#### THE MECHANISM OF PERIODATE OXIDATION OF SIMPLE SUGARS

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## THE MECHANISM OF PERIODATE OXIDATION OF SIMPLE SUGARS

By

John Christensen

#### A THESIS

#### Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

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#### ABSTRACT

The exidation of <u>D</u>-glucose by periodate has been investigated with respect to mechanism. A spectrophotometric method of following the change of concentration of periodate by measuring the optical density at 222.5 millimicrons has been developed. The mechanism of the periodate attack on <u>D</u>-glucose has been found to consist of a small ensure of random attack on anyclic glucose accompanied by exidation of the cyclic form to give 2-formyl glyceraldehyde ester. This ester then slowly hydrolymes as the rate-determining step in the reaction, followed by further exidation of the glyceraldehyde. A similar reaction has been found to take place with D-Arabinose.

The reaction of 1,3-diketones with periodate has been investigated and four 1,3-diketones have been found to react (acetyl acetone, 1,1-dimethylcyclohexan-1,3-dione, 3-methyl-2,4-pentadione and 1,4diphenyl-1,3-butadione). These involve both cyclic and acyclic compounds. The reaction has been found to produce no formic acid or formaldehyde, but it does produce carbon dioxide from unsubstituted 1,3-diketones. The reaction is more rapid with periodic acid than with sodium periodate. A mechanism for the reaction has been proposed. The stoichicmetric ratie of periodate to unsubstituted 1,3-diketones has been found to be about four to one.

The reaction of periodate with <u>D</u>,<u>L</u>-glyceraldehyde has been investigated. In reactions involving excess glyceraldehyde and in

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which final production of formic acid and formaldehyde are determined analytically, a method of calculating glycolaldehyde and glyoxal production as well as glyceraldehyde consumption has been devised. The method has been applied to the task of finding the relative rates of attack on the carbonyl-carbinol bond  $(O_1-O_2)$  and the glycol bond  $(C_2-C_3)$  in glyceraldehyde. The rate of the first has been found to exceed that of the latter by at least a factor of five.

A decrease in concentration of the glyceraldehyde-periodic acid reaction mixture produces an increase in rate of the reaction, particularly at the initial stage. A decrease in pH below that produced by the periodic produces a decrease in rate during the first stage. Addition of equivalent amounts of sodium hydroxide produces a decrease in the final stage of the reaction.

The depolymerization of the dimer of <u>D</u>,<u>L</u>-glyceraldehyde has been investigated at  $0^{\circ}$  C and the equilibrium constant has been found to be 1.0.

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#### INTRODUCTION

The Malaprade (1-3) reaction, which involves cleaving a chain between two carbon atoms, each of which carries either a hydroxyl, a carbonyl or an smino group, has been used extensively for determining the structure of unknown compounds and for quantitative analytical determinations. These uses are based on the assumption that a periodate reacts quantitatively with the 1,2 groups listed and that a reaction does not take place with other groupings. The reaction can only remain dependable as an analytical tool if the conditions under which stoichiometric reactions take place are known and can be utilized. This requires a knowledge of the mechanisms of the reactions.

In the use of periodates for either of the purposes mentioned, the progress of the reaction is usually followed either by determining the decrease in periodate or the increase in some product, or products, such as formaldehyde, formic acid or occasionally some less common product. It is therefore essential that the reaction proceed stoichiometrically.

A search of the literature has disclosed a number of anomalies with respect to the reaction which can invalidate many of the results obtained by the use of periodate and also a number of disagreements between investigators on matters of fundamental importance. It was therefore decided to investigate further the mechanism of the reaction between periodic acid and the simple sugars.

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Many contradictory statements with regard to the influence of various factors on the reaction have been noted. With respect to the simple glycols. Price and Kroll (4) found that with ethylene glycol and pinacol the reaction was more rapid in acid than in alkaline solution and that the reaction with pinacol was a general acid-base satalyzed reaction, although the variation of rate constant with pH produced a complex surve. Price and Knell (5) found the rate constants for ethylene glycol essentially independent of pH below 7 and falling off rapidly above pH 7. Heidt, Gladding and Purves (6) reported the reaction with ethylene glycol as more rapid at lower pH's, falling off up to pH 5, then rising to approximately pH 8 and assuming very low values in more alkaline media. Duke and Bulgrin (7) predicted the rate for ethylene glycel should be independent of pH in the range pH 3-7 because increased acid catalysis was offset by decreased ionisation of the periodic acid. Buist and Bunton (8) found the maximum rate seefficients for the ethylene glycol-periodic asid reaction at pH 4 with a decrease on both sides and a very sharp decrease above pH 7. Taylor, Soldano and Hall (9) for most glycols describe a broad pH range between 2.5 and 6.0 producing maximum reaction rates and a decrease at either end.

For glucose, Hughes and Nevell (10) found that periodate was consumed faster in buffered acid and in neutral solution than at pH 9.7. Speck and Forist (11) found the reaction with glucose slower in acid than in bicarbonate buffer. Fleury, Courtois and Rieder (12) oxidised aldohexoses more readily at pH 6.5 using a phosphate buffer than at

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pH 1.6, but the polyhydroxyl compounds, mannitol and sorbitol were oxidized more rapidly at pH 1.6 than at 6.5.

Greville and Northcote (13) with di, tri- and tetra-Q-methylated glucoses detected a decrease in rate with decrease in pH, but a few compounds which did not have the expected structure for normal reaction showed abnormal reactions and oxidized active hydrogens faster at pH 5 than at pH 7.5. Neumuller and Vasseur (14) with maltose, melibiose, methyl- $\alpha$ -<u>D</u>-glucoside, yeast, glucan and dextran found a second or overoxidation stage having a minimum rate at pH 3 and 4. Jeanloz (15) using methylated sugars found rates faster in weakly alkaline solution. Bell (16) with methylated sugars obtained best results at pH 7.5.

Others who found more rapid rates in the alkaline region were Reeves (17) with monosaccharides in sodium bicarbonate buffer; Grangaard, Gladding and Purves (18) with starch and cellulose; Jeanlos and Forchielli (19) with starch and hyaluronic acid; Lindstedt (20) with yeast mannan; Sprinson and Chargaff (21) with tartaric acid; Huebner, Ames and Bubl (22) with various organic compounds containing active hydrogen; Van Slyke, Hiller and MacFadyen (23) with hydroxy lysine; McCasland and Smith (24) with aminocyclanols; Jeanlos and Forchielli with chitin (25) and gluccsamine (26).

With respect to the effect of other ions on the periodate reaction, Taylor, Soldano and Hall (9) discovered that the rate of reaction of periodates in the presence of neutral salts is increased proportionally to the concentrations of the salts. Buist and Bunton (8) found rate constants dependent on ionic strength and acidity and Huebner, Ames

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and Babl (22) found considerable variation at the same pH with different buffers. A number of investigators have reported greatly increased rates and even some abnormal effects from the use of phosphate buffers. Fleury, Courtois and Bieder (12) with aldeses and Bell and Greville (27) with 3,4-di-Q-methyl-D-glucose report increased rates with phosphate. The latter also report formation of formaldehyde very late in the reaction and an early production of formic acid.

Bell (16) with 2,3- and 2.6-di-0-methyl glucose observed overexidation and production of carbon diexide. Bell, Palmer and Johns (28) noted that when the periodate to phosphate ratio was increased 3-O-methyl and 2,3,4-tri-O-methyl glucoses produced more formaldehyde and less formic acid and vice versa. They also noted an evercensumption of periodate. The total yield of formaldehyde and formic acid was approximately one mole per mole of the trimethyl glucese, so that when one increased the other apparently decreased. Lindstedt (20) using yeast mannan, catalysed by phosphate, found increased rates and everoxidation even in acid solution and showed that two thirds of the mannan molecule was exidized to carbon dioxide at pH 7 and 50° C when catalyzed by phosphate. With a variety of carbohydrates and derivatives he obtained overoxidation and in some cases carbon dioxide. Greville and Northcote (13), on the other hand, found no acceleration due to phosphate catalysis on methylated glucoses. In fact, they found the rate even faster when the solution was adjusted to the desired pH by MaCH rather than by phosphates.

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A number of workers have reported pH changes during the course of the reaction. Buist and Banton (8) found a sharp drop in pH when an excess of glycol was mixed with periodic acid and which they assumed to be due to the greater acidity of the intermediate complex between the two compounds. A further allow decrease followed, which was evidently due to the decomposition of the complex and the formation of the relatively strong iodic acid. At pH 5 they found a sharp decrease in pH followed by a allow increase. Price and Enell (5), also using ethylene glycol, over the ranges pH 1-7.5 and 10-11.5 found no changes of pH during the reaction, but they did find a change in the range 7.5-10. McCasland and Smith (2h) using aminocyclanels found a rise of 1-3 pH units during the reaction, but this was apparently due to the ammonia liberated.

Greville and Northcote (13) observed that in some methylated sugars the rate of exidation increases with the degree of methylation and that ther ate is not dependent on the number of actual or potential hydroxyl groups, but rather on the number of methoxyl radicals. For instance, the consumption of periodate by 2,3,4,6-tetra-Q-methyl-Bglucose reaches 14.5 moles, whereas, theoretically, 14 moles are required to exidise it completely to formic acid and 24 meles to carbon diexide.

Several investigators have reported inconsistencies in the use of arsenite for the determination of periodate in the solution. This is the method of Fleury and Lange (29). Hughes and Nevell (10) attributed the inconsistencies to the slow reduction of the intermediate complex

between periodate and the glycol (glucose in this case). If this is true, the arsenite method would not measure the periodate which had already formed the intermediate complex, but which had not yet exidized the glycol. They also found that the solutions after reaction with excess arsenite liberated considerable iodine upon standing. They assumed this to be the result of a slow reaction of the intermediate complex with iodide ions in the slightly alkaline solution. Such a result would naturally indicate a correspondingly larger consumption of periodate as compared with formic acid produced.

Speck and Forist (11) also observed that periodate consumption as measured by arsenite reduction appeared to proceed considerably faster than formaldehyde formation.

Van Slyke, Hiller and MacFadyen (23) obtained a similar discrepancy in working with hydroxylysine, but they attributed the results to the fact that their reaction running in acid solution was progressing slowly. Then upon adding sodium bicarbonate in order to produce a favorable pH for reaction with arsenite, the reaction with periodate took place instantly. They found the glycol-periodate reaction in sodium bicarbonate buffer to be almost instantaneous.

Schwars (30) explains the apparent high periodate consumption, when titrated by the argenite method, as being a result of exidation of active hydrogen or of iodination by free iodine liberated in the alkaline solution. This reaction might be illustrated as follows:



<sup>1,4-</sup>anhydromannitol

In this connection it will be recalled that free iodine in an alkaline solution substitutes for alpha hydrogen in a manner similar to the iodoform reaction when aldehydes or ketones are present in the reaction mixture.

Overoxidation, which may appear merely as a slight overconsumption of periodate without apparently diminishing the analytically determined products or which may in its extreme case involve oxidizing most of the products of the normal reaction to c arbon dioxide and water, appears to be a common phenomenon if one can judge by the number of references to it in the chemical literature. Overoxidations produced by phosphate catalysis has already been considered.

Brown, Dunstan, Halsall, Hirst and Jones (31) conducted their experiments on end-group determination in polysacoharides specifically to determine conditions for avoiding overoxidation. Mitchell and Percival (32) working with several methylated fructoses found overconsumption of periodate and less than theoretical yields of formaldehyde in nearly every case. Miyada (33) on the other hand obtained practically theoretical formaldehyde yields and periodate consumption with 3,4,6-di-O-methyl-D-fructore. Doerschuk (34) found in an exidation of glycerol-1-C<sup>14</sup> by periodate 0.25% of the C<sup>14</sup> present as formic acid. We attributed this to enclisation occurring in the glycol aldehyde which resulted from the first stage of the reaction. This may more likely have been the result of overoxidation of formaldehyde by the periodic acid.

Jeanlos and Forchielli (19,26) concluded from their studies on polysaccharides and glucosumine that overoxidation is a function of temperature as well as of pH and that it can be eliminated by working at  $5^{\circ}$  C or lower. They also recommended a pH of h-5 for minimizing overexidation.

Methylated sugars appear especially subject to overexidation as previously noted in reference to the work of Greville and Morthcote (13), Bell (16) and Bell, Palmer and Johns (28).

The effect of light on periodate solutions has been carefully investigated by Head and Standing (35). They found that periodate solutions will reduce to iodate and finally to free iodine upon sufficient exposure to light. Head and Hughes (36) found that daylight catalysed periodate reactions with simple organic compounds such as formic acid, formaldehyde, glycollic acid, methyl alcohol, exalic acid, ethyl alcohol, acetaldehyde. With a large excess of periodate in bright sunlight it was found possible to completely exidise formic acid in one day; otherwise a matter of days was required to produce significant exidation. Head (37), investigating the effect of daylight on periodate exidation of glycosides and cellulose, found definite · · · • • • • •

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catalysis by light. However, in the course of two hours no appreciable difference was observed between reactions run in the dark and in the daylight. In fact, in six hours the difference was small, but in terms of days it became very great. He continued his observations over periods as great as 120 days. The results of exidations extending over this period of time are quite meaningless in view of the frequent observation of overoxidation by periodate.

Head also found that a great excess of periodate tends to increase everoxidation, so much so that the curve shows no differentiation between the original Malaprade reaction and overoxidation. He also found that free iodine is produced when there is insufficient periodate.

Periodate is also known to oxidize certain sulfur containing compounds. Micolet and Shinn (38) found that periodic acid reacted with methionine and cystine, apparently by oxidation of the sulfur. Bonner and Driske (39) have shown that phenyl-B-D-thioglucopyranoside is exidized to a sulfone, in addition to the normal glycol cleavage, and that this reaction produces iodine. This iodine oxidized active hydrogen to a hydroxyl group, as shown in equation (1), thus making further reaction with periodate possible.

A number of workers have reported the reaction of periodate with active hydrogen, either with or without carbon dioxide production. The findings of Schwarz (30) have already been mentioned. Neumaller and Vasseur (14) reported a second stage of oxidation with maltose, similar to the example in equation (1), in which active hydrogen was oxidized to a hydroxyl group capable of further oxidation with periodate.

Head and Hughes (40) postulate essentially the same results on intermediate compounds in the oxidation of cellobiose. This is illustrated as follows:

(2) OHC-C-CIO OHC-C-CHO 
$$\xrightarrow{2HIO}_{R}$$
 R-O-C-CIO + HCOCH

Huebner et al. (41) reported a similar oxidation of active hydrogen in bornyl glucoside and of the hydrogen on carbon 1' in 2(1',4'anhydro-D-xylo-tetrahydroxy butyl) benzimidazole.



Neuberger (42) also reported a similar phenomenon in connection with periodate oxidation of ethyl glucosamate (together with the appearance of free iodine) as shown in equation (4)



Potter and Hassid (43) in end group determinations of starch also report exidation of the active hydrogen in the formic acid ester of tartronaldehyde. Halsall, Hirst and Jones (44) found that when an active hydrogen was exidized, such as in a methyl furaneside, iedine was formed. They also found that when methyl hexa- or pentapyramesides were exidized that normal products were obtained.

Huebmer, Ames and Bubl (22) specifically checked the reactions between certain compounds containing active hydrogen with periodate and found that a reaction took place with malonic acid, tartronic acid, mesoxalic acid, menoethyl malonate, malic acid, digitoxose, acetoacetic acid, alpha-ethylmalonic acid, oxalacetic acid, acetone dicarboxylic acid, citric acid, lactic acid, pyruvic acid, 1,4-anhydrosorbitol and ethyl oxomalonate. Many of these produced carbon dioxide in the reaction. Some were very rapid, as for example malonic acid using 3.01 equivalents of periodate; acetone dicarboxylic acid, 4.5 equivalents and acetoacetic acid, 2.5 equivalents of periodate in an hour. Significantly, they reported no reaction with acetyl acetone, because it gave off no carbon dioxide. These reactions were run at  $21^{\circ}$  C in a solution 0.04-0.06 M with respect to the substance being oxidized and containing from 1.2 to 1.6 times the molar equivalent of sodium metaperiodate theoretically necessary for complete oxidation.

Sprinson and Chargaff (21), investigating the periodate oxidation of active hydrogen, proposed the following reaction with malonic acid:

(5) HOOC-CH<sub>2</sub>-COOH 
$$IO_{4}$$
 HOOC-CHOH-COOH  $IO_{4}$  OHCCOOH +  $CO_{2}$   
 $IO_{4}$   $IO_{4}$   
 $CO_{2}$  + HCOOH

They found that although these reactions are slower than the normal Malaprade reaction between two groups consisting of carbonyls or hydroxyls, the reactions nevertheless are reasonably rapid and must be taken into consideration in the use of the Malaprade reaction. For instance, tartaric acid consumed from one to three moles of periodate in less than one hour, the amount of periodate depending on the concentration of periodate, pH and temperature.

A most interesting observation was made by Courtois and Joseph (45) who reported a slow periodate reaction with dimedone, a 1,3diketone. This substance consumed 2.67 moles of periodic acid per mole of dimedone in 96 hours.

Wolfrom and Bobbitt (46) have recently obtained reactions with a mamber of cyclic 1,3-diketones and have proposed a mechanism for the reactions. They, however, reported that acyclic 1,3-diketones did not react.

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Fleury and Boisson (47), Nicolet and Shinn (38) and Khouvine and Arragon (48) all attribute the formation of carbon dioxide to periodate cleavage of glyoxylic acid. The first of these groups of workers reports that glyoxylic acid is quantitatively transformed into formic acid and carbon dioxide in 24 hours. However, this presumably takes place at the temperature of a hot water bath, which is definitely above the temperature at which reactions of analytical significance may be run.

Therefore, in addition to the usual Malaprade reaction with periodates, at least two other types of exidation seem to exist: (1) Oxidation of so-called active hydrogen. (2) Cleavage of a carboxyl group from a carbonyl group, or to a lesser extent from a carbinol group. A knowledge of these reactions which involve unusual periodate exidations is of great value in analytical work involving periodate exidation of carbohydrates, so that periodate consumed in these reactions will not be attributed to the usual Malaprade reactions.

In addition to these reactions there are others which are more difficult to classify. Nicolet and Shinn (38) report a slow reaction with a variety of amino acids containing only amino and carboxyl groups. This perhaps can be classified under type (2) above, inasmuch as the amino group would be susceptible to hydrolysis.

The reactions of periodate on simple organic compounds such as methyl and ethyl alcohols, as reported by Head and Hughes (36), would also be difficult to classify as one of these two types. Another

reaction difficult to classify would be the one reported by Ramachandra and Sarma (49) of the oxidation of 1,2-dihydroxy-3-anthraquinone sulfonate, which is the same product produced by mild oxidising agents such as alkaline ferricyanide.

With respect to the kinetics of the reaction between periodate and the glycols, Price and Kroll (4) worked out rate constants on the basis of a second order reaction for pinacol. Price and Knell (5), in addition worked out second order rate constants at various pH's for ethylene glycol and for cis- and trans-cyclohexene glycols.

Duke (50) was able to demonstrate for the first time by working at relatively high glycol concentrations that the kinetics of the periodate-glycol reaction are compatible with the mechanism in which formation of a periodate-glycol complex is rapid and reversible, whereas transformation of this complex to iodate and formaldehyde is the rate determining step.

Periodate + glycol complex formaldehyde

Taylor (51) expanded Duke's work and found that the rate constants for the second order reaction increased as the reaction proceeded, especially at low temperatures and equal concentrations. Upon increasing the glycol to periodate ratio, the rate constants decreased markedly.

Duke and Bulgrin (7) have worked out a series of rate constants for the methylated ethylene glycols in which from zero to four methyl groups were substituted for the hydrogens on ethylene glycol. They drew the conclusion that the mechanism involved an active intermediate and that in all except the tetramethylated compound (pinacol) the rate determining step was the disproportionation of the complex. In the case of pinacol the formation of the complex was the rate determining step. This was assumed to be due to the steric hindrance of the methyl groups.

Buist and Bunton (8) have worked out a series of rate constants as affected by pH and have drawn the conclusion that the attacking species of periodate ion is monovalent. This ion could be either  $IO_{ij}$  or  $H_{ij}IO_{ij}$  with the former as the more logical choice. Taylor, Soldano and Hall (9) have given evidence from the pH effects that the attacking species must be a monovalent ion in that the calculated pH-rate curve based on a monovalent ion agrees very well with the experimental curve. However, these calculated rates when applied to pinacol and some carbohydrates do not correlate with experimental data.

Criegee, Kraft and Rank (52) early applied the theory of a lead tetraacetate ester intermediate with glycols to the periodate complex. This idea that the intermediate is an ester of the general structure



has been quite generally accepted by workers in the field. Heidt, Gladding and Purves (6) have attempted to give a mechanism for the formation of the intermediate in terms of molecular models.

Smith and Duke (53) have given a probable electronic mechanism for the reactions involving the cleavage of the carbon chain.

With respect to the method of attack by the periodate ion upon the glycol grouping, Frice and Knell (5) have postulated a back side approach of the periodate ion and in which the reaction forming the intermediate complex takes place in two stages. If the last stage is assumed to be the rate controlling step it would agree with the data presented by a number of workers, namely that the cis-glycol grouping reacts faster than the trans-glycol. Price and Knell (5) found this to be true in the case of the cis- and trans-syclohexene glycols. Floury, Courtois and Bieder (54) in comparing the rates of the reaction of periodate on aldoses and polyalcohels found the following relative reactivities:

Mannitel > Sorbitel > Mannese > Galactose > Glucose This arrangement corresponds with an arrangement in terms of decreasing numbers of adjacent carbon atoms with the same configuration.

McCasland and Smith (24) experimenting with aminocyclanols found the cis- advantage in reaction rates much less pronounced in the case of reaction with periodate than with lead tetraacetate. With cis-2aminocyclopentanol the reaction was four times as fast as with transcompound, but with cis-2-aminocyclohexanol the distinction was not pronounced. In some cases the trans-compound even reacted faster.

Certain ring structures have been used to demonstrate cis- transeffects. Dimler, Davis and Hilbert (55) found that  $\beta$ -1,6-anhydro-Dglucofurances (I) does not react with periodate and Alexander, Dimler and Mehltretter (56) similarly found that  $\alpha$  -1,6-anhydro-D-galactofurances (II) does not react either.

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The assumption has been that it is because of the trans- positions together with the two locked rings which effectively prevent any isomerisation. Klosterman and Smith (57), carrying this idea farther, synthesized the compounds L-threitan (III) and erythritan (IV) which both reacted with periodate. Nevertheless the L-threitan reacted the slowest, particularly with periodic acid. Both of them reacted slower with the periodic acid than with the sodium periodate, and with the acid the difference between them was more pronounced.

Jackson and Hudson (58), on the other hand, prepared 1,6-anhydro-B-glucopyranose (V) which reacted with the periodate. This compound also has a double ring structure and trans- grouping of hydroxyls and yet undergoes a reaction. On this basis Greville and Northcote (13) are of the opinion that it is not the trans- configuration that interferes with the reaction. However, they apparently do not take into consideration the fact that in (V) the ring system which carries the hydroxyl groups is six-membered, whereas in (I-IV) the corresponding rings are five-membered. The six-membered ring is more packered and permits greater movement of the atoms than the more rigid and planar five-membered rings as was observed in the case of McCasland and Smith (24).

Smith (59) found that the 1,4-lactone of glucosaccharic acid (VI) did not react with periodic acid in one hour, whereas the glucosaccharo-3,6-lactone (VII) reacted readily with periodic acid. Again the hydroxyl groups are on a rigid five-membered ring, which could prevent the isomerisation of trans- groups.



VI

VII

Taylor (51) discusses the effects of reactions on compounds (I-IV) and draws the conclusion that the hindrance is caused by a field effect of the oxygen, whereby the oxygen repels the negative periodate ion in a backside approach. This is a possible explanation and in the case of a six-membered ring formed by the bridgehead oxygen, such as in (V), the oxygen being further removed, could have less effect.

In most of the previous discussion of mechanism the emphasis has been mainly on the reaction between periodate and the fundamental glycol groups. Hughes and Nevell (10), however, tackled the problem of the mechanism in the reaction of periodic acid on sugars such as glucose. When they measured the progress of the reaction between sodium periodate and glucose by sodium thiosulfate titration of the iodine liberated from excess potassium iodide in acid solution, they found a fairly close correlation between periodate consumed and formic acid produced, except for a small discrepancy at the "beginning of the reaction," as they call it. Therefore they postulate a very systematic attack upon the glucose molecule, cleaving first the  $C_1-C_2$  bond, then in succession the  $C_2-C_3$ ,  $C_3-C_4$  bonds, etc. to the end of the chain, thus liberating formaldehyde as the final product. This apparently would explain the lag in the production of formaldehyde, which they found according to their data. Their first measurement was taken after 22 hours had elapsed and therefore they really did not have the data at the beginning of the reaction.

In view of the possibility of a random attack of periodate on glucose it seems strange that the attack on the  $O_1-C_2$  bond should be the exclusive mode of attack. One of the reasons for undertaking this research problem was to further check this phenomenon and if possible to determine the actual mechanism or the principles involved in preferential attacks on sugar molecules.

It is a generally accepted fact that only an extremely small percentage of the glucose molecules in a solution are present in the open chain aldehyde form.

To assume that only the open chain form reacts with periodate would seem rather presumptuous in view of the fact that polysaccharides with locked rings react practically as fast as glucose whenever soluble. Consequently, a number of investigators have assumed that possibly a formic ester may be formed in the reaction in the following manner:



Hughes, himself, in collaboration with Head (40) assumed a similar mechanism in the exidation of cellobiose and whereby it would produce a formic acid ester. The hydrolysis of such an ester could be the rate determining step of the reaction.

Morrison, Kupyer and Orten (60) assume the formation of such an ester as the reason for obtaining less than the theoretical amount of formic acid from maltose. Neumuller and Vasseur (14) and Halsall, Hirst and Jones (44) also assumed such an ester. Meyer and Rathgeb (61) found evidence of it in determining terminal end groups in starch.

Barker and Smith (62), by starting with glucose containing a methyl or a methanesulfonyl (mesyl) group substituted on carbon 3 and oxidizing it with sodium metaperiodate, obtained 4-formyl-3-methyl-D-arabinose or 4-formyl-3-mesyl-D-arabinose, respectively. These products were quite positively identified by carbon, hydrogen, methyloxy and formyloxy analyses as well as by physical constants, derivatives and infra-red spectra. Smith (63) later used the periodate reaction



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with 3-mesyl-D-glucose in a preparative reaction in which the formyl ester was used for the preparation of ribose.

Schopf and Wild (64) in oxidizing glucose with sodium periodate found that three moles of periodate reacted rapidly, but only two moles of formic acid were readily available for titration with sodium hydroxide. A third mole of sodium hydroxide was slowly consumed over a period of four days. This was explained by step A in equation (6), which consumed three moles of periodate and produced 2 moles of formic acid plus one mole of the formic ester. This ester them hydrolysed ever an extended period, thus really being the rate determining step in the reaction. They performed this reaction on a preparative scale, isolated the ester, determined its physical properties, hydrolysed it and identified the dimedone derivative as that of glyceraldehyde. When they reacted one mole of sodium periodate with one mole of glucose they recovered two-thirds of a mole of glucose. This seems to indicate that once the ring is cleaved, the bonds in the open chain are more readily available for reaction than the bonds in the cyclic structure.

This mechanism fits the experimental data determined by Hughes and Nevell (10) inasmuch as by the time they made their first analysis of the reaction mixture, a large proportion of the ester would have had an opportunity to hydrolyse, producing almost a mole of formic acid per mole of periodate. The remaining unhydrolysed ester evidently produced the small discrepancy that they noted.

It was the purpose of this investigation to elucidate the mechanism by means of which the periodate attack on glucose produced formaldehyde

at a slower corresponding rate than periodate was consumed. During the course of the investigation the formation of a formyl ester became apparent and the idea was soon corroborated by other investigators who have been cited. To investigate the further attack on glucose and other simple sugars the investigation was extended to include relative rates of reaction with various bonds in intermediate products of glucose oxidation, particularly in glyceraldehyde.

#### EXPERIMENTAL METHODS AND DATA

Apparatus and Materials

Apparatus. A Beckman, model DU spectrophotometer with 1 cm. quarts cells was used for measuring ultra-violet absorption and a Beckman, model B spectrophotometer with 1 cm. Corex cells for measuring absorption in the visible range.

<u>Materials</u>. The periodic acid, iodic acid and sodium iodate used were produced by the G. Frederick Smith Chemical Co. The formic acid, fermaldehyde, sodium bicarbonate, sodium sulfite, glyserol and annonium acetate were C. P. grade. The <u>D</u>-glucose used for these experiments was Mational Baream of Standards dextress. The <u>D</u>-arabinose was procured from General Riochemicals Inc. The acetal was a Rice Co. product.

The chromotropic acid (4,5-dihydroxy-2,7+nephthalenedisalfonic acid) was Matheson Oo., practical grade, and was purified by recrystallisation from 50% ethanol-water mixture. The glycolaldehyde was prepared by the method of Fischer and Traube (65) from dihydrox, maleic acid which was in turn prepared from tartaris acid by the method of Fischer and Feldman (66).

The asetyl acetome was an Eastman Kodak Co. product. It was redistilled and the fraction beiling in the range 135-136° was collected. Its refractive index at 18.5° was 1.4528. Methyl acetyl acetome was prepared from acetyl acetome and methyl iodide by the method of Sprague, Beckham and Adkins (67). Fhenyl acetyl acetophenome was

prepared from ethyl phenyl acetate and acetophenone by the Claisen reaction as developed by Bulow and Grotowsky (68).

Glyceraldehyde, unless otherwise stated, was prepared by the "Organic Syntheses" procedure (69). The last glyceraldehyde used was a Mutritional Biochemicals Corporation product.

The glyoxal used was a technical grade 30% solution produced by Union Carbide Corporation.

Ultra-Violet Absorption Method of Determining Periodic Acid

In order to determine the progress of periodic acid exidations it was necessary to have an analytical method for following the concentration changes of periodic acid which would not alter the course of the reaction itself as the arsenite method has been known to do. To achieve this, ultra-violet absorption curves of 0.0001 M periodic acid, 0.0001 M iodic acid solutions and mixtures of both which totaled 0.0001 M concentration of acid were plotted with the Beckman, model DU spectrophotometer. In all experimental work fresh periodic acid solutions were made daily.

Interferences. To determine whether formaldehyde, formic acid or the carbon dioxide in ordinary distilled water would interfere by means of their own absorption, a preliminary experiment was performed.

Two 0.0001 M periodic acid solutions were made, one made up with ordinary distilled water and the other with doubly distilled carbon dioxide-free water.

A third 0.0001 M periodic acid solution containing formic acid to the 0.00009 M concentration and a fourth containing formaldshyde to the extent of 0.000018 M concentration were made up. These concentrations of formic acid and formaldshyde are approximately the maximum expected when any reaction between periodic acid and glucose is complete and has been diluted to 0.0001 M concentration, based on the original periodic acid.

The maximum absorption occurred at 222.5 mm, which would then appear to be the logical point for measurement of the optical density of periodic acid solutions. A comparison of optical densities at 222.5 mu gave the following results:

0.0001 M Periodic acid in ordinary distilled water0.9820.0001 M Periodic acid in ordinary distilled water1.020.0001 M Periodic acid in ordinary distilled water1.020.0001 M Periodic acid in doubly distilled OD2-free water0.9960.0001 M Periodic acid with 0.00009 M formic acid0.9600.0001 M Periodic acid with 0.000018 M formaldehyde0.981

Apparently none of these components, carbon dioxide, formic acid or formaldehyde have any appreciable absorption at this concentration. Their effect, if any, is to lower rather than to increase the optical density. Of these, the only one which has an effect beyond the experimental error is that of formic acid.

Dixon and Lipkin (70), who developed a somewhat similar spectrophotometric method for periodate, report that in the light beam of the spectrophotometer formic acid is oxidized by metaperiodate to

carbon dioxide. This could account for the slight decrease in optical density of periodic acid solution containing formic acid. Dixon and Lipkin, however, neglected the absorption of the iodate, which was produced in the reaction. This neglect introduces an error of about 12-13% in the analysis.

Determination of absorption curves. From a 0.0001 M periodic acid solution and a 0.0001 M iodic acid solution, mixtures of the two solutions were made as shown in Table I.

### TABLE I

Periodic Acid Concentration	Iodic Acid Concentration	ML. of 0.0001	M Solution
x 10 <sup>-5</sup>	x 10-5	Periodic Acid	Iodic Acid
9	1	45.00	5.00
8	2	40.00	10.00
7	3	35.00	15.00
6	4	30.00	20.00
5	5	25.00	25.00
24	6	20.00	30.00
3	7	15.00	35.00
2	8	10.00	40.00
1	9	5.00	15.00

MIXTURES OF PERIODIC AND OF IODIC ACIDS

The experimentally determined optical densities are found in Table II and a comparison of the absorption curves of 0.0001 M periodic acid and 0.0001 M iodic acid are found in Figure 1. TABLE II

Iodie Acid 1 × 10-4 0.436 0.342 0.265 0.106 0.528 0.181 0.131 0.083 0.0<u>3</u>5 0.203 0.161 0.051 0.492 0.133 0.163 0.068 0.375 0.322 0.261 0.241 0.201 0.279 0.123 ч <mark>Ч</mark> 0.487 0.450 0.330 0.282 0.089 0.121 0.396 0.370 0.352 0.225 0.168 0.054 n N N N N 0.159 0.453 0.453 0.133 211-0 0.355 0.283 0.209 0.109 0.457 0.447 5 Å Periodic Acid Concentrations\* 0.509 0.465 0.478 0.533 0.533 0.5.0 0.428 0-340 0.252 0.128 791-0 ы Кк 0.1L6 0.1480 0.538 0.590 0.610 0.502 0.294 0.447 0.605 0.581 0.398 0.078 у У С 0.1129 0.572 0.655 0.695 0.69.0 0.665 0.453 0.162 111.0 0.577 1126-0 ы Ч 0.403 وۍل.0 0.610 0.745 0.648 0.723 0.779 0.182 6.13 113.0 0.378 7 × 20 0.378 0.640 0.830 0.198 0.780 0.858 0.725 0.573 0.855 0.418 791.0 0.098 10 × 10 0.372 0.910 0.219 0.507 0.685 0,940 0.795 0.630 0.160 0.844 0.937 ч У У И 0 1718-0 0.505 006-0 0.232 0.75 0.860 0.680 0.493 0.108 1.0 1.02 0.97 222.5 Length 파리 8 Here: 800 210 දි 22 225 230 235 32 R 260

ů OFTICAL DENSITIES OF FIRIODIC ACID AND IODIO ACID MIXTURES AT 25°

Total concentration of periodic acid + iodic acid = 1 x  $10^{-41}$ 



Figure 1. Absorption Spectra of 0.0001 M Periodic Acid and 0.0001 M Iodic Acid.

<u>Summary of possible errors</u>. From the measured optical densities of the original  $1 \ge 10^{-\frac{1}{1}}$  M periodic and iodic acids the optical density of each mixture at each wave length was calculated. These calculated optical densities were compared with the experimental optical densities and the deviation noted. This deviation was labelled plus if the observed optical density was higher than the calculated and minus if it was lower than the calculated optical density. A summary of these deviations is given in Table III.

### TABLE III

Wave-	Co	ncent	ratio	ns of	Peri	odic	Acid	x 10	5	Sum of the
length in ma	9	8	?	6	5	4	3	2	1	Absolute Errors
200	+12	0	+6	-2	+13	+12	-12	-3	-17	77
205	+9	<b>4</b> 6	-45	-48	+10	+14	-4	0	-11	147
210	+7	+10	+13	<b>+</b> 36	+10	+18	-1	+5	<del>-</del> 5	105
215	+7	+7	+13	<del>+</del> 9	+3	+14	+2	+4	-6	65
220	+8	+6	+11	<del>4</del> 9	+4	+7	+2	+6	-5	58
222.5	+4	+6	+7	+6	+5	+3	0	+4	-4	39
<b>2</b> 25	+20	+6	+18	+18	+16	<b>+</b> δ	+11	+7	-1	105
230	+8	+11	+7	<del>\$</del> 8	+7	+5	+5	+5	-3	59
235	<b>+8</b>	+8	+3	+3	+5	+4	+5	+4	0	40
240	+8	+5	<b>+</b> 8	+6	+6	<b>+</b> 6	+3	+3	-1	46
<b>25</b> 0	+5	+4	+5	+3	+5	+4	+4	+2	-1	33
260		+3		+7				+4		

## SUMMARY OF READING ERRORS

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Examination of the sums of the absolute errors indicates that the two lowest sums are at 250 mp and 222.5 mp. The former wavelength has the disadvantage of extremely small increments between the various dilutions. The latter has much larger increments and therefore a greater degree of accuracy. This illustrates the principle that measurements made at absorption peaks usually produce the greatest accuracy. From the optical densities of the original  $1 \times 10^{-4}$  periodic acid and iodic acid concentrations, values were calculated for each of the mixtures. These concentrations are compared with the experimentally determined concentrations calculated from the optical densities of the mixtures in Table IV.

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Actual Concentrations in Moles/Liter	Concentrations Calculated from Optical Densities	Per Cent Error
9.00 x 10 <sup>-5</sup>	9.05 x 10 <sup>-5</sup>	+0.55
8.00 x 10 <sup>-5</sup>	8.06 x 10 <sup>-5</sup>	+0.75
$7.00 \times 10^{-5}$	$7.08 \times 10^{-5}$	+1.14
6.00 x 10 <sup>-5</sup>	6.07 x 10 <sup>-5</sup>	+1.17
$5.00 \times 10^{-5}$	$5.06 \times 10^{-5}$	+1.20
$4.00 \times 10^{-5}$	$3.92 \times 10^{-5}$	-2.0
$3.00 \times 10^{-5}$	$3.00 \times 10^{-5}$	0.0
$2.00 \times 10^{-5}$	$2.04 \times 10^{-5}$	+2.0
1.00 x 10 <sup>-5</sup>	0.95 x 10 <sup>-5</sup>	-5.0

ERRORS IN CALCULATING CONCENTRATIONS OF PERIODIC ACID

It will be seen from Table IV that for all except the very low consentrations of periodic acid the method is good to an error of plus or minus 2%.

Application of the absorption method of determination to  $1.75 \times 10^{-14}$  M sodium periodate solutions. A 1.75 x  $10^{-14}$  molar solution of sodium periodate was made by dissolving 3.990 g. (17.5 millimoles) of periodic acid in 15 ml. of water in a 100-ml. volumetric flask, adding 79.36 ml. of 0.2309 M NaOH solution and diluting to the indicated volume. The resulting solution was 0.175 M with respect to NaIO<sub>4</sub> (or NaH<sub>4</sub>IO<sub>6</sub>). This solution was then diluted to a molarity of 1.75 x  $10^{-14}$ . It was found that sodium periodate required several days of shaking to obtain a solution and even then it did not completely dissolve. Therefore, the relatively soluble periodic acid was used.

A 1.75 x  $10^{-11}$  M solution of sodium iodate (NaIO<sub>3</sub>) was made by dissolving sodium iodate in water. Mixtures of these two solutions were made and their optical densities measured at 222.5 mm and 20° C as given in Table V.

#### TABLE V

Concentrat	ions of	Ratio by	Nole Percent		
Sodium	Sodium	Volume of Periodate	of Solute Which is	Optical	Densities
x 10-4	x 10-4	Solutions	Periodate	Observed	Calculated
1.75	0.00		100	1.72	
1.40	0.35	4:1	80	1.42	1.43
1.05	0.70	312	60	1.14	1.13
0.70	1.05	2:3	40	0.830	0.833
0.35	1.40	1:4	20	0.536	0.538
0.00	1.75		0	0.242	

OPTICAL DENSITIES OF SODIUM PERIODATE-SODIUM IODATE MIXTURES AT 222.5 mm AND 20° C.

Effect of Sedium bicarbonate on absorption of periodic acid. The altra-violet absorption curve for a 0.0002 M solution of periodic acid was constructed by measuring the optical density at various wavelengths.

To determine the interference, if any, from sodium bicarbonate, 1.000 g. of sodium bicarbonate was dissolved in 485 ml. of the above 0.002 M solution and the absorption curve was determined again. The results are shown in Figure 2. It is apparent that the ultra-violet method of periodic acid analysis can not be used on reaction mixtures buffered with sodium bicarbonate. The carbonate group appears to shift the absorption peak toward the shorter wavelengths.

It was found on several occasions that a periodic acid solution, which was treated with an equal number of moles of sodium hydroxide OPTICAL DENSITY



Figure 2. Absorption Spectra of 0.0002 M Periodic Acid With and Without Sodium Bicarbonate.

gave the same absorption as a solution of the same molarity without the sodium hydroxide.

Experimental method. For periodate determination during the progress of a reaction, aliquot samples were removed at intervals, diluted to 0.0001 M (based on the original concentration of periodic acid in the reaction mixture) and the optical density was read at the temperature indicated in the appropriate table.

Oxidation of D-Glucose

### Reaction of 0.025 M periodic acid with 0.0045 M D-glucose.

Equal volumes of a 0.050 M periodic acid solution and a 0.0090 M <u>D</u>-glucose solution were placed in 200 ml. erlemmeyer flasks and at the moment of initiation of the reaction, the solutions were rapidly poured from one flask to another. Samples were taken out as rapidly as possible and alternately for periodic acid analysis and for formaldehyde analysis.

Periodic acid analyses were performed by removing 1.00 ml. aliquot samples of the reaction mixture and diluting to 250 ml. The optical density was then measured at 25° C. and 222.5 mm.

Formaldehyde analyses were made by the method of Speck and Forist (11). Three-milliliter aliquots were removed from the reaction mixture and added to a 10-ml. volumetric flask containing 1 ml. of 0.4 M sodium sulfite. This was diluted to 10.0 ml. Of this resulting solution, 1.00 ml. was taken and added to a 50-ml. glass-stoppered welumetric flask. To this was added 0.5 ml. of 10% chromotropic acid and 5 ml. of 14 M sulfuric acid. The mixture was heated in boiling water for 30 minutes. It was then cooled, diluted almost to volume, cooled again, diluted to volume and then aerated for 30 minutes. The optical density was measured at 570 mm and compared with a previously constructed calibration curve prepared from National Bureau of Standards <u>D</u>-glucose according to directions given by Speck and Forist.

Subsequent chromotropic acid analyses for formaldehyde were made in the same way, differing only in the degree of dilution and the quantity of sodium sulfite used. These two factors were determined by the original concentration of the reaction mixture. The reaction mixture was usually diluted to such an extent as to obtain between 0.03 and 0.10 millimoles of formaldehyde per milliliter of diluted solution. The quantity of sodium sulfite was adjusted in each case to give a slight excess beyond that required to reduce all of the original periodic acid in the aliquot to iodide. In all cases, a one milliliter aliquet of the diluted solution was used for the subsequent calor development with chromotropic acid.

Table VI shows the results of the reaction mixture analyses and Figure 3 illustrates the progress of the reaction graphically.

Another reaction was run in the same way as the preceding, except that periodic acid analyses samples were read immediately instead of being allowed to stand for a time. The results of this analysis are found in Table VII and in Figure 4. A comparison of the two graphs provides definite indication of a continuation of the reaction after dilution.

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## TABLE VI

PERIODIC ACID CONSUMED AND FORMALDEHYDE PRODUCED BY THE REACTION OF 0,025 M PERIODIC ACID AND 0.0045 M D-GLUCOSE AT 25° C.

Original Quantities of Reactants: Periodic Acid, 5.00 Millimoles D-Glucose, 0.900 Millimoles

Perio	iic Acid C	Formaldehyde	Produced	
Time Intervals in Minutes*	Density	Millimoles of Periodic Acid Consumed	Time Intervals in Minutes	Millimoles of Formaldehyde Produced
1.5	0.792	1.40	2,2	0.087
4.25	0.698	1.95	6.7	0.106
7.0	0.670	2.12	8.3	0.140
10.0	0.635	2.32	13.5	0.120
20.2	0.555	2.76	21.7	0.153
31.0	0.500	3.06	33.0	0.160
40.0	0.475	3.23	42.6	0.167
55.2	0.434	3.50	57.5	0.173
87.0	0.399	3.71	90.0	0.193
119.0	0.394	3.74	122.0	0.220
181.0	0.400	3.70	184.0	0.270
310.0	0.349	3.98	315.0	0.360

\*After the periodic acid samples were diluted they were allowed to stand for from 30 minutes to 2½ hours before they were read, on the assumption that the extreme dilution had effectively decelerated the reaction. The samples were then read on the spectrophotometer at the first convenient time. It was later found that there was still a reasonably rapid reaction taking place in the diluted reaction mixture. Therefore, the time interval is actually longer than the one indicated in the table.

withese molar quantities are calculated for the entire reaction mixture.



Figure 3. Reaction of C.C25 M Feriodic Acid and C.CL5 M 2-Glucose at 25° C.

# TABLE VII

PERIODIC ACID CONSUMED AND FORMALDFHYDE PRODUCED BY THE REACTION OF 0.025 M PERIODIC ACID AND 0.0045 M D-GLUCOSE AT 25° C

Original Quantities of Reactants: Periodic Acid, 5.00 Millimoles D-Glucose, 0.900 Millimoles

Tim in	e Inter Seconds	rals Optical	Millimolog		
in	Seconds			Time intervals	Millimoles of
		Density	of Periodic	in Seconds	Formaldehyde
			Acid Consumed#		Produced#
	72	0.887	0.82	157	0.135
	250	0.860	0.99	330	0.153
	1.05	0.817	1.25	190	0.163
	562	0.785	1.1.1	640	0.179
	715	0.755	1.62	799	0.184
	006	0.712	1.87	972	0.185
	1.075	0.690	2.00	1.160	0.168
	1.235	0.675	2.09	1,396	0.189
	1.624	0.638	2.31	1.722	0.187
	1.904	0.618	2.12	1.991	0.192
	2.352	0.590	2.58	2.436	0.207
	2.827	0.565	2.71	2,919	0.200
	3.871	0.541	2.84	3,957	0.245
	4.762	0.504	3.04	4.880	0.235
	5.741	0.490	3.13	5.840	0.230
	6.843	0.470	3.26	6.993	0.256
	8.137	0.111	3.45	8.294	0.268
	9.11.18	0.435	3.42	9.550	0.282
	11.854	0.402	3.69	11.277	0.304
	14.537	0.384	3.80	14.640	0.359
	17.299	0.364	3.93	17.397	0.394
	20,096	0.346	4.03	20,188	0.426
	22.797	0.334	4.09	22.904	0.479
	25,428	0.319	4.18	25,545	0.485
	67,500	0.182	4.99	67,200	0.784
()	18 hrs.	45 min.)		(18 hrs. 40 mi	n.
	Developt	b here			
1	himer wa	ada farm			
	O DEC M				
	h UL av	40			
1	0.0001 1				
	V.UUL I				
	67.700	ecs. 0.980	0.28		

These molar quantities are calculated for the entire reaction mixture.


The original 0.250 M periodic acid which had stood as long as the reaction mixture was diluted to 0.0001 M concentration and measured. It showed a decrease in periodic acid concentration which corresponded to 0.28 millimole and which partially accounts for the 0.49 millimoles excess of periodic acid consumed, inasmuch as 4.5 millimoles represents the theoretical consumption of periodic acid. The theoretical production of formaldehyde was 0.9 millimoles. Therefore, the theoretical quantity of formaldehyde was not produced, although a slight overcon-

Reaction of 0.0875 M sodium periodate and 0.0125 M <u>D</u>-glucose. A reaction was run in which the same concentrations of sodium periodate and <u>D</u>-glucose were used as in the unbuffered reactions run by Hughes and Nevell (10), that is, 0.0875 M sodium periodate and 0.0125 M <u>D</u>-Glucose. Equal quantities of the two solutions with twice the above molarities were quickly mixed and aliquot samples withdrawn at intervals for periodate and formaldehyde analyses as before. The results of the reaction are shown in Table VIII and in Figures 5 and 6.

In interesting observation was made during the course of this reaction. When sodium periodate was used in the oxidation of D-glucose and the aliquots were diluted for optical density measurements, the diluted samples of reaction mixture reacted faster than the undiluted mixture. That is, diluted samples which had stood for some time had a lower optical density than samples which were freshly taken and diluted. With the periodic acid reaction, just the opposite was true. The examples given in Table IX will illustrate this tendency.

## TABLE VIII

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# PERIODATE CONSUMED AND FORMALDFHIDE PRODUCED BY THE REACTION OF 0.0875 M SODIUM PERIODATE AND 0.0125 M D-GLUCOSE AT 20° C

Original Quantities of Reactants: Sodium periodate, 8.75 Millimoles <u>D</u>-Glucose, 1.25 Millimoles

Periodic	Acid Con	sumed	Formaldehyd	le <b>Produce</b> d
Time Intervals in Seconds	Optical Density	Millimoles of Periodic Acid Consumed#	Time Intervals in Seconds	Millimoles of Formaldehyde Produced*
82	1.50	1.30	154	0.156
240	1.38	2.00	327	0.156
491	1.28	2.65	560	0.176
673	1.23	2.95	742	0.176
814	1.18	3.25	912	0.192
1,074	1.16	3.40	1,152	0.190
		***	1,332	0.192
1,630	1.09	3.80	1,703	0.192
2,072	1.07	3.90	2,144	0.206
3,538	1.01	4.25	3,604	0.200
4,367	1.00	4.30	4,450	0.203
6,533	0.957	4.55	6,707	0.218
9,326	0.922	4.75	9,405	0.224
13,473	0.903	4.85	13,578	0.218
15,216	0.883	4.95	<b>15,</b> 310	0.204
<b>22,</b> 133	0.858	5.10	22,237	0.236
26,003	0.845	5.15	<b>26,</b> 085	0.256
28,580	0.832	5.25	28,701	0.280
32,473	0.820	5.30	32,570	0.296
35,010	0.816	5.40	35,187	0.312
74,355	0.755	5.70	74,497	0.500

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	1	'eri	odic .	Acid Cons	umed		For	naldehyd	le Produced
TU	ne Ir	nter	vals	Cptical Density	Millimoles of Periodic Acid Consumed*	Tim	e In	tervals	Millimoles of Formaldehyde Produced*
32	hrs	. 17	min.	0.716	5.95	32 1	hrs.	19 min.	0.662
كلا	hrs.	30	min.	0.675	6.15	144 I	hrs.	32 min.	0.800
56	hrs.	. 17	min.	0.703	6.00	56 1	hrs.	18 min.	0.912
67	hrs.	38	min.	0.691	6.10	67 1	brs.	35 min.	0.982
79	hre.	. 25	min.	0.665	6.25	79 1	hrs.	30 min.	1.012
92	hrs.	5	min.	0.662	6.25	<b>91</b> I	brs.	37 min.	1.07
128	hrs.	. LO	min.	0.665	6.25	128 1	hrs.	10 min.	1.11
139	hrs.	10	min.	0.651	6.30	139 1	hrs.	10 min.	1.14
189	hrs.	. 10	min.	0.653	6.30	189 1	hrs.		1.16
						11 0	d <b>ays</b>		1.16

TABLE VIII (CONTINUED)

**Elenk** 1.71

These molar quantities are calculated for the entire reaction mixture.



Figure 5. Reaction of 0.0875 M Sodium Periodate and 0.0125 M D-31ucose at 20° C.

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## TABLE IX

## COMPARISON OF REACTION RATES HEFORE AND AFTER DILUTION

Sodium Periodate Reaction		Opti	cal Dens	ities	
Sample taken at 6533 secs.	<u>0 hr.</u>	<u>3/4 hr.</u>	43 hrs.	$\frac{9\frac{1}{2}}{\text{hrs}}$ .	<u>21 hrs</u> .
(See Table VIII) and which stood diluted for the specified time.	0.957	0.830	0.785	0.750	0.680
Samples taken after 6533 seconds + the specified time and freshly diluted.		0.992	0.858	0.806	0 <b>.7</b> 55
Sample takon at 32 hrs. 17 min.	<u>0 hr</u> .		<u>113 h</u>	rs.	
the specified time.	0.716		0.658		
Sample taken after 32 hrs. 17 mi + the specified time and freshly diluted.	n.		0.675		
Periodic Acid Reaction	<u>0 hr</u> .	<u>31 min</u> .	115 m11	<u>a. 4 hr</u>	s. 17 min.
Sample taken at $l_2^{\frac{1}{2}}$ min. (See Table VI) and which stood diluted for the specified time.	0 <b>.7</b> 92	0.762	0.747	,	0.603
Samples taken after 12 min. • the specified time and freshly diluted.		0.698	0.670	ſ	0.400
Rample taken at 55 min	0	min.	18 m	in.	
and which stood diluted for the specified time.	0.	434	0.42	7	
Sample taken a fter 55 min. + the specified time and freshly diluted			0.399	9	

### Oxidation of D-Arabinose

The reaction was conducted at the following concentrations: 0.0200 molar in periodic acid and 0.0045 molar in <u>D</u>-arabinose. To start the reaction the two solutions, each twice the above concentration, were quickly mixed in equal volumes. Samples were removed as before for periodic acid and formaldehyde analyses.

Periodic acid analyses: One-milliliter aliquots were taken and diluted to 200 ml.

Formaldehyde analyses: Two-milliliter aliquots were removed and added to one milliliter of 0.4 M sodium sulfite and the whole to 10.00 ml. Of this solution, 1.00-ml. aliquots were taken for the chromotropic acid determination as given previously.

The results of the analyzes are given in Table I and in Figure 7.

#### TABLE X

PERIODIC ACID CONSUMED AND FORMALDENYDE PRODUCED BY THE REACTION OF 0.0200 M PERIODIC ACID AND 0.0045 M D-ARABINOSE AT 25° C.

Original Quantities of Reactants: Periodic acid, 4.0 Millimoles D-Arabinose, 0.9 Millimoles

Periodi	c Acid Co	named	Formaldehyd	e Produced
Time Intervals	Optical	Millimoles	Time Intervals	Millimoles of
in Seconds	Density	of Periodic	in Seconds	Formaldehyde
		Acid Consumed#		Produced#
65	0.680	1.67	109	0.056
170	0.587	2.08	226	0.056
318	0.538	2.29	386	0.064
473	0.485	2.53	560	0.052
653	0.461	2.66	712	0.072
816	0.451	2.71	882	0.076
1,220	0.433	2,80	1,286	0.080
1.800	0.425	2.84	1.872	0.092
2,550	0.416	2.88	2,631	0.116
3.550	0.409	2.92	3.650	0.146
5.200	0.395	2.99	5.300	0.182
7.400	0.373	3.10	7.500	0.220
8,200	0.370	3.11	8.300	0.236
10.107	0.357	3.18	10.200	0.252
15.655	0.328	3.31	15.765	0.384
19,155	0.309	3.39	19.850	0.456
22.700	0.297	3.11	22.800	0.1.92
25.850	0.287	3.1.8	26.000	0.5/2
20.000	0.278	3.53	29,150	0.572
30,850	0.271	3.56	30.932	0.591
70.000(20 hrs.	)0.229	3.7)	72.000	0.828
h dame	0.215	3.83	li dere	0.810
4 days	1.01	ل ۱۰ ق	4 ugys	V+040

These molar quantities are calculated for the entire reaction mixture.



### Reactions of Periodic Acid with Various Compounds Which are Not Sugars

<u>Glycolaldehyde</u>. A check on the purity of the compound by periodate exidation and formaldehyde analysis, using the method originated by Speck and Forist (11) showed it to be about 90% pure.

Periodis acid in 0.0050 M concentration was allowed to react with glycelaldehyde in 0.0045 M concentration. This was done by rapidly mixing equal volumes of 0.0100 M periodic acid (2.0 millimoles) and 0.0090 M glycelaldehyde (1.8 millimoles) solutions. Analyses were made by the same methods used in the preceding reaction.

The first periodate sample removed (after 121 seconds) indicated a periodic acid consumption of 1.81 millimoles and the first formaldehyde analysis (189 seconds) indicated 1.58 millimoles of formaldehyde. The formaldehyde production did not increase with time to any appreciable extent. This production of formaldehyde was 88% of theory, which compared with a purity of about 90% indicates a reaction practically complete in 189 seconds or less. Even if this compound were available in a pure form it would be quite difficult to measure with respect to reaction rates. Its rate of oxidation must be extremely rapid.

<u>Glycerol</u>. A reaction between glycerol and periodic acid gave results very similar to the glycelaldehyde. The solution at the beginning of the reaction was 0.555 M with respect to periodic acid and 0.250 M with respect to glycerol. The reaction was run at  $0^{\circ}$  C and the periodate was titrated with standard sodium thiosulfate solution.

Formaldehyde samples were run, as previously described, by the chromotropic acid method. The results of the reaction are given in

Table II.

## TABLE XI

## PERIODIC ACID CONSUMED AND FORMALDENIDE PRODUCED BY THE REACTION OF 0.555 M PERIODIC ACID AND 0.250 M GLICEROL AT 0° C.

Original Quantities of Reastants: Periodic acid, 16.67 Hillimoles Olycerol, 7.50 Hillimoles

Period	He Acid Cons	nmed	Formaldehyde Produced			
Time Intervals in Seconds	Millimoles Periodic Acid Con- sumed*	Per cent of Theoret- ical	Tine Intervals in Seconds	Millimoles Periodic Acid Con- sumed=	Per cent of Theoret- ical	
278	14.6	97.8	101	14.1	93.7	
1,288	14.9	99.4	1,186	14.8	98.4	
2,401	15.1	101.0	2,304	14.8	98.4	

No further increase was observed beyond these samples

These molar quantities are calculated for the entire reaction mixture.

Elycerol also shows a very rapid reaction with periodic acid, too rapid for kinetic determination by the usual titration methods.

Another trial using a solution 0.0100 M with respect to periodic acid and 0.0045 M with respect to glycerol, but otherwise under the same conditions, gave the results shown in Table XII. · · · ·

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#### TABLE XII

PERIODIC ACID CONSUMED AND FORMALDEHYDE PRODUCED BY THE REACTICE OF 0.0100 M PERIODIC ACID AND 0.0045 M GLYCEROL AT 0° C.

Original Quantities of Reactants: Periodic acid, 2.00 Millimoles Glycerel, 0.900 Millimoles

Period	ic Acid Cons	umed	Formaldehyde Produced		
Time Intervals in Seconds	Millimoles Periodic Acid Consumed*	Per. cent of Theoret- ical	Time Intervals in Seconds	Millimoles Periodic Acid Consumed*	Per cent of Theoret- ical
187	1.08	60.2	96	1.15	63 <b>.7</b>
996	1.69	94.0	844	1.64	91.0
1,775	1.75	97.2	1,661	1.76	97.8
2,522	1.78	98.8	2,409	1.76	97.8
3,517	1.77	98 <b>.5</b>	3,381	1.76	97.8
7,708	1.78	99.0	7,522	1.76	97.8
22,037	1.78	99.0	21,757	1.74	96.6

These molar quantities are calculated for the entire reaction mixture.

It is difficult to see how the formaldehyde produced and the periodic acid consumed can differ at the same time interval. Therefore these differences must be due to analytical errors. A duplicate run of the same reaction gave similar results.

There appears to be a slower reaction in the more dilute solution as compared with the more concentrated solution.

Trimethylene Glycel. On the chance that periodic acid might form a complex with some compounds which it could not cleave, a number of likely compounds having 1,3-difunctional groups were combined with periodic acid in solution and the reaction mixture examined in the spectrophotometer for changes in absorption, either of the periodic acid absorption or for the appearance of new bands.

A technical grade of trimethylene glycol was used and any propylene or ethylene glycols were destroyed by treatment with sodium periodate solution. The excess periodate was reduced by sodium sulfite and the resulting solution was passed through a Dowex 50-12X (100-200 mesh) eation exchange column which was charged with hydronium ions. The solution was next passed through a Dowex 2X 10 (50-100 mesh) anion exchange column charged with carbonate ions. The solution was passed through repeatedly until it no longer gave a test for iodide ion and until it was neutral. The water and other low boiling impurities were distilled off at 117-120 mm. pressure and finally the trimethylene glycol was distilled over at  $115-119^{\circ}$  C.

Fifty milliliters of a 0.2 M solution of trimethylene glycol was mixed with a similar volume of 0.2 M periodic acid solution. Samples of the resulting solution, 0.1 M with respect to each component were examined for possible evidence of a complex as were dilutions of the original mixture to concentrations of 0.01 M, 0.001 M and 0.0001 M. A formaldehyde determination was performed on the reaction mixture.

About 0.1 millimole of formaldehyde was found to be present. This evidently originated from some unremoved 1,2-glycol. The optical density of the 0.0001 M solution at 222.5 mp indicates the disappearance of 0.6 millimoles of periodic acid. This would leave a net of 0.5

millimoles of periodic acid unaccounted for. This is such a small fraction of the total that inasmuch as no absorption which could be due to a complex was found it may be assumed that trimethylene glycol does not form a complex with periodic acid and the disappearance of this periodic acid could have been due to an impurity in the glycol.

<u>Acctorylacetone</u>. This compound was examined in the same way as the trimethylone glycol. After standing for four hours the mixture still had its original content of periodic acid and no unusual absorption peaks were found in the range 200-350 mp.

<u>Thyl Acctometate</u>. This also gave no evidence of any decrease in periodate when checked in the same way.

<u>Acetyl sostone</u>. Again equal quantities of 0.2 M acetyl acetone and 0.2 M periodic soid solutions were mixed, thus making the resulting mixture 0.1 N with respect to each component. After allowing the mixture to stand two hours at room temperature, then diluting one milliliter of the mixture to one liter and checking its absorption in the ultra-violet it was found that about one half of the periodic soid had been reduced. Upon measuring the absorption curve of a corresponding 0.0001 M acetyl mostone solution, it was found that in the reaction mixture the mostyl mostone absorption had also decremed. Acetyl acotone has an absorption peak at about 273 mm. The remotion mixture was allowed to stand for a total of 22 hours at which time the periodic acid had quantitatively disappeared. In fact, the absorption was slightly less than what would be expected from the remaining iodic acid. At the end of 22 hours about one half of the acetyl acotone

absorption, as measured at 273 mp, had disappeared. The results of the reaction are shown in Figure 5.

Therefore, there definitely seems to be a reaction between acetyl acetone and periodic acid.

The entire reaction was repeated using a 0.2 M sodium periodate solution instead of periodic acid. A similar reaction except that it was such slower, was observed. The results of this reaction may be seen in Figure 9.

After samples had been removed for optical density measurements at the end of  $3\frac{1}{2}$  and 27 hours, the reaction mixture was allowed to stand in the dark at room temperature. After he days a small flaigy brownish-yellow precipitate was observed in the flack. It was filtered out, washed with water and dried. It had the odor of iodoform, but its melting point was found to be  $113^{\circ}$  C and very sharp thus disproving any suspicion of its being iodoform (N.P.  $113^{\circ}$  C). A Beilstein test indicated the presence of a halogen. The compound may have been tri-iodo acetic acid (H.P.  $150^{\circ}$  C) (72), although at the time this possibility was not suspected and therefore the compound was not identified.

An attempt was made to determine the stoichiometry of the reaction between acetyl acetons and periodic acid by the titration of samples from a reaction mixture 1/60 M with respect to acetyl acetone and 5/60 M with respect to periodic acid. Samples of the reaction mixture were added to a solution containing excess potassium iodide, sodium bicarbonate and a measured quantity of standard godium ergenite solution.





After standing 15 minutes the samples were titrated with standard iodine solution. The endpoints were not stable and the blue starchiodine color at the endpoint disappeared on standing. After some time the titration mixtures had the odor of iodoform. Evidently in the slightly alkaline reaction mixture the iodoform reaction was proceeding parallel to that of the titration.

Another reaction with the same concentration was undertaken at  $25^{\circ}$  C and the periodic acid was checked by means of its ultra-violet absorption at 222.5 mp after the reaction mixture had been diluted to 0.0001 M, based on its original periodic acid concentration. At the end of the various time intervals the following optical densities and ratios of moles of periodic acid per mole of acetylacetone were ebserved and compared with a 0.0001 M periodic acid blank solution which had an optical density of 1.02.

Time Interval	Optical Density	Moles of Periodic Acid Reduced
		Moles of Acetyl Acetone
4 hrs. 16 min.	0.945	0.4
10 hrs.	0.790	1.41
21 hrs. 35 min.	0.701	1.93
30 hrs. 45 min.	0.697	1.96
51 hrs.	0.650	2.23
76 hrs.	0.612	2.46
7 days 41 hrs.	0.549	2.80

At the end of seven days the mixture was yellow with free iodine as evidenced by the blue color obtained when starch was added to the

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sample. Therefore the iodine had been reduced farther than to the iodate stage.

Rereading of diluted samples of the reaction mixture indicated that there was very little reduction taking place at the extreme dilution necessary for periodic acid measurement.

An attempt was made to identify some of the products of the reaction of periodic acid with acetyl acetone under the same reaction conditions as the two previous trials. Formaldehyde tests were run at intervals by means of chromotropic acid, but no color was obtained, thus indicating that no formaldehyde was formed in the reaction.

Indeform tests were made at the end of 2 hours,  $18\frac{1}{2}$  hours,  $31\frac{1}{4}$  hours,  $52\frac{1}{2}$  hours and 10 days. Indeform was produced each time, except the last time, but each time the quantity produced became progressively less.

The reaction mixture was also tested for formic acid. The procedure used was that given by Feigl (71). Two drops of reaction mixture was placed in a test tube and to it was added 2 drops of 3N hydrochloric acid and a slight excess of magnesium powder (Too much magnesium causes the test to fail, even on a known.). Three milliliters of 14 M sulfuric acid and a little chromotropic acid were then added and the test tube heated in boiling water for 10 minutes. No selor was obtained.

When one drop of formic acid was added to 50 ml. of water and this solution tested by the above method, a violet color was obtained.

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Even after adding sufficient periodic acid to make the solution 5/60 M with respect to periodic acid a violet color was obtained. Therefore, periodic acid does not interfere with the test for formic acid and fermic acid does not appear to be one of the products of the reaction.

In a subsequent reaction mixture, which was 0.2 M with respect to periodic acid and 0.04 M with respect to acetylacetone and in which the periodic acid consumption was measured by titrating the samples in a potassium iodide-sulfuric acid solution, the consumption of periodate in moles per mole of acetyl acetons was as follows:

After 17 hrs. 25 min. of reaction time--2.9 moles of periodate per mole of acetyl acetone.

After 46 hrs. 20 min. of reaction time--3.52 moles of periodate per mole of acetyl acetone.

At the end of 6 days free icdine had appeared.

These quantitative determinations show values approaching that of four moles of periodate per mole of acetyl acetone.

<u>Dimedone (1,1-dimethylcyclohexan-3,5-dione</u>). Since the reaction of periodic acid had taken place so readily with acetyl acetone, a 1,3-diketone, it was decided to try dimedone, also a 1,3-diketone. Due to its alight solubility in water it was necessary to run this at lower concentrations than the acetyl acetone. The reaction was run at  $25^{\circ}$  C in a solution that was 0.0125 M with respect to both dimedone and sodium periodate.

Measurements of periodate consumption were made by diluting the reaction mixture 2.00 ml. to 250 ml. and by measuring the optical density of the resulting 0.0001 M solution at 222.5 mm. The entire

absorption spectrum was also determined for the mixture at the end of  $\frac{1}{4}$   $\frac{3}{4}$  hours and at the end of  $23\frac{1}{4}$  hours, as well as for a 0.0001 M dimedone solution. The results of the reaction are shown in Figure 10. After  $\frac{1}{4}$   $\frac{3}{4}$  hours the reaction mixture showed an optical density of 0.428, corresponding to 0.71 moles of periodate consumed per mole of dimedone. After  $23\frac{1}{4}$  hours an optical density of 0.244 was obtained, which corresponds to 0.98 moles of periodate consumed.

Two attempts were made to determine the stoichiometry of the periodate reaction with dimedone. One was conducted at  $25^{\circ}$  C and in which the dimedone was 0.01 M and the periodic acid was 0.05 M and in which the periodic acid was determined by measurements of the optical density after dilution to 0.0001 M in terms of the original concentration of periodic acid.

The following results were obtained in the first cases

Time Interval	Moles of Periodate Per Mole of Dimedone
h hrs. 16 min.	1.17
10 hrs. 30 min.	2,00
22 hrs. 35 min.	2.58
31 hrs. 20 min.	2.82
52 hrs.	3.14
752 hrs.	3.36
7 days 42 hrs.	3.80

The other was run at room temperature with 0.025 M dimedone and 0.1667 M periodic acid and the periodate consumption was determined by sodium thiosulfate titration.



Absorption Spectra of the Reaction Mixture of 0.0125 M Dimedone and C.0125 M Sodium Periodate, Diluted to 0.0001 M. Pigure 10.

The second trial gave the following results:

Time Interval	Moles of	Periodata	Per Mole	of	Dimedone
1 day		4	•06		
2 days		հ	•31		
6 days		5	•00		

The sixth day the reaction was brown and contained iodine. This iodine was filtered off and the remaining iodates and periodates were reduced by bubbling sulfur dioxide through the solution. Sodium bicarbonate was used to partially neutralize the acids in the reaction mixture in order to obtain a more complete reduction. The solution was then concentrated in a vacuum and extracted with small portions of ether. The ether was evaporated and a small quantity of crystals appeared. These were recrystallized twice from three drops of distilled water each time. The melting point of the crystals was found to be  $101-102^{\circ}$  C. This corresponds reasonably well with the melting point for 3,3-dimethyl glutaric acid which is given by Heilbron (73) as  $103-10h^{\circ}$  C.

In a subsequent preparative attempt, similar crystals were obtained from a solution 0.025 M in dimedone and 0.117 M in periodic acid and which was allowed to react 95 hours before filtering off the iodine and extracting with ether. In this case the iodine was accompanied by a tarry organic substance which lodged in the funnel. This time 0.730 g. of crude crystals were obtained from 600 ml. of reaction mixture, which originally had contained 2.100 g. of dimedone. After these were recrystallised from water the melting point was found to be  $99-100^{\circ}$  C.

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<u>Methyl acetyl acetone</u>. A solution which was 0.2 M with respect to periodic acid and 0.04 M with respect to methyl acetyl acetone was allowed to react at room temperature and the periodic acid consumption was determined by sodium thiosulfate titration. The periodic acid consumption was found to be as follows:

Time Interval	Moles of Periodate Per Mole of Methyl Acetyl Acetone
174 hours	1.5
45 3/4 hours	1.6
6 days	2.06

It is evident that this reaction neither proceeds as fast nor consumes as much periodate as the reaction with unsubstituted acetyl acetone. Iodine crystals were noticed in the reaction mixture after 25 days.

<u>Phenyl acetyl acetophenone (C(H5CH2COCH2COC6H5)</u>. The ultra-violet absorption spectrum of this compound was determined as a 0.00001 M solution in methyl alcohol as a solvent. See Figure 11. Absorption of this diketone was too great in the region of 222.5 mp to permit use of the spectrophotometric method of determination of periodate. The relative insolubility of phenyl acetyl acetophenone makes a quantitative reaction in aqueous solution very difficult.

To produce a reaction, 5 ml. of 0.2 M phenyl acetyl acetophenone in methyl alcohol solution was mixed with 5 ml. of 0.4 M periodic acid. Upon contact with the water the phenyl acetyl acetophenone immediately began to precipitate. When the flask was shaken, large bubbles of gas came from the solution. The flask was put on the shaker for 14 hours


more opportunity to react than in the portion having a lower concentration of periodic acid. To overcome this mixing error a Pyrex mixing tube was made in which the two arms connected by a ground glass joint, were constructed at an angle of about 120° to each other. It was then possible to cool the selutions separately in each arm and at the moment of mixing quickly turn the tube so that the two solutions would both be rapidly combined. By quickly turning it back and forth several times, mixing was done very efficiently.

The second error appearing in reactions utilizing less than theoretical amounts of periodic acid, was caused by unreacted glyceraldehyde. Where there was an appreciable amount of glyceraldehyde remaining unconsumed, a brown color appeared in the chromotropic acid determination of formaldehyde. Normally a reddish-violet color is produced in this determination. The substance producing the brown color also exhibited fluorescence. This phenomenon was reported by Thermton and Speck (74) as a product of the reaction of sulfuric acid with glyceraldéhyde. It had also proviously been reported by Eegriwe (75).

To evercome this error, the method developed by Nash (76) for the determination of fermaldehyde was checked in the presence of glyceraldehyde and of glyccal and found to be free from interference by these compounds. The method was standardized by means of resublimed hexanethylene tetramine as the source of a known quantity of formaldehyde, as worked out by MacFadyen (77). The standardization curve was constructed from six point determinations, which produced a straight lime.

The procedure used to determine formaldehyde by the Nash method was as follows:

Aliquot samples of the reaction mixture containing no excess periodate were diluted until the solution contained from one to four micrograms of fermaldehyde per milliliter. Five milliliters of this dilute solution were mixed with 5 ml. of the reagent and allowed to stand for 5 hours at room temperature. The optical density was then measured at 412 mm against a blank made up at the same time as the sample and made by mixing 5 ml. of the reagent and 5 ml. of water. The observed optical density was compared with the standard curve and the concentration of formaldehyde was calculated.

In order that the reactions of glyceraldehyde might be better understood, its depolymerisation reaction was studied. The crystalline <u>D,L-glyceraldehyde</u> exists as a dimer. Wohl (78) lists the molecular weight of glyceraldehyde as 164 one hour after dissolving the crystals, as 102 after one day and as the calculated molecular weight of 90 for the monomer after two days at room temperature, but he failed to give the concentration of the solution. Dimerization, such as this, could have an appreciable effect on the reaction with periodic acid. Therefore, the experimental molecular weight and the equilibrium constant were found at 0° C by the method of change of freezing point.

Inasmich as glyceraldehyde is very difficult to dissolve it was not found practical to follow the course of depolymerization with a rate study. A mixture of 0.450 g. D.L-glyceraldehyde in 15.00 ml. of carbon dioxide-free doubly distilled water was placed on the shaker

at room temperature for about one hour until all the crystals were dissolved and then cooled to  $0^{\circ}$  C. This was then a 0.3 molal solution. The freezing point was measured with a Beckman thermometer and compared with the freezing point of the water.

In two trials the equilibrium constant for the depolymerisation reaction was calculated at 1.03 and 0.99 respectively and the calculated percentage of dimer at equilibrium was 30.9% and 31.6% respectively. In the second case equilibrium was reached in 2 hours and 25 minutes after mixing, but in the first case a somewhat longer time was required.

By the use of this equilibrium constant a 0.1 M glyceraldehyde solution at equilibrium at  $0^{\circ}$  C would have approximately 16% of the solute in the form of the dimer.

Three series of glyceraldehyde oxidations with an excess of periodic acid or sodium periodate, were run at  $0^{\circ}$  C. Table XI shows the effect of a change of concentration on the reaction. In this case the ratio of periodic acid to glyceraldehyde was kept constant at 10:9, that is, 10% of the periodic acid was in excess above that which was theoretically required for exidation of the glyceraldehyde. It is evident from the table that an increase in concentration produces a decrease in rate during the first stage of the reaction and an increase during the last stage.

Table XII shows the effect of concentration on the reaction of sodium periodate on glyceraldehyde at  $0^{\circ}$  C, still maintaining the same ratio of periodate concentration to glyceraldehyde concentration. Again an increase in concentration produced a decrease in the rate of

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## TABLE XI

	<b>22.2</b> 10 M	2 Millimoles 0 illimoles 0.25	.555 M Periodic A O M Olyceraldehyd	cid 9	
Periodic	Acid Co	nsumption	Formalde	hyde Pro	duction
Time Interval 1n Seconds	Milli- molea*	Per cent Theoretical	Time Interval in Seconds	Milli- moles*	Per cent Theoretical
220	14.37	71.8	103	6.68	68.8
1,378	15.22	76.1	1,271	7.30	73.0
2,525	15.43	77.1	2,437	7.44	74-4
8,092	16.70	83.5	7,976	7.70	77.0
15,052	17.47	87.4	14,937	8.22	82.2
21,940	18.44	92.2	21,780	8.74	87.4
25,202	18.47	92.3	25,080	9.02	90.2
22 <sup>1</sup> / <sub>2</sub> hrs.	19.86	99.3	222 hrs.	9.56	95.6

# EFFECT OF CONCENTRATION ON THE REACTION OF PERIODIC ACID AND CHICERALDEHYDE AT O° C

"These molar quantities are calculated for the entire reaction mixture.



	htal biolitida	0.00 M Feriadic	1111moles 0/	10 M	
notion Per cent Decretical	reifie Profi vel Milli- malem	asno roJul astr signasa hir	reiter Per one Beoretion	a co stal a Lerron fa Naeren 10	Periodi Time Inferv in Seconda
74.6	3.36	601 848	6.0079.3	8.9814	192
78.2	3.52	*** 1.s037	4.28000	8.9833	1,122
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### TABLE XII

	10 Mi 4.5	llimoles 0.100 Millimoles 0.0	M Sodium Perioda 45 M Clyceraldehy	te de	
Periodat Time Interval in Seconds	e Consum Milli- moles#	ption Per cent Theoretical	Formalde Time Interval in Seconds	hyde Pro Milli- moles#	duction Per cent Theoretical
159	7.06	78.5	93	3.48	77-4
974	7.20	<b>79.</b> 9	900	3.71	82.5
1,870	7.33	81.5	1,796	3.80	84.5
2,777	7.25	80.6	2,708	3.90	86.7
4,770	7.35	81.6	4,645	3.82	84.9
10,597	7.55	83.9	10,490	3.91	86.9
21,065	6.83	75.9	20,911	3.98	88.5
22,784	7.46	82.9			
27,200	7.52	83.6	29,795	3.94	87.6

# EFFECT OF CONCENTRATION ON THE REACTION OF SODIUM PERIODATE AND GLICERALDEHYDE AT O<sup>O</sup> C

These molar quantities are calculated for the entire reaction mixture.



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the reaction at the initial stage and also in the more advanced stage. In fact, with more concentrated solutions and sodium periodate it is difficult to approach theoretical yields.

Table XIII illustrates the effect of extra acid (perchloric acid) or base (sodium hydroxide) on the reaction mixture 0.100 M with respect to periodic acid and 0.045 M with respect to glyceraldehyde. It may be seen from the data given that an increase in acidity decreased the rate at the beginning of the reaction and increased it toward the end. The addition of an equivalent number of moles of sodium hydroxide, that is changing periodic acid to sodium periodate, does not affect the reaction greatly at the initial stage, but does decrease the rate at the last stages until it is difficult to achieve a theoretical yield.

In all of the preceding series involving glyceraldehyde, the periodate was determined by the sodium thicsulfate method and the formaldehyde by the chromotropic acid method.

A series was run in which less than the theoretical amounts of periodate was used. In this series the formaldehyde was determined by the Mash method using acetyl acetone, and the formic acid was determined by titration of the reaction mixture with sodium hydroxide. This formic acid determination is possible in the case where no periodic acid remains at the end of the reaction and the remaining iodic acid is definitely monovalent. Aliquots of the reaction mixture ware titrated for total acidity and the formic acid was determined by subtracting the known equivalents of iodic acid.

To check the validity of titrating formic sold in the presence of lodic sold, standard solutions of each were made and titrated in

# TABLE XIII

EFFECT	OF	ACIDITY	ON	THE	REAC	TICH	OF	0.100	MP	TRIODIC	ACID	AND	0.045M
	(	GLYCERAL	OPH	DE I	NT OU	C, 1	10	MILLIM	OLES	PERIODI	C AC	D,	
		1	4.5	MILI	LIMOL	79 G	LYC	FRALDE	HIDE	° AT 0° (	)	•	

Re	action M	ixture Contain	Berchloric Aci	a 0.277	ſ
Periodic	Acid Co	nsumed	Formalde	hyde Pro	duced
<b>Time Interval</b> In Seconds	Milli- moles#	Per cent Theoretical	Time Interval in Seconds	Milli- moles*	Per cent Theoretical
188	5.29	58.8	100	2.68	59.1
1,188	5.98	66.5	1,070	3.13	69 <b>.5</b>
2,174	6.24	69.4	2,090	3.39	75.3
3,727	6.76	74.0	3,655	3.59	79.8
7,130	7.46	82.9	7,043	3.68	81.8
13,261	7.99	88.7	13,164	4.11	91.3
20,690	8.31	92.4	20,600	4.02	89.4
17 hrs	8.91	95.9	17 hrs	4.52	100.4

"These molar quantities are calculated for the entire reaction mixture.

the presence of each other. Mixtures consisting of 10.00 ml. of standard formic acid and 20.00 ml. of standard iodic acid were mixed and titrated. Two such mixtures were titrated and the volumes of standard sodium hydroxide required to titrate the formic acid in the presence of iodic acid were calculated. The formic acid in the mixture required 3.96 and 3.91 ml. of base respectively as compared with 3.93 ml. and 3.94 ml. for 10.00 ml. of formic acid solution alone. Therefore, the method appears to be valid for the determination of formic acid.

These reactions involving less than theoretical amounts of periodate were run at 25° C. The periodate and the glyceraldehyde solutions were mixed rapidly and allowed to remain in an amber flask at the specified temperature for two hours before the analyses were made.

An attempt was made to determine glyoxal in a reaction mixture by precipitation with semicarbazide hydrochloride, but no precipitate of the sparingly soluble disemicarbazone was obtained, even with a deficiency of periodate. Therefore, any glyoxal produced in the reaction must be of such a small quantity so that its semicarbazone is within its solubility limit in the reaction mixture.

Table XIV gives the results of the series of reactions involving less than theoretical amounts of periodic acid. In this table the original glyceraldehyde and periodic acid were determined by weight and the formaldehyde and formic acid by the methods previously described. All other quantities were calculated by methods which will be discussed later.

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	RESULTS	OF THE REAC	TICN OF EXC	ESS GLICERA	LUERINDE WI	IH PERIODI	C ACID	
Conc. 1n ]	Molarities	a A	tio Per Mol	e af Period	ic Acid			Minimu
<b>Uy</b> ceral- dehyde	Perdodic Acid	Original Clycerel- dehyde	Ulyceral- dehyde Consumed	HCHO Produced	HC00H Produced	Gly oxal Produced	Glycolal- dehyde Produced	E112
OLD GLYCE	RALDERYDE PR	M TA CTART	.S.U.					
0.2	0°0	5.0	<b>0.</b> 805	0.125	66*0	10.0	0.76	6.08
0.1	0.1	1.0	0.67	15.0	6ó•0	10.0	0•34	0.938
FRESHLY R	ECHISTALLIZE	O GIYCERALD	HANDE					
1.0	1.0	1.0	0.64	0.38	C.93	0.02	0.26	0.67
1.0	0.2	5.0	16.0	0-075	66° U	0.012	0.85	12.0
NUTRITION	AL BUOCHTAILC	ALS CORP. G	IXCERALINGHY	周				
Series in Acid is d	which conce ecreased	mtration of	<b>Uycerel</b> de	hyde 1s kep	t constant	and concer	ntr <b>ation</b> of	Periodic
	<b>L.</b> 0	1.0	0.61 0.61	0 <b>.52</b> 0 <b>.52</b>	0.86 0.86	77.0	0.03 0.03	0 <b>.162</b> 0.162
<b>1.0</b> *	0-02	5.0	0.615	כנון-ס	0.97	0.03	0.20	0.483
1.0	10-0	10-0	0.72	0*30	0.98	0*025	0.48	1.42
1.0	0.005	20.0	0.81	0.225	0.965	0-036	0.585	2.61
1.0	0-0025	10°0	0.868	6661.0	1.00	0.000	0.732	5.38

TABLE XIV

kept	
3	
Acid	
Clyceraldebyde to Parlodis	te decreased progressively
y	4
pncentrations	concentration
ð H	ţ.
ratio o	a milch
the	777
Ð	
ł	57. 22
Ę	* *
Series	oonstal

0.2	0-04	5.0	0.64	14.0	0.95	0.05	0.23	0.57
1.0#	0-02	5.0	0.615	كليا•0	0.97	0.03	0.20	0.483
0.05	10-0	5.0	61.0	0.28	0.98	<b>710.</b> 0	0.46	1.67
Series of	identical c	concentration	s in which 1	the pH is ve	ried			
1.0	0.02 + 0	1.1 5.0	0.615	0.385	1.00	0.00	0.23	0.598
1.0+	0°05	5.0	0.615	كليلــ٥	16-0	<b>60-</b> 0	0.20	0.483
0.1	0.02M Na	101, 5.0	0.662	6،43	16.0	<b>0</b> •09	0.23	0.546
Series in of Olycers	which the c ldehyde is	<del>oncentrati</del> on varied	of periodic	o acid is k	spt constant	t and the	concentrat	lon
0.2	0-02	10.0	0.754	0.265	C. 932	0.018	0.439	1.84
<b>1.0</b> *	0.02	5.0	0.615	كديا.0	6.97	0.03	0.20	0.483

\* These are identical runs, the data of which are repeated for comparison in the individual series.

0.0097

0.965 0.035 0.005

0.515

0.52

1.0

0.02

0.02

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In order to better understand the glyceraldehyde oxidation a series of reactions was performed on glyoxal, one of the intermediates in glyceraldehyde oxidation. A 5% glyoxal solution was distilled, the distillate thoroughly mixed and used as the basis for a series of reactions in a solution 0.045 M with respect to periodic acid. One solution was made 0.3 M with perchloric acid, a second was used as it was and the third had an equivalent amount of sodium hydroxide added to it to produce monosodium periodate. The progress of the reaction was followed by sodium thiosulfate titrations for periodate consumed. This change of pH produced no significant differences in the rates of reaction.

#### DISCUSSION

#### D-Glucose

It is apparent from the results of the exidation of glucose that the production of formaldehyde does not keep pace with the consumption of periodate. This fact was also reported by Speck and Forist (11), whe obtained practically theoretical consumption of periodate while the formaldehyde production was still less than two-thirds of the theoretical value. Haghes and Nevell (10) noted that when the periodate was determined with sodium thiosulfate there was a reasonably close correlation between the periodate consumed and the formic acid produced, but when the periodate was determined by means of argenite the periodate consumption far exceeds the formic acid production.

Maghes and Nevell attempted to explain this by assuming that the argenite did not reduce the complex which was formed between glucose and periodate and that therefore the argenite method analyzed only the free periodate. If a reversible reaction is assumed between the

## Periodate + glucose \_\_\_\_\_ complex

periodate and the glucese to form the complex (whether or not this complex is an intermediate between the reactants and the products or whether it is an unproductive complex makes no difference in this case.), it would be expected that the complex would disappear within the 15 minute waiting period when exposed to excess arsenite. The procedure in this method was to add sodium bicarbonate, an excess of

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standard argenite solution and then after 15 minutes to back-titrate the excess of argenite. The argenite should then by reacting with the free periodate have reversed the reaction, causing the disappearance of the complex. If the complex is an intermediate in the reaction and if its disproportionation is very rapid, as there is good reason to believe, the complex would disappear even more rapidly. Therefore, this explanation of the discrepancy does not seem very convincing.

This anomaly with respect to the arsenite determination of periodate may be explained by the greatly increased rate of periodate oxidation in sodium bicarbonate buffer as reported by Speck and Forist (11). During the brief interval after adding the sodium bicarbonate and before adding the arsenite the reaction is accelerated to the extent that considerably more periodate has been consumed than was really the case at the time of removal of the sample, whereas the formaldehyde or fermic acid determinations which do not involve sodium bicarbonate additions do not show this acceleration. This explanation was advanced by Van Slyke, Hiller and MacFadyen (23) for the oxidation of hydroxy lymine.

If the open chain aldehyde form of glucose were the only form attacked and assuming a random attack upon the bonds, a mole of formaldehyde ought to be obtained for each five moles of glucose. This is not found experimentally by any method of analysis. There may be preferential attacks on particular bonds within an open chain molecule, but there is essentially no information on this at present. However, it is not very likely that such preferential attacks would

delay the theoretical production of formaldehyde as long as has been ebserved, especially in the presence of excess periodate.

It became apparent early in the investigation that the periodate attack on the cyclic hemiacetal form of glucose would yield the formyl glyceraldehyde as shown in equation (7) and that this might be a relatively stable substance.



Evidence for this ester has been ably presented by Barker and Smith (62) and by Schopf and Wild (64). Hydrolysis of the ester would then slowly take place, thus really becoming the rate-determining step.

This mechanism fits the experimental facts better than any which has previously been considered. One fact which equation (7) does not explain is the almost immediate appearance of a noteworthy quantity of formaldehyde. This quantity is larger than would be explained on the basis of the proportion of glucose present in the acyclic form at a given instant and which would then be oxidized directly in that form. In explanation of this formaldehyde production, it is possible for the equilibrium between the cyclic and acyclic forms to continue even

after an attack by periodate on the cyclic form, previding the  $C_1-C_2$ bond has not been cleaved. This may be illustrated by equation (6).



A periodate attack on the products formed in equation (8) would then yield the same immediate quantities of formaldehyde and formic acid as if the acyclic form had been attacked directly.

Experimental evidence has been found for this mechanism. Upon examination of Figures 3-6 it will be observed that the initial portion of the reaction is represented by a region in which the slope of the lines representing the rates of periodate consemption and formaldehyde production is comparatively great. This changes in the course of the reaction to eventually produce an almost straight line with little slope. The steep initial portion evidently represents the attack on the open chain aldehyde form of glucose as well as the A stage in equation (7).



By examination of a typical case such as the one represented by Table VII and Figure 4 it will be noted that the formaldehyde production surve becomes practically a straight line after about 900-1000 seconds. Consultation of the table indicates 0.185 millimoles of formaldehyde produced in 972 seconds, which represents the same number of millimoles of glucose oxidized. Assuming this to be oxidized in the open chain form, this would represent a periodaic consumption of 0.925 millimoles. assuming a ratio of five to one. Having originally started out with 0.900 millimoles of D-glucose and having exidised 0.185 millimoles in the open chain form, there would remain 0.715 millimoles to be exidized as the cyclic form. Using the ratio of 3 millimoles of periodate per millimole of cyclic D-glucose in the rapid A stage of the equation, this world represent the consumption of 2.145 millimoles of periodate, er a total consumption of 3.07 millimoles of periodate for both the open chain form and stage A of the cyclic form. Table VII indicates that this quantity of periodate was consumed at approximately the end of 5000 seconds. Consulting Figure 4 it will be seen that from slightly beyond this point the periodate consumption curve becomes nearly a straight line. The slight difference between the calculated point of 3.07 millimoles and the indefinite point where the change occurs is very likely due to the slow hydrolysis of the formyl ester which releases more glyceraldehyde to consume periodate.

A similar calculation based on the data in Table VIII and Figure 5 indicates a total consumption of 4.05 millimoles of periodic acid for the oxidation of the open chain and the initial stage of the reaction with the cyclic compound.

The data in Table VI and the accompanying Figure 3 will hardly lend itself to this type of analysis because the delay in reading the periodate samples has changed the appearance of the periodic acid consumption curve.

## D-Arabinose

The reaction of <u>D</u>-arabinose with periodic acid may be considered on a basis very similar to that of <u>D</u>-glucose, forming however, a formyl ester of glycollaldehyde instead of glyceraldehyde.



The formaldehyde production curve is very nearly straight from the beginning. Therefore, take the value of 0.056 millimoles (See Table I) for the first formaldehyde reading, which is assumed to arise from an exidation of open chain aldehyde molecules. This value would then indicate a corresponding periodate consumption of 0.224 millimoles and would suggest that 0.844 millimoles of the arabinose would be exidised in the cyclic form. This latter reaction would require 2.532 millimoles of periodate to produce the ester stage or a total periodate



consumption of 2.756 millimoles. This is very close to the definite break in the periodate consumption curve. (See Figure 7).

Therefore, it appears very probable that the oxidation of D-arabinose proceeds by the mechanism indicated, although definite proof would depend on the isolation and characterisation of the formyl ester of glycollic aldehyde.

## 1,3-Diketones

Courtois and Joseph (45) were apparently the first to report the reaction of periodate with a 1,3-diketone in a statement that periodate exidises dimedone (1,1-dimethyl cyclohexan-3,5-dione) (No data on periodate consumed or the products formed were given). Very recently Wolfrom and Bobbitt (46) have reported observations which clearly show that cyclic 1,3-diketones in general undergo a specific oxidation with periodate, but that this phenomenon does not extend to the acyclic 1,3-diketones. Although not as elaborate as that of Wolfrom and Bobbitt the present investigation has also elucidated the course of the dimedone reaction. Moreover, it has shown, contrary to Wolfrom's report, that acyclic 1,3-diketones (acetyl acetone, 3-methyl-2,4pentadione and 1,4-diphenyl-1,3-butadione) undergo oxidation. In general, the reaction of 1,3-diketones proceeds without formation of formic acid or formaldehyde. Carbon dioxide is formed and monoor dicarboxylic acids (depending on the structure of the ketone) are the products. These facts have led to proposal of the following mechanism for the reaction.



As an alternate mechanism for equation (12) the following possibilities may be suggested:

HH 000 000 \* \* \* + 103 I0<u>).</u> R-C-C-C-R (14) + HOH R-C-C=C-R000 00 . . . \_\_\_\_\_R-C-C-OH + R'COOH + IO3 (15) IO], R-C-C-R + HOH

The latter scheme is that proposed by Wolfrom and Bobbitt. They suggest exidation of the encdiclone form to the trione and subsequent exidation of the latter substance, because they observed that exidation

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of 2,3-dihydroxy-2-cyclohexenone occurred rapidly with compution of three moles of periodate per mole and also that 1,2,3-cyclohexanetrione rapidly consumed 2 moles of periodate per mole. Apparently the kete acid is the final intermediate in the reaction since phthalonic acid was isolated as its aniline derivative from an exidation of 1,3-indandione.

The exact nature of the carboxylic acids obtained from asyclic 1,3-diketones was not determined in the present investigation so that the question of whether these mechanisms embrace the oxidation of all 1,3-diketones remain to be settled.

Wolfrom and Bobbitt included the effect of pH in their investigations, but it should be pointed out that they conducted their investigations on the effect of pH by means of phosphate buffers. These buffers have a tendency to obscure the effect of pH by the effect of the phosphate ion on the reaction.

A similar effect may occur in exidations involving the bicarbenate ion. Speck and Forist (11) found greatly increased rates in the presence of sodium bicarbonate. Moreover, reactions of periodis acid when treated with an equivalent amount of sodium hydroxide do not appear to react in the same way as in the presence of sodium bicarbonate. An examination of Figure 2 indicates that there is a shift of the periodate absorption toward lower wave lengths as affected by the addition of sodium bicarbonate. This might indicate a change in the periodate ion, which may affect the rate of the oxidation reaction. Equivalent amounts of sodium hydroxide produce no change in the
absorption spectrum of periodic acid. This is a matter that might well be investigated in the future.

Wolfrom and Bobbitt also determined periodate by the sodium bicarbonate-argenite method, which in the process of this investigation has been shown to give results that are not always dependable in the presence of excess reagent containing active hydrogen. The iodoform reaction mixture becomes too highly competitive with the analytical reaction. Therefore, the thiosalfate method or the absorption method have both been found superior to the argenite method for determining periodate in reaction mixtures containing 1,3-diketenes.

## D,L-Glyceraldehyde

<u>General reaction</u>. Insample as glyceraldehyde has been proved to be an intermediate product in the periodate oxidation of glucose, its reaction with periodate is of great importance. It is also a very simple sugar.

In spite of its apparent simplicity, its reaction with periodate involves competition between four simultaneous reactions. Moreover the presence of glyceraldebyde dimer may possibly complicate the situation.

The reactions taking place in the exidation of the memomer may be represented by the following schematic representation:



The symbols  $P_1$ ,  $P_2$ ,  $P_3$  and  $P_{l_1}$ , which will be used throughout the succeeding discussion to represent the various molar quantities of periodic acid used in the four reactions. In addition  $r_1$ ,  $r_2$ ,  $r_3$  and  $r_{l_1}$  may be allowed to represent the rates of these respective reactions. Thus  $r_1:r_3$  represents the ratio of the rates of reaction 1 to that of reaction 3.

In reaction mixtures containing an excess of periodic acid, an increase in concentration, produces a decreased rate in the first stage of the reaction and a somewhat increased rate in the last stage. The same general trend is observed in an oxidation with sodium periodate with the exception that in this case the decreased rate with increased concentration holds to the end of the reaction and it is difficult to get the reaction to run to completion under the conditions of these experiments. An increase in acidity of the solution also decreases the rate of the first stage of the reaction, but increases the rate of the last stage. In an acid solution the theoretical endpoint is quite readily attained, but in nearly neutral solution such as is the case with sodium periodate the reaction is not complete.

These may all be pH effects if low pH's cause a decrease in initial rate. However, this would hardly explain the decrease in rate due to increased concentration when using sodium periodate, inasmuch as this solution is only slightly acid. There is, of course, a decrease in pH as the reaction proceeds and liberates formic acid. If a low pH decreases the reaction then it appears difficult to understand why this last stage of the sedium periodate reaction should decrease more than that of corresponding reactions involving periodic acid, which is considerably more acidic. There is the probability that the last stage may represent reactions 2 and 4 which may possibly be catalyzed by acid.

However, reaction h represents the oxidation of glyoxal, which gave no evidence of any appreciable difference due to acidity when it was oxidized by itself. In the reactions involving excess glyceraldehyde (Table XIV) there appears to be a very slight accumulation of glyoxal when using sodium periodate, but this difference is so small as to be almost negligible as compared with the greater differences in completion of the reaction as indicated in Tables XII and XIII.

<u>Development of relationships when glyceraldehyde is present in</u> <u>excess</u>. When considering the quantities involved in the reaction containing excess glyceraldehyde, an interesting series of relationships is found. Knowing the original molar quantities of glyceraldehyde and periodic acid and the production of formaldehyde and formic acid

by analysis and knowing by periodate titrations that the periodate reaction is complete under these conditions, it has been found possible to calculate all the other quantities involved. By reference to equation (19) and by expressing all products and reactants in molar quantities the following relationships are obviously true:

Glyoxal produced =  $P_3 - P_{l_1}$ Formaldehyde produced =  $P_3 + P_2$ Formic acid produced =  $P_1 + P_2 + 2P_{l_1}$ Periodic acid consumed (total) =  $P_1 + P_2 + P_3 + P_{l_1}$ Glyceraldehyde remaining = Original glyceraldehyde -  $P_1 - P_3$ Glyceraldehyde consumed =  $P_1 + P_3$ Glyoxal produced = Periodic acid (total) - formic acid Glyceraldehyde consumed = Formaldehyde + glycolaldehyde Glycolaldehyde produced  $+ P_1 - P_2$ 

From the preceding relationships the following equations may be developed:

Glycolaldehyde produced = 2 Periodic acid (total) - 2 HCHO - HCOOH Glycolaldehyde produced = 2 Glycxal - 2 HCHO + HCOOH

Olyceraldehyde consumed · Periodic acid (total) - HCHO + glyexal

With the above relationships it is possible to calculate the molar quantities of all the products in the reaction. Since in all of these relationships there are four unknowns  $(P_1, P_2, P_3 \text{ and } P_1)$  and since there are only three independent equations (All other relationships are dependent upon the three.), the problem has no direct mathematical solution based on the data available. However, if the ratio of

glyceraldehyde to periodic acid is increased progressively to infinity a limiting ratie of  $r_1$  to  $r_3$  ought to be reached since the relative smounts of periodic acid represented by  $P_2$  and  $P_{l_1}$  will tend to become less and  $P_1$  and  $P_3$  will represent practically the entire amount of periodic acid. Their ratio will then represent the ratio of the rates of reaction 1 to reaction 3. The column headed "Minimum Ratio of  $r_1 r_3$ " in Table XIV represents the smallest possible ratio of  $P_1$  and  $P_3$ (that is, the ratio  $r_1 r_3$  must be equal to or greater than this value) and is calculated by means of an equation, which is developed as follows:

- (a) Let  $r_3 = 1$ .
- (b) Then the ratio of r, ir, may be called x.
- (c) Then glyceraldehyde consumed  $(\frac{x}{x+1})$  = glycelaldehyde, if  $r_2$  is neglected.
- (d)  $r_2$  may be neglected ealy if  $\frac{glycoraldehyde}{periodic acid} = \infty$ .
- (e) But x determined from equation (c) should approach an asymptote as the ratio of glyceraldehyde increases.
- (f) Solving (c) for x: x = glycolaldehyde glyceraldehyde consumed - Glycolaldehyde

It is evident from the column headed "Minimum Ratio of  $r_1:r_3$  that  $r_1$  is more rapid than  $r_3$  and that when the competition for periodate ions becomes sufficiently great the relative rates become more prominent as shown by the above ratio and qualitatively by a comparison between the relative quantities of formic acid and formaldehyde or by glycolaldehyde and glyoxal.

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Effect of a change in pH. By considering in Table XIV the differences which are caused by a change in pH, it is found that an addition of perchloric acid produces small increases in the ratio of formic acid to formaldehyde, of glycolaldehyde to glycxal and in the minimum ratio of  $r_1 i r_3$ . These would all indicate a relatively increased rate for reaction 1 or a relatively decreased rate for reaction 3. It would, of course, be possible for the added sold to increase or to decrease the rates of both reactions. The results obtained with excess periodate (see Table XIII) which indicate a general rate decrease for the first stage of the reaction when the pH has been decreased by the addition of perchloric acid, would support the idea of a general decrease in the rates of reactions 1 and 3. If this is the case, there must be a relatively greater decrease of  $r_3$  than of  $r_1$ .

However, in Table XIV a change of pH does not seem to affect the glycolaldehyde produced. Therefore, if  $r_3$  is decreased more than  $r_1$  a greater quantity of periodic acid ought to have an opportunity of reacting as  $P_1$ , thus producing a larger quantity of glycolaldehyde. Hevertheless, the quantity of glycolaldehyde produced does not seem to insrease. Therefore the rate of reaction of glycolaldehyde ( $r_2$ ) may be increased by an increase in acid and decreased by an increase in pH. This decrease in  $r_3$  and insrease in  $r_2$  could at least agree with the results obtained in Table XIII.

This suggested increase in reaction rate for glycolaldehyde in acid solution might profitably be checked, providing glycolaldehyde of sufficient purity were available. As has been mentioned, a small

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quantity of the impure compound was prepared from tartaric acid, but two subsequent attempts at preparation produced no yield. With glycolaldehyde available, a comparison of reaction rates in reaction mixtures of various pH's should then throw some light on the problem.

Ruffered solutions have not been used in any of these experiments, because of the effect of the buffers on the reaction. Comparisons of reactions run at various pH's maintained by buffers are practically valueless. Such comparisons may show only the effects of the buffers used rather than effects of pH.

Effect of a change in concentration without change of glyceraldshyde to periodate ratio. The effect of changes in concentration on the reaction of periodic acid and glyceraldehyde may be due to accompanying pH changes, that is, a more concentrated reaction mixture would necessarily have a lower pH. By consideration of the variations in a reaction when the concentrations are decreased without changing the ratio of glyceraldehyds to periodate (see Table XIV), it will be seen that dilution causes a general increase in the ratio  $r_1 \cdot r_3$ . This is also borne out by the increased ratio of formic acid to formaldehyde and of glycolaldehyde to glyoxal. Both of these latter ratios would be increased by a relative increase in  $r_{ij}$ , which would increase the yield of glycolaldehyde and decrease the yield of glyoxal. It must be remembered that any conclusions drawn from Table XIV can give only a comparison of relative rates and can not give absolute rates.

The data in Table XI using an excess of periodic acid indicate a faster general reaction at greater dilution, especially with respect to formaldehyde production, which could be interpreted as a more rapid reaction of glycolaldehyde  $(r_2)$  or an increase in  $r_3$ . However, comparison with the data in Table XIV shows that at greater dilutions there is some increase in glycolaldehyde. This is hardly compatible with a more rapid reaction of glycolaldehyde  $(r_2)$  unless accompanied by a somewhat greater increase in  $r_1$ .

An increase in  $r_3$  without a corresponding increase in  $r_4$  would mean an accumulation of glyoxal. This is in harmony with the evidence. This reasoning now presents two alternatives, which could take place upon dilution, either one or both of which may take place:

1. In increase in  $r_1$  with a somewhat smaller increase in  $r_2$ .

2. An increase in  $r_3$  without a corresponding increase in  $r_{j_1}$ .

The second of these alternatives does not seem quite as logical as the first inasmuch as the ratio  $r_1:r_3$  appears to increase with dilution. The first alternative does not explain the small increase in glyexal. Therefore, the most logical explanation would be a combination of the two. This conclusion is also in agreement with the increased glyceraldehyde consumption at greater dilutions.

Effect of increasing glyceraldehyde concentration. By increasing the glyceraldehyde concentration while maintaining periodic acid concentration, the pH is kept nearly constant. It will be observed then that the formaldehyde and glycxal yields are decreased and formic acid and glycolaldehyde yields are increased many fold. This shows that  $r_1$  · · · ·

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is considerably greater than  $r_3$  and that an increase in glyceraldehyde concentration merely amplifies the opportunity for reactions 1 and 3 to take place rather than 2 or 4. The fact that part of the glyceraldehyde is in the form of a dimer (In a 0.1 M solution at 0° C and at equilibrium about 16% is in the form of the dimer.) would tend to counteract this effect, but apparently even this is not great enough to provide opportunity for reactions 2 and 4 in solutions containing high ratios of glyceraldehyde to periodic acid.

Effect of increasing periodic acid concentration. The series in which the concentration of glyceraldehyde is kept constant and the concentration of periodic acid is varied emphasizes the same general trend as in the preceding series. However, it also would be affected by the change of pH as the concentration of periodic acid varies. Therefore, conclusions based on this series are not as clear, although the general trend emphasizes the effect of the increasing glyceraldehyde: periodic acid ratio rather than it does the effect of pH.

One fact which has been proven without doubt in these series of reactions is the much greater rate of attack by periodate on the carbonyl-carbinol bond  $(C_1-C_2)$  than on the glycol bond  $(C_2-C_3)$ . The ratio of these two rates can not be definitely determined, but that it is greater than five can be stated with a considerable degree of certainty.

## TIMMARY

1. A spectrophotometric method for determining the periodate ion in reactions involving the Malaprade reaction has been developed. The method is based on measurement of the optical densities at 222.5 millimicrons. Simple sugars, formaldehyde and formic acid do not interfere. Bicarbonates do interfere.

2. The mechanism of periodate exidation of <u>D</u>-glucose has been elucidated. A small but noteworthy fraction of the glucose is exidized in the open chain form and the remainder in the cyclic form, producing an ester, 2-formyl glyceraldehyde, which then slowly hydrolyses. This hydrolysis stage is the rate-determining step in the reaction. D-arabinose has been found to follow a similar mechanism.

3. Periodate oxidation has been found to take place with 1,3-diketones, both cyclic and acyclic. The following compounds have been found to react: acetyl acetone, 1,1-dimethylcyclohexan-3,5-dione, 3-methyl-2,4-pentadione and 1,4-diphenyl-1,3-butadione. The reaction produces carbon dioxide with unsubstituted 1,3-diketones, but no formaldehyde or formic acid. A mechanism for the reaction has been postulated.

4. A method of calculating glycolaldehyde and glycoxal produced and glyceraldehyde consumed has been worked out for reactions involving

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excess glyceraldehyde and in which formic acid and formaldehyde have been determined by analysis. This method has also been applied to a determination of the relative rates of attack on the bonds of glyceraldehyde.

5. The equilibrium constant for depolymerization of the <u>D</u>,<u>L</u>-glyceraldehyde dimer has been determined at  $0^{\circ}$  C and has been found to be 1.0.

6. A study of the reaction of periodate with D,L-glyceraldehyde has produced the following conclusions:

- a. The bond between the first and second carbon atoms in glyceraldehyde is attacked at least five times as rapidly as the bond between the second and third carbon atoms.
- b. A decrease in concentration of the reactants produces an increase in the rate of reaction, especially at the initial stage.
- c. An increase in acidity also decreases the first stage of the reaction. Addition of an equivalent quantity of sodium hydroxide to the periodic acid in the reaction mixture shows a slower final stage of the reaction.

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