[CONTRIBUTION FROM THE IOWA AGRICULTURAL EXPERIMENT STATION]

Studies on the Schardinger Dextrins. V. Periodate Oxidation[1]

BY DEXTER FRENCH AND ROBERT L. MCINTIRE[2]

Structural studies on the α- and β-Schardinger dextrins[3,4,5] have indicated that these are cyclic, symmetrical molecules containing six and seven glucose residues, respectively, united through α-1,4-glycosidic bonds. In view of the paucity of chemical evidence on the structure of the γ-dextrin,[6] it seemed advantageous to carry out a study on the periodate oxidation behavior of this substance, in comparison with the better characterized α- and β-dextrins.[7]

Initially attention was directed to the stoichiometry of the reaction: the number of moles of periodate consumed per glucose residue, the examination of the oxidation products for formic acid and formaldehyde, and exploration for conditions necessary for complete oxidation without excessive overoxidation. Figure 1 shows that a 10% excess of periodate leads smoothly to a consumption of one mole of periodate per glucose residue in cycloheptaamylose; this excess was then used in most subsequent experiments.

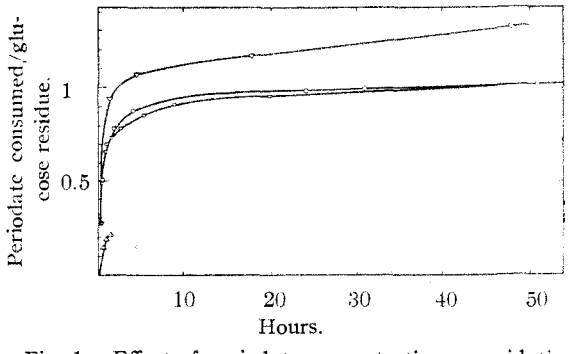

Fig. 1.—Effect of periodate concentration on oxidation of β-dextrin at 27°, moles of periodate per glucose residue: ▽, 3.36; O, 1.10; □, 1.06; △, 0.22.

Figures 2 and 3 illustrate the progress of periodate oxidation as measured by periodate consumed and by change in optical rotation. From these graphs it is apparent that the α-, β- and γ-dextrins are very nearly parallel in their behavior with regard to both periodate consumption and rotatory change. No detectable formaldehyde or formic acid was produced by oxidation of any of the Schardinger dextrins. The close similarity in oxidation behavior together with the failure to produce formic acid or formaldehyde make it appear likely that the γ-dextrin has a cyclic structure analogous to the α- and β-dextrins.[6c]

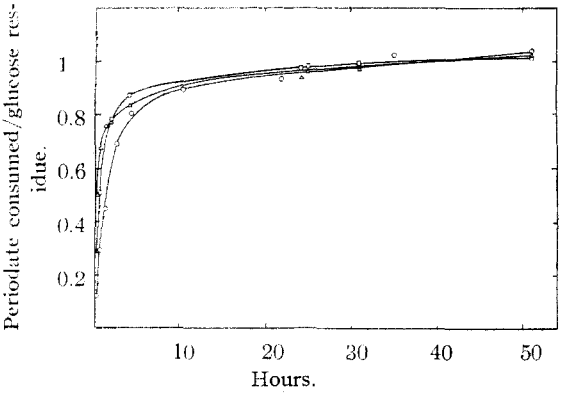

Fig. 2.—Periodate oxidation of the Schardinger dextrins at 27°: O, α-; □, β-; △, γ-, initial periodate was 1.1 moles per glucose residue.

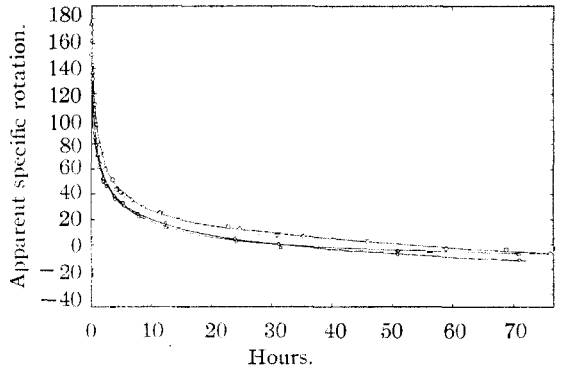

Fig. 3.—Changes in optical rotation during periodate oxidation of the Schardinger dextrins at 27°: O, α; □, β; △, γ; initial periodate 1.1 moles per glucose residue.

In the second part of this study an attempt was made to analyze the kinetics of the oxidation reactions. In contrast to the situation observed with ethylene glycol,[8] the low value of the equilibrium constant for periodate complex formation together with the low carbohydrate concentration available, due to limited solubility, render the initial kinetics approximately second order (actually about 0.9 order in each reactant at the concentrations used).

Let S = Schardinger dextrin, P = periodate, G = glycolic groups remaining in the initial and all subsequent oxidation products, A = dialdehyde groups, and n = the number of glucose

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(2) From the M.S. thesis of Robert L. McIntire, Iowa State College, 1948.

(3) Freudenberg and Meyer-Delius, *Ber.*, **71**, 1591 (1938).

(4) Karrer and Nageli, *Helv. Chim. Acta*, **4**, 169 (1921).

(5) French and Rundle, THIS JOURNAL, **64**, 1651 (1942).

(6) (a) French, Levine, Pazur and Norberg, *ibid.*, **71**, 353 (1949); (b) Freudenberg and Jacobi, *Ann.*, **518**, 102 (1935); (c) Freudenberg and Cramer, *Z. Naturforsch.*, **3B**, 464 (1948).

(7) Myrbäck and Järnström, *Arkiv Kemi*, **1**, 129 (1949); *C. A.*, **43**, 6986 (1949).

(8) Duke, THIS JOURNAL, **69**, 3054 (1947).

residues per molecule of Schardinger dextrin. Quantities in italics represent concentrations. If one symbolizes[9] the over-all oxidation by the set of consecutive second order equations

$$S + P \longrightarrow (n-1)G + A \quad (1)$$
$$G + P \longrightarrow A \quad (2)$$

the following rate equations should hold

$$dS/dt = -k_1SP \quad (3)$$
$$dP/dt = -k_1SP - k_2GP \quad (4)$$
$$dG/dt = (n-1)k_1SP - k_2GP \quad (5)$$

Following a method recently outlined[10] kinetic equations of this type may be integrated by introducing the parameter $\theta = \int_0^t P dt$. Since $d\theta = P dt$, equations 3–5 become

$$dS/d\theta = -k_1S \quad (6)$$
$$dP/d\theta = -k_1S - k_2G \quad (7)$$
$$dG/d\theta = (n-1)k_1S - k_2G \quad (8)$$

Integrating equations 6–8 as a set and setting $x = k_1\theta$ and $y = k_2/k_1$, we obtain

$$(P_0 - P)/nS_0 = 1 - \frac{e^{-x}}{n} - \frac{(n-1)}{n(y-1)}(ye^{-x} - e^{-xy}) \quad (9)$$

The value of θ at any time was determined by graphical integration of $\int_0^t P dt$ and thereby the relationship between P and θ established. Calculated values of $(P_0 - P)/nS_0$ using a range of y values were then plotted against log x and the resulting curves compared with a template of the experimental data relating $(P_0 - P)/nS_0$ to log θ. Once having an approximate value of y, curves varying by 0.05 y unit were constructed in this neighborhood and compared with the experimental curve. The value of $y = k_2/k_1$ was obtained by interpolating the experimental curve between the calculated curves giving the *best fit*, and k_1 was directly calculable from the lateral displacement of log x on the log θ scale. The experimental graphs together with the calculated curves giving best fit are presented in Fig. 4.

From this comparison of calculated curves with experimental points it appears obvious that (a) there is a considerable hindrance to the first oxidative step and (b) although subsequent oxidation proceeds more rapidly for a time it again slows down so that the complete kinetic analysis would require more terms. The agreement between the curves is within the experimental error up to the stage of oxidation at which approximately three glucose residues have been converted to dialdehyde structures. Beyond this point it seems reasonable that the rigidity of the original cyclic Schardinger dextrin ring has collapsed, leaving the aldehyde groups in positions favorable

(9) The representation of the *kinetics* of the reaction by second order equations is not meant to imply that the *mechanism* is second order.

(10) French, THIS JOURNAL, **72**, 4806 (1950).

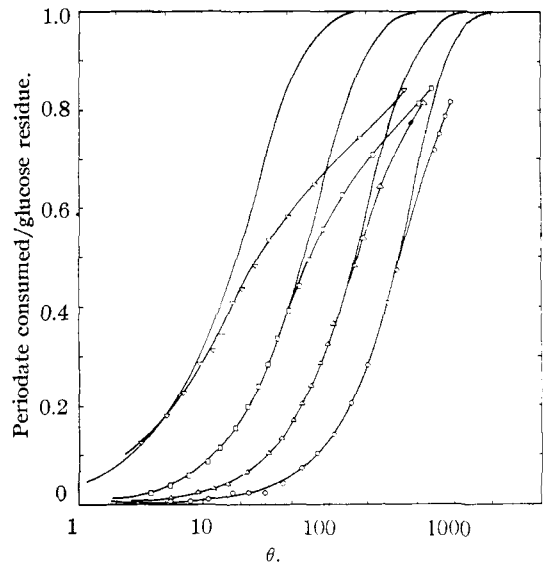

Fig. 4.—Rate curves for the periodate oxidation of the Schardinger dextrins at 4°: O, α; Δ, β; □, γ; ▽, amylodextrin; $\theta = \int_0^t$ (periodate) dt (see text).

for the formation of intramolecular hemiacetals and thus slowing down the rate of periodate attack by preventing periodate complex formation. The possibility that accumulating iodate tends to slow down the reaction by forming unreactive glycol complexes has been ruled out by measuring the oxidation rate in the presence of added iodate. No change in rate was observed, even with a three-fold ratio of iodate to periodate.

In the case of amylodextrin, the calculated curve was based on the assumption that all the glycol groups react independently and with equal ease. The experimental deviation from this type of curve indicates clearly that as the reaction proceeds the average kinetic constant decreases, again possibly due to intramolecular hemiacetal formation.

It is rather striking that the rate of oxidation increases markedly through the series α-, β- and γ-dextrin. Furthermore, inhibition of the initial oxidative step diminishes in the same direction (nk_2/k_1 = 3.6, 1.8 and 1.6, respectively). Even so, the γ-dextrin still shows much hindrance to oxidative attack in comparison to amylodextrin, and we conclude tentatively that this hindrance is due to and to some extent a measure of the relative rigidity of the Schardinger dextrin rings.[11]

Experimental

Methods and Material.—The Schardinger dextrins were prepared in this laboratory following the method previously outlined.[6] The Nägeli type amylodextrin was prepared by treating potato starch granules at room temperature for several months in 15% sulfuric acid followed by removal of the acid, thorough washing and repeated

(11) A similar hindrance has been noted in the acid hydrolysis of the Schardinger dextrins; see French, Levine and Pazur, *ibid.*, **71**, 356 (1949); Swanson and Cori, *J. Biol. Chem.*, **172**, 797 (1948).

crystallization from 60% methanol. The average chain length from reducing value was 14.4 glucose units and the material was completely converted to maltose by β-amylase. Measured amounts of standardized solutions of the dextrins were allowed to react with measured quantities of periodate under various conditions. Analyses for the concentration of unreacted periodate were carried out by titration of the iodine liberated on addition of excess potassium iodide and standard arsenite to the bicarbonate buffered solution. Tests for formic acid were made by destroying the periodate with an excess of propylene glycol and titrating with 0.1 *N* sodium hydroxide to the phenolphthalein end-point. The alkali titrations of 15-ml. aliquots of the oxidation mixtures were equivalent to those obtained on periodate blanks, indicating that no formic acid was produced. The dimedon test[12] did not reveal the presence of formaldehyde in any of the digests.

Total Consumption of Periodate and Rotational Changes.—Conditions necessary to avoid over-oxidation were ascertained by treating 0.01 *M* β-dextrin with varying amounts of sodium periodate at room temperature. The reaction was followed by measuring the periodate consumption (Fig. 1) as well as the change in optical rotation; when an excess of oxidant was present the apparent rotations dropped to about −11°, then very gradually rose 3 to 4°.

The addition of buffers to the oxidation mixtures caused wide differences in the optical rotations observed. With either sodium or potassium periodate, no added buffer, and *p*H about 4, the rotation dropped to −11°. In the presence of a *p*H 6.5 potassium phosphate buffer the final rotation was 75–78°, and with sodium periodate buffered with acetic acid–sodium acetate to *p*H 4.1 or 5.5 the final rotation was 50°. Since no appreciable amount of acid was produced in the reaction, there was very little shift in the *p*H of reaction mixtures without additional buffer. This effect of buffers on rotation was not investigated further.

Following the preliminary experiments with β-dextrin, solutions of α-, β- and γ-dextrins were oxidized under identical conditions with 1.1 moles of sodium metaperiodate/glucose residue. The oxidations were carried out at 28° without added buffer. The rotational changes and periodate consumption are plotted in Figs. 2 and 3. After four days, the consumption of periodate was not greater than 1.03 moles/glucose residue.

Kinetic Analysis.—The order of the initial part of the reaction at 4° was determined in 25-minute oxidations with beta dextrin:

S_0	P_0	$-\Delta P/25$ min.	
0.00254	0.01660	0.00037	
.00254	.04975	.00100	Order in periodate = 0.90
.00506	.01660	.00070	Order in Schardinger dextrin = 0.92

Oxidation of dextrin–periodate mixtures (dextrins *ca.* 0.04 *M* with respect to glucose residues) was then followed at 4° over 2–3 days, the amount of periodate consumed determined by titrating aliquots, and the data used for the determination of the rate constants as explained above. The values of the second order rate constants k_1 and k_2 so obtained are: α, $k_1 = 9.0$, $k_2 = 5.4$; β, $k_1 = 33$, $k_2 = 8.3$; γ, $k_1 = 105$, $k_2 = 21.0$; all expressed as liters × mole^{-1} × sec.$^{-1}$ × 10^{-5} at 4°, with an uncertainty of about 5% of the stated values for each constant. The curves calculated for these constants together with the experimental points appear in Fig. 4. Comparable data for a Nägeli-type amylodextrin of average chain length approximately 14.4 glucose residues are compared (Fig. 4) with a curve calculated on the assumption that all the glucose residues are oxidized at the same rate; $k_{av.} = 65 \times 10^{-5}$ liters × moles^{-1} × sec.$^{-1}$.

Effect of Iodate on Reaction Velocity.—To each of five flasks was added sufficient sodium periodate and β-dextrin so that the initial concentration after dilution was 0.0133 *M* and 0.00161 *M*, respectively, together with sodium iodate to the extent of 0, 0.0056, 0.0140, 0.0280 and 0.0420 *M*. After 61 min. at 4° the remaining periodate was determined to be the same in each flask: 0.0126 ± 0.0001 *M*.

Summary

Oxidation of the α-, β- and γ-dextrins with sodium periodate has been characterized as producing no formic acid or formaldehyde and consuming one mole of periodate per glucose residue. The kinetics of the reaction have been analyzed by an approximate method and up to about 40% total oxidation agree with curves calculated for an initially hindered reaction followed by a more rapid oxidation. The rate of the initial oxidation increases in the order: alpha, beta, gamma. The initial oxidation of the γ-dextrin is still subject to an inhibition not characteristic of a low molecular weight amylodextrin.

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(12) Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

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Studies on the Schardinger Dextrins. VI. The Molecular Size and Structure of the γ-Dextrin[1]

BY DEXTER FRENCH, DORIS W. KNAPP AND J. H. PAZUR

The application of crystallographic procedures for the determination of molecular size to the α- and β-dextrins indicated that these are composed of six and seven glucose residues, respectively.[2] A similar study has now been directed toward the establishment of the size of the γ-dextrin,[3] previously regarded by Freudenberg[4] as a cyclic heptasaccharide. In the present study it was not found possible to determine unambiguously the number of glucose units per molecule of γ-dextrin by crystallographic procedures alone, but in addition it was necessary to examine

(1) Journal Paper No. J-1782 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1116; supported in part by a grant from the Corn Industries Research Foundation.

(2) French and Rundle, THIS JOURNAL, **64**, 1651 (1942).

(3) French, Levine, Pazur and Norberg, *ibid.*, **71**, 353 (1949).

(4) (a) Freudenberg and Jacobi, *Ann.*, **518**, 102 (1935); (b) Freudenberg, Plankenhorn and Knauber, *Chem. and Ind.*, 731 (1947); (c) *Ann.*, **558**, 1 (1947); (d) FIAT Report No. 1096 (duplicate publications). (e) A late publication, Borchert, *Z. Naturforsch*, **3b**, 464 (1948), presents X-ray evidence that the γ-dextrin is either a tetrasaccharide or an octasaccharide. (f) A tetrasaccharide structure is not favored by Freudenberg and Cramer, *ibid.*, **3b**, 464 (1948), as being sterically unlikely as well as being out of line with the trend in optical rotations established by the α- and β-dextrins. Freudenberg and Cramer now accept the hexasaccharide character of the α-dextrin and the heptasaccharide character of the β-dextrin.