REACTION OF DL-GLYCEROSE WITH PERIODIC ACID

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REACTION OF DL-RAMNOSE WITH PERIODIC ACID

By

Jarvin Hedlund

AN ABSTRACT

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AN ABSTRACT

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The rates of cleavage by periodic acid of the two carbon-to-carbon bonds in DL-glycerose have been investigated by a method which involves carrying out the reaction in the presence of large excesses of DL-glycerose and determining the concentrations of formic acid and formaldehyde produced. The rate of scission of the bond between C_1 and C2 has been found to be approximately five times greater than that of the cleavage between C2 and C3 at 30°. At 20° the rate of the former reaction was observed to be nearly ten times greater than that of the latter.

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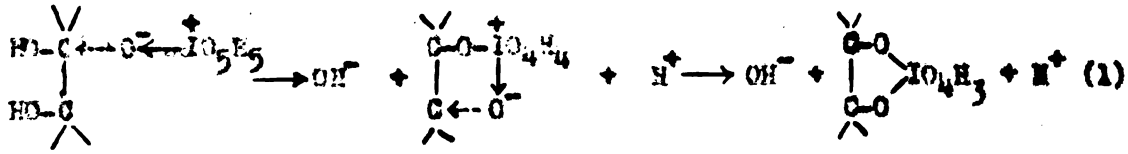
INTRODUCTION

The cleavage by periodic acid of carbon-to-carbon bonds between carbon atoms which carry either oxygen atoms (as hydroxyl groups or as carbonyl groups) or amino groups, was first discovered by Malaprade[1] in 1928. Since its discovery, this reaction has found numerous applications in quantitative determinations and it has been used extensively and successfully for investigation of various structural problems of the sugars. Since the development of these applications has been recently reviewed[2], this introduction will be limited to discussion of the kinetics and the mechanism of the Malaprade reaction.

The first precise kinetic study of periodate oxidation was carried out on pinacol (because of its conveniently measured reaction rate) by Price and Kroll[3]. Price and Kroll observed that this reaction follows simple second order kinetics (first order in pinacol and first order in periodate). They also found that the value of the second order constant varies greatly with the pH of the reaction mixture. In an acid medium this rate constant was found to be directly proportional to the hydrogen ion concentration; in basic media it was proportional to the square root of the hydroxide ion concentration.

Price and Knell[4] carried out similar experiments with ethylene glycol and cis- and trans-cyclohexene glycols. No complex variation in rate with pH was found in the reaction of periodate with these substances. On the acid side, the rate constant for ethylene glycol was found to be essentially independent of the pH. On the basic side it was roughly

proportional to the hydrogen ion concentration, or the inverse of the hydroxide ion concentration. With respect to the cyclohexene glycols, Price and Knell found that cis-cyclohexene glycol is oxidized more rapidly than is the trans-isomer. To explain this observation they suggested a bimolecular inversion mechanism involving the formation of a cyclic diester of periodic acid (Equation 1). Such an intermediate had



(1)

been previously proposed by Criegee[5] who noted that periodic acid, like lead tetraacetate, should act as a coordination center. Formation of such an ester should proceed most readily when the two hydroxyl groups are cis. This idea of an intermediate diester has been generally accepted by the workers in the field.

Duke[6] reinvestigated the periodate-ethylene glycol reaction somewhat later and found that the kinetics of this reaction must be explained by either of two mechanisms: (1) the first order decomposition of the periodate-glycol complex as the rate determining step, or (2) direct oxidation of the glycol by periodate (first order each in periodate and in glycol) in which formation of an unproductive periodate-glycol complex is a reversible side reaction. Furthermore, Duke found that the rate of this reaction is nearly independent of hydrogen ion concentration in the pH range of 3 to 7. In order to explain the absence of a dependency of rate on pH under these conditions, Duke later referred[7] to a suggestion of Waters[8] to the effect that hydrogen ion catalysis in

this range is offset by decreasing ionization of H_5IO_6 to the reactive species, $H_4IO_6^-$.

In an effort to observe the effect of methyl substitution on the reaction rate and to understand the unusual effect of hydrogen ion in the periodate-pinacol reaction, Duke and Bulgrin[7] carried out a kinetic investigation of the periodate oxidation of ethylene glycol, propylene glycol, meso- and levo-butylene glycols, trimethylethylene glycol and pinacol. Duke and Bulgrin found that all of these substances, except pinacol, behaved similarly to ethylene glycol, which is to say that the data were in accordance with the following expression:

$$IO_4^- + \text{glycol} \overset{\text{rapid}}{\rightleftharpoons} \text{cyclic intermediate} \overset{\text{slow}}{\longrightarrow} \text{products} \quad (2)$$

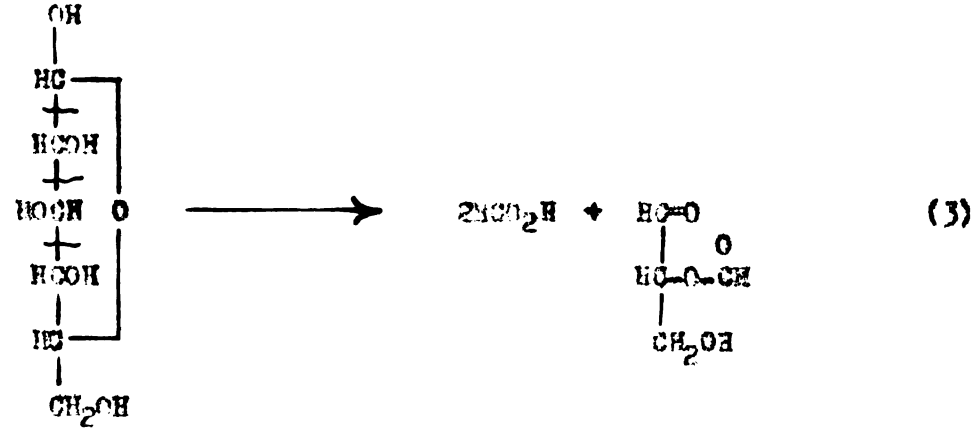
For the pinacol reaction, Duke and Bulgrin assumed that the oxidation proceeds through a cyclic intermediate. However, in this instance, the rate determining step is the formation of the complex between the glycol and periodate. This slowness of complex formation was attributed to steric hindrance by the four methyl groups which force the hydroxyl groups out of a favorable orientation for coordination with periodate.

The periodate oxidation of carbohydrates is seldom a simple reaction on account of the polyfunctionality of these substances which may permit the occurrence of both competitive and consecutive reactions. Hence, progress to a complete understanding of these oxidations has been slow and somewhat limited.

Investigation of the mechanism of the periodate oxidation of carbohydrates has been hampered by the rapid rates of many of these reactions.

Thus, Christensen[9] found that it was nearly impossible to measure the rates of the initial stages of several aldose-periodate reactions—even at low temperatures and concentrations—by following the changes in periodate titers. As far as it is known such rates have never been accurately determined.

In spite of the rapidity with which periodate cleaves some bonds in carbohydrates, there are often slow phases in the complete oxidation of these substances. For example, this behavior has been noted for the periodic acid oxidations of D-glucose[10,11] and D-xylose[11], both of which are quite slow in their final stages. However, these slow steps do not involve direct reactions between periodic acid and the substrate, but, instead, they result from the slowness of the hydrolysis of intermediate products formed in the initial oxidations. Thus, Schopf and Wild[12] found that the rate determining step in the last part of the reaction of D-glucose with periodate is the hydrolysis of 2-O-formyl-D-glycerose (Equation 4) which is formed in the scission of the bonds between C1 and C2, C2 and C3, and C3 and C4 (Equation 3). Schopf and Wild firmly



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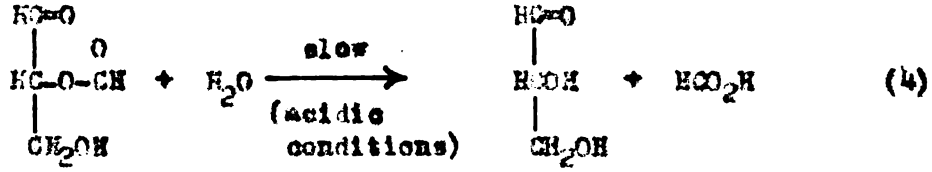

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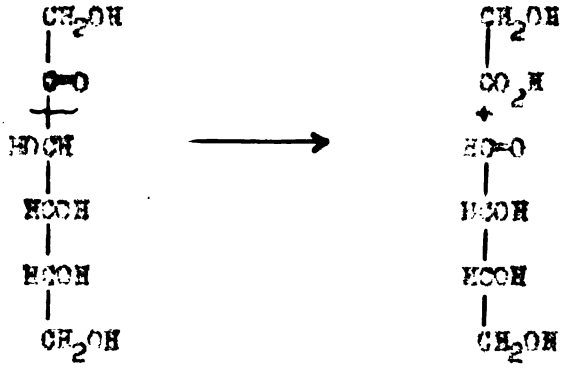
established this fact by isolation of 2-O-formyl-D-glycerose from D-glucose-periodic acid reaction mixtures.

Information about the relative rates of periodate oxidation of the bonds between C1 and C2, and between C2 and C3 of ketoses have been available from the time of the first applications of the Malaprade reaction to these substances. For example, the consumption of 4.85 moles of periodate, instead of 5.0 moles, by 1 mole of fructose clearly indicates that the rate of cleavage of the C_1-C2 bond exceeds that of scission of the C2-C3 bond. If the rates of these cleavages were equal, the consumption of periodate per mole of fructose would be 4.5 moles, since glycolic acid formed by oxidation of the C2-C3 bond (Equation 5) is not attacked by periodate at any significant rate.



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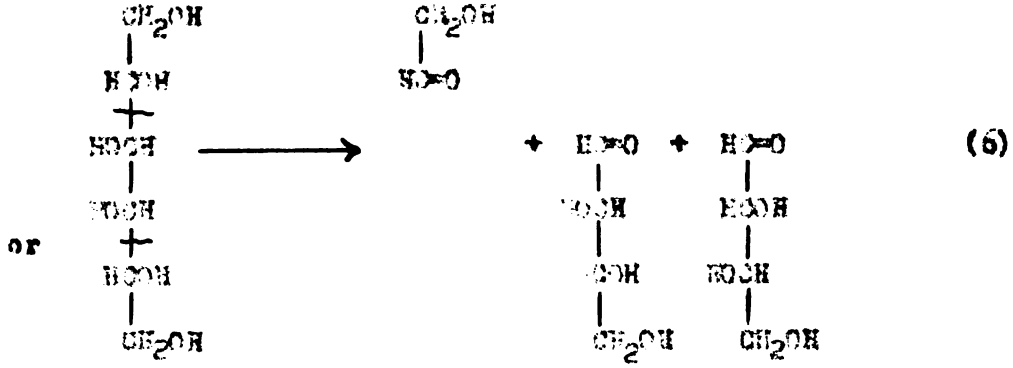
Nevertheless, these data from ketose oxidations provide only qualitative knowledge of the relative rates of the periodate oxidation of

the different kinds of bonds within carbohydrates. It is only very recently that some progress has been made in the quantitative determination of these effects. Christensen[9] carried out an investigation of the relative rates of cleavage of the two carbon-to-carbon bonds in DL-glycerose by a technique which involved large ratios of the concentration of DL-glycerose to that of periodic acid. Christensen experienced some difficulty in obtaining reproducible results—apparently because of insufficiently rapid mixing of the reactants. Notwithstanding this, his data indicated that, at 25°, the cleavage of the C_1-C2 bond is approximately five times as fast as that of the C2-C3 bond (for intact DL-glycerose).

Recently, Schwarz[13] has carried out a similar investigation in which large excesses of galactitol (dulcitol) and mannitol were oxidized with periodate. Schwarz's experiments indicate preferential oxidation of three-glycol groups, since the galactitol oxidation afforded principally a tetrose (Equation 6), whereas the mannitol reaction gave mainly glycerose (Equation 7).



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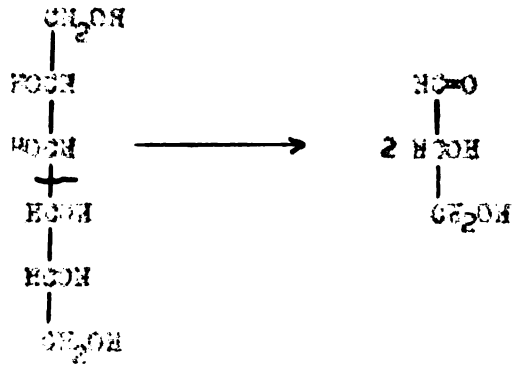

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The present investigation was an attempt to extend the work of Christensen on the D-glycerose-periodic acid reaction.

EXPERIMENTAL

Apparatus. A Beckman model B spectrophotometer equipped with Corex cells was used for optical density measurements. A line-operated Beckman pH meter was used for all acid-base titrations.

Chemicals. The DL-glycerose employed in these experiments was either prepared according to the "Organic Syntheses Procedure"[14] or was a Nutritional Biochemicals Corporation product. The periodic acid was a product of the G. Frederick Smith Chemical Co.. Sodium bicarbonate, ammonium acetate, potassium iodide, arsenious acid and sodium acetate were all c.p. grade. The hydroxylamine hydrochloride and acetylacetone were Eastman Kodak best grade.

Preparation of Standard Curves of Formaldehyde Concentration versus Absorbance. The procedure used to determine formaldehyde was the one developed by Nash[15]. Resublimed hexamethylene tetramine was hydrolyzed as recommended by MacFayden[16] in order to prepare a formaldehyde solution of known concentration. The standard curve was constructed from five-point plots according to the following procedure: The reagent was prepared by dissolving 2 gram formula weights of ammonium acetate, 0.05 mole of acetic acid, and 0.02 mole of acetylacetone in water and diluting the solution to a volume of 1 liter. Five milliliters of this reagent and 5 milliliters of a solution (of known concentration) containing not more than 8 micrograms of formaldehyde per milliliter were mixed and allowed to stand for 5 hours at room temperature. The absorbance

was then measured at 412 millimicrons against a blank which was made up by mixing equal volumes of reagent and water at the same time that the solution of formaldehyde and reagent were mixed.

Purity of the DL-Glycerose. The purity of the DL-glycerose was checked by oxidation with periodic acid. One milliliter of 0.2 M glycerose was added to 15 milliliters of 0.0485 N periodic acid. This reaction mixture was allowed to stand for four hours at room temperature and was then neutralized with excess sodium bicarbonate. To this mixture was added 2 milliliters of 5 percent potassium iodide. This was followed immediately by the addition of 10.00 milliliters of 0.13 N sodium arsenite. After 15 minutes, the excess sodium arsenite was titrated with 0.0092 N iodine to a starch end point. The periodate consumption was 99 percent of the theoretical amount for glycerose.

Periodic Acid Oxidation of DL-Glycerose. The concentration of periodic acid was kept constant in all of these experiments but the ratio of the initial glycerose concentration to that of periodic acid was varied from 1.25 to 10.0. The calculated amount of DL-glycerose was placed in the outer section of a special mixing vessel and dissolved in 20 milliliters of water. Five milliliters of 0.4 M periodic acid was placed in the central section. This mixing vessel was made by fixing a glass tube 3 centimeters high in the center of a 150-milliliter erlenmeyer flask. By placing the DL-glycerose and periodate solution in the separate sections, it was possible to bring them into equilibrium with the thermostat before mixing and to achieve nearly instantaneous mixing by sinking the flask.

The two solutions were kept in the thermostat for 1 hour before mixing, in order to allow for monomerization of the crystalline cyclic dimer, which is incapable of reacting with periodic acid. On standing in aqueous solution the dimer dissociates into the monomer--although not completely. After mixing the solutions, the reaction vessel was allowed to remain in the thermostat for one-half hour before carrying out the analyses.

The formaldehyde concentrations in these mixtures were determined by the Nash[15] method. The formic acid was determined by titration of the reaction mixture with sodium hydroxide for total acidity (using the line-operated Beckman pH meter to determine the end point) and by subtracting the known equivalent of iodic acid from the total acidity.

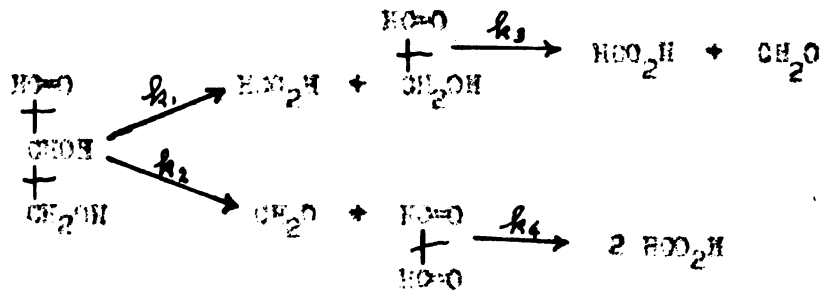
In order to indicate the effect of temperature on the relative rate of oxidation of the two bonds the same experiments were repeated at 20° C.

Determination of glycerol in the Periodate-M-Glycerose Reaction Mixture by Paper Chromatography. Twenty milliliters of 0.25 M M-glycerose solution was mixed rapidly with 5 milliliters of 0.4 M periodic acid solution. This reaction mixture was allowed to stand for one-half hour to ensure completion of the reaction. An excess of standard arsenite sodium solution and 2 milliliters of 5 percent potassium iodide solution were then added to this mixture. After 15 minutes, 0.5 gram of hydroxylamine hydrochloride and 1 gram of sodium acetate were added and the resulting mixture was allowed to stand for 4 hours. At the end of this time, this mixture was saturated with sodium chloride

and extracted with eight 30-milliliter portions of ethyl ether. The combined extracts were dried overnight over anhydrous sodium sulfate. The ether was then removed under vacuum in order to avoid overheating the residue. The residue was dissolved in 0.2 milliliter of water and a few drops of the solution were placed on a strip of Whatman No. 1 filter paper. The solvent used for paper chromatography was water-saturated n-heptanol. After allowing the solvent to rise for 18 hours, the strip was dried in the air and sprayed with a 5 percent solution of nickelous chloride. On exposing the resulting damp chromatogram to ammonia gas, the nickelous glyoxime complex appeared as a yellow spot. This chromatogram was compared with another which was prepared in the same way from a known solution of glyoxal. The positions of the spots on the chromatograms from the known and unknown mixtures were identical.

RESULTS AND DISCUSSION

As Christensen[9] noted, the reaction of periodic acid with DL-glycerose in the presence of excess glycerose (Equation 8) is capable of giving



(8)

information about the rates of scission of the two carbon-to-carbon bonds in this substrate. However, in this system there are four principal products, formic acid, formaldehyde, glycolaldehyde and glyoxal, in addition to iodic acid and unreacted glycerose. Such a mixture obviously requires five independent analytical methods for determining its composition. The formic acid produced is determinable by titration of the reaction mixture for total acidity and by subtracting the known equivalent of iodic acid from this total. The formaldehyde concentration can be measured with high precision by the Nash procedure[15]. But, at the present, there are no direct methods for the determination of glyoxal, glycolaldehyde and DL-glycerose in such mixtures. Hence, the complete analysis of this system could not be employed for determining the various oxidation rates.

Nevertheless, the determination of formic acid and formaldehyde concentrations were useful in determining the relative rates of oxidation

of the bonds in glycerose. When the reaction of glycerose with periodic acid goes to completion, the ratio of the concentration of formic acid produced to that of formaldehyde is equal to 2 (Equation 9). On the

$$\begin{matrix} HC{=}O \\ | \\ CHOH \\ | \\ CH_2OH \end{matrix} + 2\ IO_4^- \longrightarrow 2HCO_2H + CH_2O + 2\ IO_3^- \qquad (9)$$

other hand, if, by adding excess glycerose, the reaction can be restricted to the oxidation of intact glycerose molecules only, the ratio of formic acid to formaldehyde becomes the ratio of the rate of scission of the C_1-C_2 bond to that of the bond between C_2 and C_3 ($k_1 : k_2$). Obviously, this ratio may be greater than, less than, or equal to 2.

In the present experiments, on increasing the ratio of DL-glycerose to periodic acid in the oxidation mixtures, the ratios of formic acid to formaldehyde approached a limiting value equal nearly to 4 at 30° (Table I) and approximately equal to 10 at 20° (Table II). This means that in glycerose the oxidation of the bond between the aldehyde group and the secondary carbon is about four times faster than cleavage of the bond between the secondary carbon and the primary carbon. The presence of unoxidized glyoxal in such reaction mixtures was confirmed by converting this substance to its dioxime and identifying this derivative by paper chromatography.

It is worth noting that increasing the temperature of the glycerose-periodic acid reaction mixture produces a greater increase in the rate of the slower reaction. This behavior was expected, since, if the

TABLE I.

Effect of increasing the Ratio of the Initial Concentration of D-Glycerose to that of Periodic Acid at 30°.

Initial periodic acid concentration Moles/liter	D-Glycerose concentration Moles/liter	Concentration of formic acid produced Moles/liter	Concentration of formaldehyde produced Moles/liter
0.082	0.0	0.0076	0.01356
0.082	0.4	0.064	0.01556
0.084	0.2	0.0680	0.01352
0.089	0.2	0.0660	0.01348
0.080	0.1	0.0704	0.01916
0.083	0.1	0.0696	0.01748
0.083	0.05	0.0692	0.03436
0.083	0.05	0.0692	0.03436

TABLE II

Effect of Increasing the Ratio of the Initial Concentration of DL-Glycerose to that of Periodic Acid at 20°.

Initial periodic acid concentration Moles/liter	DL-Glycerose concentration Moles/liter	Concentration of formic acid produced Moles/liter	Concentration of formaldehyde produced Moles/liter
0.084	0.4	0.0736	0.00084
0.053	0.2	0.0704	0.01072
0.084	0.2	0.0708	0.00972
0.093	0.1	0.0748	0.01972
0.078	0.1	0.0744	0.01900
0.085	0.05	0.0672	0.03372
0.088	0.05	0.0672	0.03748

activation energy is lower for cleavage of the C1-C2 bond than it is for cleavage of the C2-C3 bond, the rate of the latter reaction should be affected more by a change in temperature than that of the former.

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