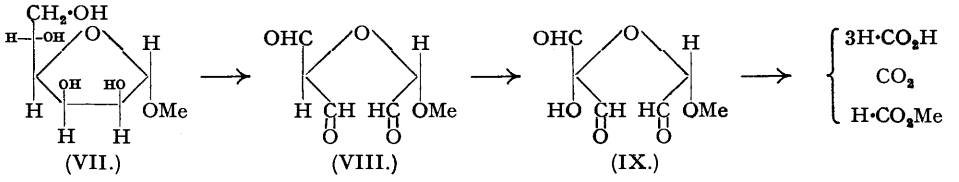
two carbonyl groups to a hydroxyl group (V). This would result in the formation of a substance which in its hydrated form contains hydroxyl groups on adjacent carbon atoms and would



undergo further oxidation with periodate with the formation of an ester of oxalic acid (VI). Once this stage had been reached the ester, which would be unstable in water, could hydrolyse to oxalic acid and a product which could then undergo further oxidation to formic acid and an ester of formic acid.

The stage in which the aldehyde (IV) is converted into the α-hydroxy-aldehyde (V) has been investigated by Sprinson and Chargaff (*J. Biol. Chem.*, 1946, **164**, 443). These workers demonstrated that substances such as malonic acid and its derivatives—substances which contain a hydrogen atom combined to a carbon atom situated between two carbonyl groups  —are all capable of being oxidised to the corresponding hydroxy-compound which may then undergo further oxidation [cf. the work of Neuberger (*J.*, 1941, 47) on the oxidation of ethyl *N*-benzoylglucosamate].

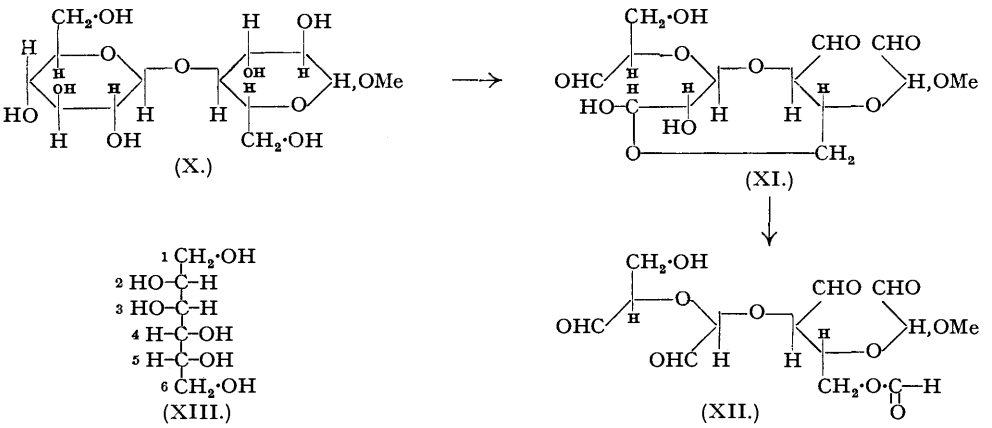
We have found that whenever this secondary type of oxidation occurs free iodine is ultimately produced and the amount of acid in the solution falls. The mode of origin of the iodine is obscure, but the simplest explanation would be to ascribe its formation to the well-known reaction between sodium iodide and sodium iodate in acid solution, the sodium iodide arising in turn from reduction of sodium iodate during oxidation on the activated carbon atom. A typical example of the grouping which undergoes further oxidation, with liberation of free iodine, is shown in (VIII) which is the intermediate product obtained by the periodate oxidation of α-methylmannofuranoside (VII). Attack at the activated hydrogen would yield (IX) which would readily undergo further oxidation giving 3 mols. of formic acid, 1 mol. of methyl formate, and 1 mol. of carbon dioxide.



The rate of oxidation and rate of formation of formic acid varies with the configuration of the glycoside. Those glycosides, such as the arabo-, galacto-, and manno-pyranosides, which contain *cis*-hydroxyl groups, are oxidised relatively quickly, whilst those glycosides which contain *trans*-hydroxyl groupings, such as the glucopyranosides, are oxidised more slowly (cf. " Organic Reactions ", Vol. 2, p. 353, Wiley, New York). The presence of *cis*-hydroxyl groups, however, is not the only factor which decides the rate of formation of formic acid This is dependent also upon the rate of oxidation of the α-hydroxy-aldehyde, which is a comparatively slow reaction. Furthermore, the methylglycosides of maltose and cellobiose, both of which contain glucopyranose residues only, give formic acid at different rates. For example, methylmaltoside yields 1 mol. of formic acid in about 100 hours, whilst the cellobioside gives only 0·86 mol. in this time and 300 hours are necessary for the liberation of one equivalent of acid. This low yield of formic acid from methylcellobioside (X) could be explained by the intermediate formation of a lactol grouping such as (XI) which on further oxidation would yield not free formic acid but a formyl ester (XII). An examination of the model of the methylcellobioside molecule (and similarly of the sucrose molecule) reveals that the hydroxyl groups are so situated in space that lactol formation can readily take place, whereas the configuration of the groups in the molecule of the methylmaltoside is unfavourable for the formation of lactol groupings.

This oxidation of an intermediate lactol with formation of an ester of formic acid instead of free formic acid receives support from a study of the oxidation of mannitol. The oxidation of this hexahydroxy-alcohol yields two mols. of formaldehyde and formic acid. A quantitative

estimation of the formic acid, however, showed that the yield of this acid reached 95% of 4 mols. under the standard conditions and was not complete until the reaction had proceeded for about



400 hours. The primary oxidation of mannitol (XIII) may occur in one of five places in the molecule. It may give either (*a*) a pentose, (*b*) a tetrose, or (*c*) glyceraldehyde as the main reaction product. Other things being equal, reactions (*a*) and (*b*) will predominate. The pentose and tetrose which result from this oxidation may exist either as *aldehydo*-sugars or as ring structures. Should oxidation of the ring structure or lactol form occur, then a formic ester will be produced with a consequent lowering in the yield of titratable formic acid. On standing in water these esters will undergo hydrolysis and further oxidation, and a consequent slow rise in the titration figure will result. This formation of ester groupings from the oxidation of a lactol is small, since in no case have we encountered a yield of formic acid of less than 90% of the theoretical in our experiments on the oxidation of the sugar glycosides under the standardised conditions. In the oxidation of the reducing sugars, however, low yields of formic acid are produced when using our standardised reaction conditions. In certain instances, however, side reactions, with the formation of iodine, explain, in part, the low yields of formic acid produced in this complex oxidation reaction.

Experimental.

Oxidation with Sodium or Potassium Periodate. General Procedure.—Reactions were carried out in diffused light at 15—20° (with shaking when potassium periodate was used), in 500 c.c. stoppered bottles which had been cleaned with chromic acid and steamed out.

Sodium metaperiodate was prepared from sodium paraperiodate by recrystallisation from nitric acid. It contained no free acid since on addition of excess of ethylene glycol to its aqueous solution, sodium iodate neutral to methyl-red was formed. The ethylene glycol required was purified by distillation over solid potassium hydroxide. It was neutral to methyl-red.

The material (sufficient to give *ca.* 10 mg. of formic acid) was weighed out into a 500 c.c. stoppered bottle and dissolved or suspended in water. Excess of approximately 0·3M-sodium periodate was then added, followed by potassium chloride (5 g.) (if the oxidation was to be carried out with potassium periodate) and water to the requisite volume (usually 120 c.c.). Portions (either 10 or 20 c.c.) of the solution were withdrawn at intervals, excess of ethylene glycol was added to remove the residual periodate, and the formic acid was then determined either by titration with 0·01N-sodium hydroxide, using methyl-red as incidator, or after addition of potassium iodide by determination of the liberated iodine with 0·01N-thiosulphate. Blank experiments showed that under these conditions sodium iodate, formaldehyde, and ethylene glycol do not interfere with the estimation of formic acid and that the formic acid produced is not destroyed.

Oxidations with sodium metaperiodate. (1) α-Methylglucoside (87 mg.) was dissolved in water (115 c.c.), and sodium periodate solution (15 c.c.; 0·2M) was added. Portions of the solution (25 c.c.) were removed at intervals; ethylene glycol (approximately 0·1 c.c.) was added, and the formic acid titrated after a few minutes with 0·01N-sodium hydroxide. Titres: 6·3 c.c. (6 hours), 8·3 c.c. (22½ hours), 8·35 c.c. (47 hours), equivalent to 99·5% recovery.

After completion of oxidation a portion of the solution (50 c.c.) was extracted continuously with ether until the final extracts were neutral. The extracts were concentrated, and then required 18·8 c.c. of 0·01N-sodium hydroxide equivalent to 108% recovery of formic acid. The sodium formate obtained on evaporation of the neutralised solution gave, on heating with sulphuric acid (*d* 1·84) in a gas analysis apparatus, carbon monoxide corresponding to 99·4% of the theoretical yield of formic acid. The three different methods of determination gave reasonably concordant results, and the determination *via* carbon monoxide proves that the acid produced is formic acid.

(2) β-Methylmaltoside monohydrate (489·5 mg.) was dissolved in water (140 c.c.), and sodium periodate solution (60 c.c.; 0·275M) was added. The solution was left at 15—20°, samples (10 c.c.) were

withdrawn at intervals, ethylene glycol was added, and the solution titrated with 0·01N-barium hydroxide. At the same time the consumption of periodate and the change of optical rotation were determined.

Time (hours).	$[\alpha]_D^{19.5°}$.	Formic acid produced (mols. per mol. of maltoside).	Consumption of periodate (mols. per sugar residue).
0	+176°	nil	nil
0·6	− 33	0·48	2·58
1·5	− 45	0·65	2·84
5·1	−100	0·89	2·97
30	− 62	1·19	3·61

The reaction was not finished after 30 hours, since formic acid continued to be produced. The last figure for formic acid (1·19 mols.) is considerably more than the theoretical value (1·0 mol.). High results were obtained also with other disaccharide derivatives, and the occurrence of over-oxidation even under carefully controlled conditions rendered the use of sodium periodate unsatisfactory for quantitative work, except when the reagent was employed in very slight excess.

Oxidations with potassium metaperiodate. (3) Mannitol was oxidised to show that formaldehyde and formic acid were unaffected by the oxidation conditions used above. The mannitol (28 mg.) was dissolved in water (110 c.c.) containing sodium periodate (11 c.c.; 0·236M) to which potassium chloride (5 g.) had been added. Titration of the liberated acid after addition of excess of glycol was made at intervals on portions of the solution. Found, mols. of formic acid per mol. of sugar: 3·56 (3 hours), 3·60 (98 hours), 3·80 (145 hours), 3·80 (194 hours), 3·88 (266 hours), 3·96 (338 hours), 3·96 (580 hours). Two other experiments gave similar results.

(4) α-Methyl-*d*-glucoside (81 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium periodate (10 c.c.; 0·27M) was added. At intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of methylglucoside: 0·28 (1 hour), 0·43 (5½ hours), 0·69 (23 hours), 0·90 (51 hours), 0·91 (72 hours), 0·94 (170 hours), 0·97 (220 hours), 1·04 (460 hours).

(5) α-Methyl-*d*-galactopyranoside (111 mg.) was dissolved in water (110 c.c.), and potassium chloride (5 g.) and sodium periodate (10 c.c.; 0·236M) were added. The bottle and contents were then shaken and at intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of methylgalactoside: 0·95 (67 hours), 0·99 (120 hours), 1·01 (192 hours), 1·01 (230 hours), 1·03 (500 hours).

(6) α-Methyl-*d*-mannopyranoside (82 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium periodate (10 c.c.; 0·282M) was added. The bottle and contents were shaken and at intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of methylmannoside: 0·67 (68 hours), 0·85 (121 hours), 0·93 (168 hours), 0·97 (211 hours), 0·95 (289 hours), 0·96 (405 hours).

(7) β-Methyl-*l*-arabopyranoside (89 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium periodate (10 c.c.; 0·236M) was added. The bottle and contents were shaken and at intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of glycoside: 0·97 (47 hours), 0·99 (91 hours), 0·99 (143 hours), 1·01 (165 hours), 1·01 (215 hours), 1·02 (281 hours), 1·04 (362 hours), 0·99 (506 hours).

(8) α-Methyl-*d*-xylopyranoside (107 mg.) was oxidised as described for the oxidation of the arabinoside. Found, mols. of formic acid per mol. of glycoside: 0·71 (47 hours), 0·89 (91 hours), 0·93 (143 hours), 0·98 (165 hours), 0·98 (215 hours), 1·00 (287 hours), 1·02 (362 hours).

(9) β-Methylmaltoside monohydrate (249·7 mg.) was dissolved in water (70 c.c.) containing potassium chloride (2·5 g.), and sodium periodate solution (30 c.c., 0·275M) was then added. The whole was placed in a stoppered bottle which was continuously shaken. At intervals samples (10 c.c.) were withdrawn, and after addition of ethylene glycol the formic acid was titrated with 0·01N-barium hydroxide using methyl-red as indicator. Found, mols. of formic acid per mol. of maltoside: 0·13 (1¾ hours), 0·53 (20 hours); 0·75 (43 hours), 0·83 (66 hours), 0·92 (91 hours), 1·01 (163 hours); 1·03 (192 hours), followed by a very slow rise. One mol. of formic acid is liberated per mol. of maltoside in 150 hours.

(10) β-Methylcellobioside (106 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium periodate (10 c.c., 0·28M) was added. At intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol of cellobioside: 0·69 (43 hours), 0·84 (91 hours), 0·87 (144 hours), 0·88 (185 hours), 0·89 (244 hours), 0·91 (291 hours).

(11) Amygdalin (173 mg.) was oxidised as described above (cf. Courtois and Valentino, *Bull. Soc. Chim. biol.*, 1944, **26**, 469). Found, mols. of formic acid per mol. of glycoside, 1·05 (47 hours), 1·28 (91 hours), 1·53 (143 hours), 1·83 (165 hours), 1·90 (215 hours), 2·03 (287 hours), 2·08 (362 hours), 2·00 (506 hours).

(12) Sucrose (111 mg.) was dissolved in water (105 c.c.) containing potassium chloride (5 g.), and sodium periodate (15 c.c.; 0·307M) was added. At intervals samples (20 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of sucrose: 0·84 (170 hours), 0·84 (195 hours), 0·92 (267 hours), 0·91 (315 hours), 0·91 (431 hours).

(12) Zinc borneol glucuronoside (110 mg.) was dissolved in water (105 c.c.) containing potassium chloride (5 g.), and sodium periodate (5 c.c., 0·285M) was added. At intervals samples (20 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of compound: 2·34 (162 hours), 1·74 (188 hours), 1·40 (210 hours), 1·06 (307 hours). The solution became brown owing to the separation of iodine and oily droplets of bornyl formate were observable.

(13) The methyl ester of α-methylgalacturonoside (21·95 mg.) was dissolved in water (200 c.c.) containing potassium chloride (5 g.), and sodium periodate (5 c.c., 0·308M) was added. At intervals samples (20 c.c.) were withdrawn and titrated. Found, mols. of formic acid per mol. of sugar: 0·22 (3 hours), 0·79 (25 hours), 1·23 (49 hours), 1·70 (74 hours), 2·18 (117 hours), 3·74 (171 hours), 4·22 (219 hours), 4·22 (335 hours).

(14) Lactose hydrate (94 mg.) was dissolved in water (100 c.c.) containing potassium chloride (5 g.)

and sodium periodate (20 c.c., 0·2M) was added. At intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of acid per mol. of lactose hydrate : 1·85 (42 hours), 2·87 (95 hours), 3·27 (142 hours), 3·68 (185 hours), 4·13 (263 hours), 2·41 (427 hours) (iodine was liberated at this stage with a resultant fall in acid titre).

(15) Lactal (160 mg.) was dissolved in water (100 c.c.) containing potassium chloride (5 g.) and sodium periodate (20 c.c.; 0·28M) was added. At intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of acid per mol. of lactal : 0·87 (42 hours), 1·00 (95 hours), 1·09 (142 hours), 1·17 (185 hours), 1·50 (263 hours), 2·06 (427 hours).

(16) α-Methyl-*d*-mannofuranoside (106 mg.) was dissolved in water (110 c.c.) containing potassium chloride (5 g.), and sodium periodate (10 c.c.; 0·236M) was added. At intervals samples (10 c.c.) were withdrawn and titrated. Found, mols. of acid per mol. of glycoside : 1·5 (47 hours), 2·0 (91 hours) (at this stage iodine began to be liberated and the alkali titre fell), 0·43 (143 hours) (all the periodate had then been converted into iodate and iodine).

(17) Oxalic acid dihydrate (27·02 mg.) was dissolved in water (115 c.c.) containing potassium chloride (5 g.), and sodium periodate solution (5 c.c.; 0·3072M) was added. At intervals samples were withdrawn and titrated. Found, mols. of oxalic acid : 0·43 (95 hours), 0·34 (120 hours), 0·31 (144 hours), 0·18 (430 hours), 0·12 (602 hours).

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