of *aldehydo*-D-glucose pentaacetate[30] in a mixture of 80 ml. of U.S.P. chloroform and 20 ml. of methanol, cooled to 0–5°, was added 460 ml. of an absolute solution of diazomethane (2.5 molar ratio) in ether. The mixture was maintained at room temperature for 40 hr. Decolorizing carbon was then added and the mixture was warmed and filtered. The filtrate was concentrated by a stream of dry air. An ether solution (60 ml.) of the resulting sirup deposited crystalline material in two crops; 14.6 g. (combined), m.p. 84–90°. Upon extensive fractionation from aqueous alcohol and from acetone–ether–petroleum ether, pure material was obtained; yield 4.6 g., m.p. 91–92°, $[\alpha]^{21}\text{D}$ +7° (*c* 3.9, U.S.P. $CHCl_3$); X-ray powder diffraction data[19–21]: 10.05m, 8.29vw, 6.86vs (1,1), 6.22w, 5.44m, 4.88vs(1,1), 4.61vw, 4.17vw, 3.90m, 3.71w, 3.57vw, 3.33s(3), 3.07vw, 2.91w.

The pure substance reduced Fehling solution and was readily soluble in chloroform, acetone and hot ethanol, moderately so in ether and hot water and was insoluble in petroleum ether and cold water. It gave a light yellow precipitate with the characteristic odor of iodoform on treatment of an alkaline solution with iodine in potassium iodide. The crude product contained small amounts of a higher melting material which was not further investigated.

Anal. Calcd. for $C_7H_9O_6(CH_3CO)_5$: C, 50.49; H, 5.98; CH_3CO, 53.2. Found: C, 50.34; H, 5.93; CH_3CO, 53.2.

(b) **From 1-Deoxy-1-diazo-*keto*-D-*gluco*-heptulose Pentaacetate.**[33]—To a solution of 1.32 g. of crystalline 1-deoxy-1-diazo-*keto*-D-*gluco*-heptulose pentaacetate[9] in 15 ml. of chloroform was added 4 ml. of 47% hydriodic acid. Gas evolution was immediate and the mixture turned dark red. After about 5 min., when gas evolution had ceased, the chloroform solution was washed with water, sodium thiosulfate solution and again with water. The sirup obtained on solvent removal, under reduced pressure, from the dried chloroform extract, was dissolved in 10 ml. of benzene and chromatographed on a Micro Cel C[34] column (4.4 × 19.5 cm.) using 200 ml. of benzene:*t*-butyl alcohol (100:1 by vol.) as developer. Alkaline permanganate streaking[29] revealed two zones located at 3–5.5 and 9–12.5 cm. from the column top.

The top zone yielded a light yellow sirup which resisted crystallization. The bottom zone was eluted with acetone and evaporation of the eluate gave a bright yellow sirup which crystallized on scratching. This was recrystallized from ether; yield 370 mg. (two crops). Recrystallization was effected from ether and ether–petroleum ether (b.p. 30–60°); m.p. 91–92°, $[\alpha]^{20}\text{D}$ +6° (*c* 4.8, abs. $CHCl_3$), X-ray powder diffraction data identical with the product described in (a) above. The crude material was contaminated with a higher melting material which was not further investigated.

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[33] Preliminary experiments were carried out in this Laboratory by Drs. S. M. Olin and B. S. Wildi.

[34] A synthetic magnesium silicate produced by Johns–Manville Co., New York, N. Y.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION[1]]

Chlorous Acid Oxidation of Periodate Oxidized Cornstarch[2]

BY B. T. HOFREITER, I. A. WOLFF AND C. L. MEHLTRETTER

RECEIVED JULY 1, 1957

Periodate oxystarches prepared from cornstarch and containing from 5 to 100% of dialdehyde units have been quantitatively converted to the corresponding dicarboxyl derivatives by oxidation with chlorous acid. The reactions take place in aqueous acid medium and the products are isolated in good yield by precipitation with alcohol. Following investigation of reaction variables, preferred conditions found for the oxidation were: 1 *M* sodium chlorite, 0.5 *M* acetic acid, mole ratio sodium chlorite/aldehyde of 4, 25° and 3 hours reaction time.

The present study is part of an extensive program for the investigation of periodate oxystarches and their derivatives. Interest in these materials was stimulated by the development at this Laboratory of an economic electrolytic process for their preparation.[3,4] A recent publication[5] has described in some detail the physical and chemical properties of periodate oxystarches having a wide range of dialdehyde contents. Although undegraded oxidation or reduction products of periodate oxystarches have not been prepared *per se*, quantitative reduction is employed analytically,[6] and both oxidation and reduction, followed by hydrolysis, have been used for structural studies and preparation of monomeric materials.[7–9]

The purpose of the present work was the preparation of a series of carboxylated derivatives through selective oxidation of the carbonyl groups in periodate oxidized cornstarches. This objective was accomplished, and the products, referred to as dicarboxyl starches, are polyelectrolytes which have both theoretical and practical interest. For example, comparison of the pasting characteristics of dicarboxyl starches, both as free acid and as the sodium salt with those of the parent dialdehyde starches, will provide information on the influence of the various functional groups either on granule gelatinization or on viscosity characteristics of pastes derived from the respective polymeric materials. Further, the dicarboxyl starches in their general structural relationship to such natural and synthetic hydrophilic colloids as alginates, pectins, vegetable gums and carboxymethylated polysaccharides should possess similar valuable physical properties.

Chlorous acid was chosen as the oxidant because of its known specificity for the oxidation of aldehyde to carboxyl groups in acid medium,[10] a reaction condition particularly desirable because of the known sensitivity of periodate oxystarches to alkali. As established by Jeanes and Isbell[10] for the course of reaction of chlorous acid with

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[1] One of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

[2] Presented at 130th Meeting of the American Chemical Society, Atlantic City, N. J., September 16–21, 1956, Abstracts p. 14-D.

[3] W. Dvonch and C. L. Mehltretter, THIS JOURNAL, **74**, 5522 (1952).

[4] C. L. Mehltretter, J. C. Rankin and P. R. Watson, *Ind. Eng. Chem.*, **49**, 350 (1957).

[5] J. W. Sloan, B. T. Hofreiter, R. L. Mellies and I. A. Wolff, *ibid.*, **48**, 1165 (1956).

[6] J. C. Rankin and C. L. Mehltretter, *Anal. Chem.*, **28**, 1012 (1956).

[7] Allene Jeanes and C. S. Hudson, *J. Org. Chem.*, **20**, 1565 (1955).

[8] J. W. Sloan, C. L. Mehltretter and I. A. Wolff, Abstracts, 128th Meeting of the American Chemical Society, Minneapolis, Minn., September, 1955.

[9] J. K. Hamilton and F. Smith, THIS JOURNAL, **78**, 5907 (1956).

[10] Allene Jeanes and H. S. Isbell, *J. Research Natl. Bur. Standards*, **27**, 125 (1941), RP 1408.

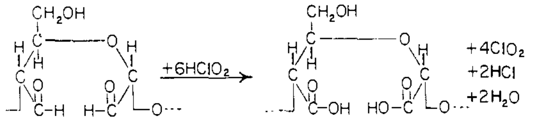
TABLE I

PREPARATION AND ANALYSIS OF DICARBOXYL STARCHES

Dialdehyde[a] units, %	Oxidation conditions: Molarity ClO_2^-	Oxidation conditions: Molarity HOAc	Oxidation conditions: Mole ratio ClO_2^-/–C(=O)–H	Oxidation conditions: Reacn. time, hr.	Yield, %	Sodium, %: Product first isolated	Sodium, %: Completely neutralized	Sodium, %: Theory[b]	Dicarboxyl units, %: A[c]	Dicarboxyl units, %: B[d]
5.0	0.10	0.11	5.4	24	100	0.09	. . .	. . .	2.1	. . .
5.0	.79	.70	40	24	. . .	.74	1.20	1.39	4.7	4.3
10	.21	.22	5.4	22	97.4	.18	. . .	. . .	8.3	. . .
10	.76	.70	11.3	24	. . .	1.58	2.19	2.72	9.5	8.0
20	.42	.44	5.6	24	92.1	4.85	5.03	5.21	21	19
40	.76	.70	5.0	24	97.1	8.24	. . .	. . .	39	. . .
40	1.57	1.40	10.2	24	94.3	9.16	9.52	9.60	42	40
40	1.57	1.40	10.2	48	. . .	9.56	9.82	9.60	44	41
60	1.40	1.26	3.4	24	69.2	. . .	12.93	13.37	61	58
77	1.57	1.40	5.3	18	86.4	. . .	16.98	16.17	78	82
98–100	1.57	1.40	4.0	24	76.1	18.00	19.78	19.49	100	102

[a] In starting material. [b] Calculated on the basis of dialdehyde content, assuming 100% conversion to dicarboxyl. [c] Calculated from % Na and exchange equivalent. [d] Calculated from % Na only, on completely neutralized material.

simple aldoses, the oxidation of the repeating unit of dialdehyde starch may be expressed as



Considerable study[11–17] has been given in the past to the chlorite oxidation of oxycelluloses mainly from the standpoint of carbonyl analysis, or as a means for increasing their alkali stability. During the course of this investigation, Davidson and Nevell[18] reported a parallel study of the chlorous acid oxidation of periodate oxycelluloses. However, the series of oxycelluloses used did not cover the range of dialdehyde content of our oxystarches nor were the extents of conversion to the carboxylated products as complete. This difference doubtless is caused by the nature of the reacting materials. Application of our chlorous acid oxidation procedure to a periodate oxycellulose (92% dialdehyde) resulted in only 83% conversion of carbonyl to carboxyl.

Experimental

Oxystarch Preparation.—As previously described[5] periodate oxystarches of varying extents of oxidation from 5–100% (dialdehyde units/100 units) were prepared from commercial non-defatted cornstarch using unbuffered sodium metaperiodate in starch slurries maintained at 0°. In general these oxidations were carried out for 24 hr. The dialdehyde contents were determined from the amounts of periodate consumed during preparation and in addition by one or more of the following analytical methods: hydroxylamine,[5] quantitative alkali consumption[19] and sodium borohydride.[6]

Oxidation of Dialdehyde Starches.—Oxidations were conducted in open beakers or flasks immersed in a water-bath at 25 ± 1°. The reaction mixtures consisted of periodate oxystarches slurried in solutions containing acetic acid and sodium chlorite. For one series of oxidations (Table I) a wide range of reaction variables was employed. Nevertheless, quantitative or near quantitative conversion of carbonyl to carboxyl was obtained in all instances.

Another series of oxidations of oxystarches was carried out under the following preferred reaction conditions: 1 *M* $NaClO_2$, 0.5 *M* acetic acid, mole ratio chlorite/aldehyde of 4 and a reaction temperature of 25°. Quantitative oxidations again resulted as shown in Table II.

TABLE II

RATE OF CHLORITE OXIDATION FOR VARIOUS PERIODATE OXYSTARCHES

Dialdehyde units, %	Dicarboxyl units, %, Oxidation time, hr.: 0.25	1	3	24	Conversion, % (at 3 hr.)
10	8.2	9.4	9.9	10	99
20	14	18	21	21	105
40	33	40	40	41	100
61	52	58	58	58	95
74	68	72	73	71	99
94	87	88	91	92	97

As the reactions progressed, the suspensions turned from a light yellow to a deep red color with evolution of a considerable quantity of chlorine dioxide gas. At the conclusion of the reaction nitrogen was bubbled through the reaction mixture to remove all but traces of chlorine dioxide.

Recovery of Dicarboxyl Starches.—Oxidized products were precipitated by pouring the reaction mixture, with rapid stirring, into 2 volumes of absolute ethanol. For those levels of oxidation at which the granule form persists (20% and below), the filtered dicarboxyl starches were then washed repeatedly with aqueous alcohol solutions (80–90% ethanol) until free of chloride. Initial precipitation with ethanol resulted in gummy products in those instances in which the dicarboxyl starch was either partially or completely dispersed in the reaction mixture. These were redispersed in a minimum quantity of water and reprecipitated at a higher alcohol concentration (80–90%) by pouring into the alcohol with rapid stirring. Several reprecipitations generally were required to obtain a negative test for chloride.

The products obtained by alcoholic precipitation were almost completely in the form of sodium salts (Table I) although precipitated from an acid solution. In later preparative work the reaction mixtures were adjusted to alkaline *p*H (*ca.* 8.3) and the completely neutralized materials then isolated as described previously. In all cases, the dicar-

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[11] H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **29**, 131 (1942), RP 1491.

[12] G. F. Davidson and T. P. Nevell, *J. Textile Inst.*, **39**, T102 (1948).

[13] R. E. Reeves and F. E. Darby, Jr., *Textile Research J.*, **20**, 172 (1950).

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[19] B. T. Hofreiter, B. H. Alexander and I. A. Wolff, *Anal. Chem.* **27**, 1930 (1955).

boxyl starches were dried in a vacuum desiccator over calcium chloride.

Rate of Reaction.—Rate studies to determine optimum oxidation conditions were carried out with representative members of the periodate oxystarch series. Such experiments were conducted in 150-ml. beakers using 5 g. of periodate oxystarch. In all runs the reactants were introduced simultaneously and the reaction timed from that point. The change in *p*H was followed, and aliquots were withdrawn through an inverted pipet and the products isolated as previously described. Figure 1 illustrates the results for the oxidation of a 40% oxystarch. Under the preferred conditions of oxidation, the reaction is complete in less than one hour. Also, the rate of decrease in reducing power and the rise of *p*H parallel the increase in carboxyl content. The reducing power measured by the Somogyi method[20] is expressed arbitrarily as mg. glucose per 100 mg. of sample. The very slight amount of residual reducing power possibly is due to hydrolysis in the alkaline copper reagent. Table II shows the rate study results for oxystarches having from 10 to 94% dialdehyde content. It can be seen that differences between the 3- and 24-hr. results are within the experimental error of the determination.

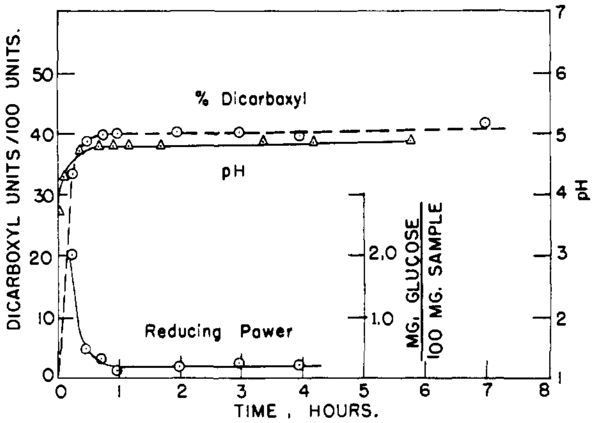

Fig. 1.—Chlorous acid oxidation of 40% dialdehyde starch.

Analysis.—Exchange equivalents (mg. of product per meq. of acid in a Dowex 50[21] resin column eluate) were determined by cation deionization of solutions having sufficient concentration to provide reliable titrations. The solutions were obtained by heating water slurries of the products on a steam-bath for approximately 5 minutes. The more highly oxidized materials required shorter periods of heating for dispersion. Titrations were carried out using 0.1 *N* sodium hydroxide with phenolphthalein as indicator. Identical end-points were obtained by potentiometric titration. Direct titration of the pasted products using 0.1 *N* sodium hydroxide yielded the neutralization equivalents.

Calculations.—Dicarboxyl contents were calculated from the various analyses as follows: The relationship between percentage sodium content and percentage dicarboxyl for a completely neutralized product is

$$\% \text{ Na} = \frac{(46)(\% \text{ dicarboxyl units})(100)}{(236)(\% \text{ dicarboxyl units}) + 162(100 - \% \text{ dicarboxyl units})} \quad (2)$$

where 236 and 162 are the molecular weights of sodium dicarboxylate and anhydroglucose units, respectively. This relationship assumes complete conversion of carbonyl to carboxyl, but could be applied to partially reoxidized products without significant error since the difference in molecular weight between a dialdehyde unit and an anhydroglucose unit is small. Equation 2 may be rearranged as

$$\% \text{ dicarboxyl units} = \frac{[\% \text{ Na}]\ [16{,}200]}{4600 - [\% \text{ Na}]\ [74]} \quad (3)$$

However, most analyses were performed on products in which the carboxyls were not completely neutralized, as with those obtained in the first series of oxidations illustrated in Table I. For these calculations, both the exchange equivalent and the neutral equivalent were required. Consider, as an example, the following relationship used to calculate the dicarboxyl content of a product whose original dialdehyde content was known to be 40%

$$\text{Exchange equiv.} = \frac{(162)\ (60) + 192y + 236x + 160\ [40 - (x + y)]}{2\ (x + y)} \quad (4)$$

where y is the number of free-acid dicarboxyl units per 100 units and x is the number of neutralized dicarboxyl units per 100 units. A relationship of x and y is readily obtained from the data since the ratio of exchange equivalent to neutral equivalent is identical to $y/(x + y)$. Alternatively, a relationship between x and y for the partially neutralized products can be derived from the sodium analysis and the exchange equivalent or neutral equivalent.

Investigation of Reaction Variables. Ratio of Chlorite/Aldehyde.—The instability at low *p*H of chlorous acid is well known; therefore, additional chlorite beyond that required by the stoichiometry of the reaction must be provided. To determine the applicability of equation 1, and hence the minimum chlorous acid required for the oxidations of periodate oxystarch, the following experiments were carried out: A 60% periodate oxystarch was oxidized at 25° using 1 *M* sodium chlorite, 0.5 *M* acetic acid and a reaction time of 24 hr. to ensure complete reaction. Mole ratios of chlorite/aldehyde were varied from 0.5 to 5.0. Figure 2 shows the plot of the various mole ratios *versus* the per cent. of theoretical conversion. The progressive deviation from the theoretical curve probably is due to several factors which include: first, decomposition of chlorous acid and, consequently, the presence of insufficient quantities; and, second, the possible loss of small quantities of carboxyl-rich fragments through solubilization. Also, it should be pointed out that variation of the sugar concentration, in the case of chlorous acid oxidation of xylose, was shown by Wilson and Padgett[17] to result in changes in the stoichiometric ratio. However, under our particular reaction conditions, verification of the theoretical mole ratio of 3[10] (chlorite/aldehyde) is strongly indicated for the chlorous acid–oxystarch reaction.

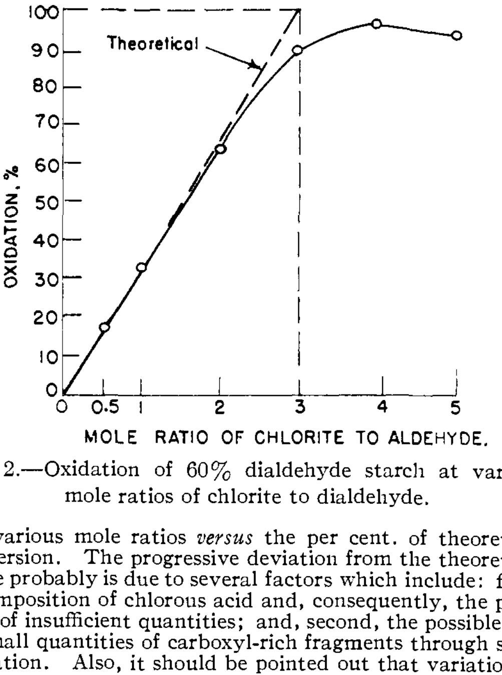

Fig. 2.—Oxidation of 60% dialdehyde starch at various mole ratios of chlorite to dialdehyde.

Reaction *p*H.—Chlorous acid oxidation in the case of an aldose was found by Launer, *et al.*,[22,23] to be first order with respect to both aldose and chlorous acid concentration. It might be expected that the periodate oxystarch and chlorous acid system would react in a similar fashion. In all of the oxidations it was noted that the *p*H increased as the reaction progressed, in fact, paralleling the increase of carboxyl content (Fig. 1). The change of *p*H with reaction time is illustrated in Fig. 3 for a 60% periodate oxystarch oxidized at

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(20) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(21) Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

(22) H. F. Launer, W. K. Wilson and J. H. Flynn, *J. Research Natl. Bur. Standards*, **51**, 237 (1953), RP 2456.

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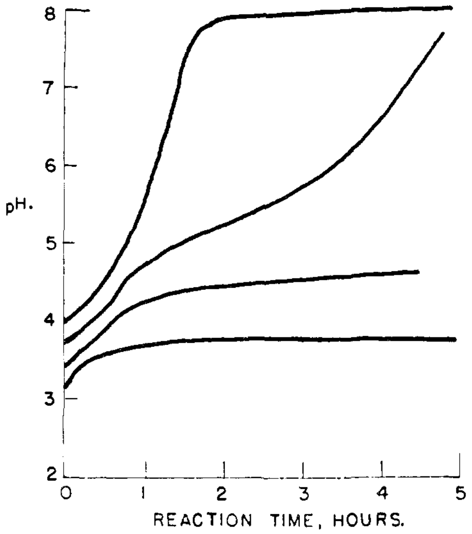

Fig. 3.—Change of *p*H as a function of initial acidity during oxidation of 60% dialdehyde starch.

various initial *p*H values (obtained by varying the amounts of acetic acid) but with other conditions held constant. The chlorous acid oxidation (Equation 1) results in consumption of protons and, consequently, a minimum level of total acidity must be present for complete reaction. Our results indicate that this requirement is met when the mole ratio chlorite/acetic acid is at least 2.

Discussion

Chlorous acid has proved to be an excellent oxidizing agent for the quantitative conversion of dialdehyde starches to the corresponding dicarboxyl starches. Analyses for carbonyl and carboxyl reveal the highly specific action of this oxidant which minimizes degradation of the dialdehyde starch. Retention of the granule form at dicarboxyl contents of 20% and below as well as the ability to form highly viscous aqueous dispersions is evidence that slight, if any, molecular degradation occurs during the oxidation. The solution properties as well as other physical and chemical characterizations of these materials are now under investigation and will be described separately.

Acknowledgment.—The authors wish to acknowledge the assistance of Mr. Darrell D. Ebbing in many of the preparations and analyses required and of Mrs. Clara E. McGrew for the sodium analyses.

PEORIA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oxidation of Amylopectin with Hypochlorite at Different Hydrogen Ion Concentrations[1,2]

BY ROY L. WHISTLER AND RICHARD SCHWEIGER

RECEIVED AUGUST 28, 1957

The principal organic products from oxidation of corn starch amylopectin with hypochlorite at different *p*H values are glyoxylic and D-erythronic acids. These products are obtained in maximum yield at a *p*H slightly above 7. Reaction is envisioned as formation of a carbonyl on carbon C_2 or C_3, enediol formation involving C_2 and C_3, addition of hypochlorous acid and subsequent further oxidation to cleave the carbon–carbon bond and produce a dicarboxylated unit in the amylopectin chain.

Oxidative reactions are of importance to industries processing polysaccharides but are of greatest importance to those industries producing starch and cellulose. Starch is often purposefully oxidized to give it new and useful properties while cellulose is unintentionally oxidized when pulps and cellulosic textiles are bleached. The most common oxidant, used in these instances, is sodium hypochlorite. Although chlorine is often used in bleaching cellulose it is commonly added to an alkaline system where it is transformed to hypochlorite. As a consequence, some importance is attached to the establishment of the mechanism through which hypochlorite reacts with polysaccharides. One of the first steps must be the determination of the nature of the oxidation products formed. Earlier,[3] it has been shown that hypochlorite at *p*H 9 and 11 reacts with corn amylose and with methyl 4-*O*-methyl-D-glucopyranosides at 25° to produce extensive cleavage of anhydro D-glucose units between carbon atoms 2 and 3. This evidence is based on isolation of D-erythronic acid, its γ-lactone, glyoxal and glyoxylic acid.

The present work provides information concerning the effect of *p*H on the oxidation rate and on the type and amount of oxidation products when hypochlorite reacts with amylopectin. Corn amylopectin is used in place of corn amylose to avoid retrogradive effects. Although amylopectin molecules are branched, the degree of branching is not great and most of the glycosidic linkages are of the α-D-(1 $\rightarrow$ 4) type which almost exclusively form the glycosidic linkages of amylose. It is not expected, therefore, that the mechanism of amylopectin oxidation should be greatly different from that of amylose.

The rate at which hypochlorite oxidizes amylopectin in solution is greatly influenced by hydrogen ion concentration. When amylopectin at 1.25% concentration in water is treated with sodium hypochlorite at a level of two moles of oxidant per mole of anhydro D-glucose unit, the oxidant is consumed at a rate indicated in Fig. 1. This graph, or a plot of the period of time for half consumption of

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[1] Paper presented before the joint Meeting of the Division of Carbohydrate Chemistry and Division of Cellulose Chemistry at the 131st Meeting of the American Chemical Society at Miami, Florida, April, 1957.

[2] Journal Paper No. 1160 of the Purdue Agricultural Experiment Station.

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