The optical rotation of the product and the high purity of the Δ^6-cholestenyl acetate used along with our experience that the lithium aluminum hydride deacetylation of steroid acetates gives a very pure product, suggested that the Δ^6-cholestenol prepared was a pure compound.

Dilution Experiments with Cholesterol-7β-*t*.—In order to determine whether part of the radioactivity of the cholesterol-7β-*t* synthesized above was due to tritium-containing Δ^6-cholestenol (specifically, Δ^6-cholestenol-5α-*t*), a small amount (0.0101 g.) of cholesterol-7β-*t* was mixed with Δ^6-cholestenol (0.3447 g.) and the acetate of the mixture was submitted to purification to remove the 7β-tritiocholesteryl acetate. The acetate was prepared by dissolving the mixture in 4 ml. of pyridine, treating it with 1 ml. of acetic anhydride for 36 hours at 42°, and working up the product in the usual way. The activity of the material was determined at the various purification stages. The data obtained are: cholesterol-7β-*t* had an activity of 3550×10^6 d.p.m./mmole[26] (disintegrations per minute/millimole). The mixture of the two alcohols should have an activity of 101×10^6 d.p.m./mmole. The Δ^6-cholestenyl acetate obtained after one crystallization from 10 ml. of 95% ethanol had an activity of 94.2×10^6 (the same units, d.p.m./mmole, are used throughout). Subsequent crystallizations were carried out in absolute alcohol. A second crystallization gave a product with an activity of 63.6×10^6, three more crystallizations gave a product with an activity of 25.2×10^6. Addition of 9.5 mg. of cholesteryl acetate to the 188 mg. of the product and two more crystallizations from alcohol gave Δ^6-cholestenyl acetate with an activity of 12.2×10^6.

The above data indicate that of the activity 101×10^6, less than 12.2×10^6 is due to tritiated Δ^6-cholestenol. In fact, the trend observed in these data suggests that continued purification would have lowered this limit even further.

Purification of Cholesterol-7β-*t* from Traces of Δ^6-Cholestenol-5α-*t*.—A mixture of 0.054 g. of cholesterol-7β-*t* (m.p. 148–148.8°) and 0.015 g. of Δ^6-cholestenol was dissolved in 0.35 ml. of ether and 1 ml. of acetic acid. The solution was allowed to stand for 10 minutes and an additional 1.65 ml. of acetic acid was added. It was cooled to 18° and was treated with 0.29 ml. of a bromine solution in acetic acid containing some sodium acetate (0.68 g. of bromine and 0.31 g. of sodium acetate in 6 ml. of acetic acid). The reaction mixture was placed in a bath at 3° and, after 4 minutes, the crystalline cholesterol-7β-*t* dibromide which had precipitated out was collected by filtration and was washed with cold acetic acid.

The crude dibromide was stirred with 1 g. of zinc dust and 0.1 ml. of acetic acid in 25 ml. of ether for 1 hour and 25 minutes. The reaction mixture was worked up in the usual way to give 0.035 g. of crude alcohol, m.p. 145–149°, which on crystallization from ethanol–water gave 0.024 g. of cholesterol-7β-*t*, m.p. 148–149°.

URBANA, ILL.

(26) 1 millicurie is equivalent to 22.2×10^8 d.p.m.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Alkaline Degradation of Periodate-oxidized Starch[1]

BY ROY L. WHISTLER, P. K. CHANG AND G. N. RICHARDS

RECEIVED DECEMBER 6, 1958

Periodate-oxidized whole corn starch is treated with oxygen-free sodium hydroxide solution and with lime-water at room temperature. The degradation products are mainly acids, among which glycolic, DL-2,4-dihydroxybutyric and formic acids predominate. Carbon dioxide also is produced and a Cannizzaro rearrangement product is detected. The mechanism of the degradation reactions is discussed.

For some time it has been recognized that oxidized polysaccharides containing carbonyl groups readily undergo depolymerization in alkali with formation of acidic products. This effect is of importance in the industrial processing of both cellulose and starch. In particular, the chemical behavior of periodate oxystarch is of current interest because methods for producing this modified starch have become available at reduced cost through improved regeneration of periodate.[2]

Alkali-lability of periodate oxystarch often has been noted[3–5] and the formation of acids during alkaline degradation has been attributed to Cannizzaro rearrangements.[4,5] However, recent work[6] on monosaccharide analogs of periodate oxidation products of starch and cellulose have indicated that the alkaline degradation proceeds predominantly by way of a β-alkoxycarbonyl elimination at C5 of the original D-glucose unit. This has been confirmed in the case of periodate oxycellulose,[7] which yields glycolic and DL-2,4-dihydroxybutyric acids as the major acidic products of alkaline degradation.

This is a report on the major acidic products which result from treatment of periodate-oxidized whole starch with sodium hydroxide or lime-water. Glycolic, DL-2,4-dihydroxybutyric and formic acids are identified as the predominant acidic products in both short-term and long-term alkali treatments, and their approximate relative yields under various conditions are shown in Table I.

It is concluded that the degradation is similar to that occurring with periodate oxycellulose and takes place predominantly as shown (I → V + VI). The 1→6 links of the amylopectin component would be expected to have little effect on the final products since these linkages become part of a glyoxal hemiacetal structure in the intermediate III and this type of structure is known to be alkali-labile. The α-alkoxycarbonyl group in intermediate II is known to inhibit further alkaline rearrangements under normal circumstances,[8] but in the completely oxidized starch, the group R is equivalent to intermediate IV. The degradation of IV as shown will therefore remove the α-al-

(1) Journal Paper No. 1027 of the Purdue University Agricultural Experiment Station, Lafayette, Ind.

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

(3) E. M. E. Fry, E. J. Wilson and C. S. Hudson, *ibid.*, **64**, 872 (1942).

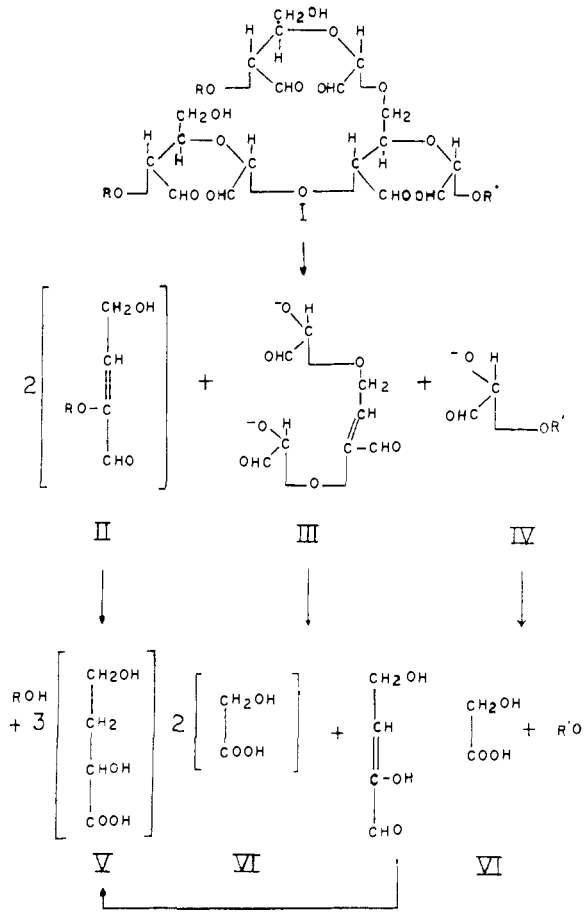
(4) B. T. Hofreiter, B. H. Alexander and I. A. Wolff, *Anal. Chem.*, **27**, 1930 (1955).

(5) D. M. W. Anderson, C. T. Greenwood and E. L. Hirst, *J. Chem. Soc.*, 225 (1955).

(6) D. O'Meara and G. N. Richards, *Chemistry & Industry*, 40 (1958); *J. Chem. Soc.*, 1204 (1958).

(7) D. O'Meara and G. N. Richards, *ibid.*, 4504 (1958).

(8) J. Kenner and G. N. Richards, *ibid.*, 2921 (1956).



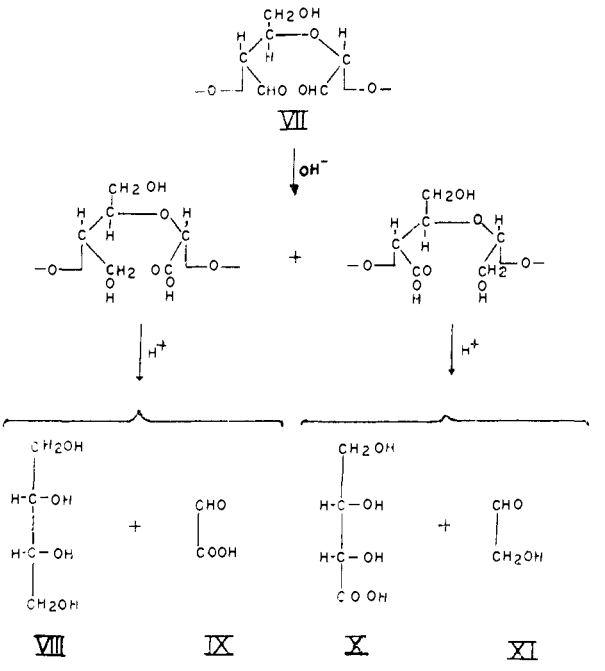
koxycarbonyl group in II and so permit rearrangement to DL-2,4-dihydroxybutyric acid (V).

Competing reactions undoubtedly occur, however, since the yields of V and VI are much less than the theoretical. The most important by-product is an acidic, polymeric material of high equivalent weight, which possibly results from complex polymerizations of low molecular weight intermediates.

Some Cannizzaro rearrangement occurs and this also will reduce the yields of V and VI. This type of reaction has been detected previously in alkaline treatment of periodate-oxidized model compounds[3,6] and presumably can occur as shown without causing chain scission. The results of the work on model compounds suggest that an intermolecular rearrangement of this type is unlikely. The Cannizzaro rearrangement in oxystarch is detected by acidic hydrolysis of an insoluble residue from the lime-water degradation. Erythronic (X) and glyoxylic (IX) acids and erythritol (VIII) are detected by paper chromatography and the latter is isolated in crystalline form. Similar chromatographic evidence also is obtained by hydrolysis of an oligosaccharide produced by the action of sodium hydroxide on oxystarch.

Although the presence of calcium ions in the alkaline degradation of carbohydrates often yields simpler mixtures because of specific cationic catalysis,[9] no such effect is noted with periodate

(9) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 3019 (1957), and earlier references.



oxystarch and even lower yields of DL-2,4-dihydroxybutyric acid were obtained in lime-water than in sodium hydroxide.

As in the case of periodate oxycellulose, the yield of glycolic acid is always higher than that of DL-2,4-dihydroxybutyric acid and this is probably due to an alternative mode of degradation which yields glycolic but not DL-2,4-dihydroxybutyric acid. In view of the arguments expressed above, the intermediates II, III and IV are expected to

TABLE I

PRODUCTS ISOLATED FROM ALKALINE DEGRADATION OF OXYSTARCH AT 25°

	Yield[a]			
	A	B	C	D
Neutral products	0.25*	0.32*	0.10*	0.11*
Total acids	.84	.63	1.80	2.15
Volatile acids	.09	.10	0.22	0.39
Formic acid	.05	.08	.20	.35
Glycolic acid	.41	.31	.75	.86
DL-2,4-Dihydroxybutyric acid	.19	.10	.39	.20
Unknown resinous acid (R_1 0.0–0.25)	.2*	.2*	.1*	.1*
Supposed Cannizzarro rearrangement product (R_1 0.29)	.1*	.1*	.1*	.1*

[a] Yield of products in equivalents per mole of oxidized D-glucose unit except those marked with an asterisk where yields are expressed in g. per g. of oxystarch. A = short-term sodium hydroxide treatment; B = short-term lime-water treatment; C = long-term sodium hydroxide treatment; D = long-term lime-water treatment.

degrade mainly as shown. It seems most probable therefore, that side reactions occur by alternative degradations of the original oxystarch I. In this respect the ketene acetal hydrolysis mechanism, proposed by Pacsu[10] for this type of degradation, is of interest, since this would yield glycolic, but

(10) E. Pacsu, *Text. Res. J.*, **15**, 354 (1945); *Fortschr. Chem. org. Naturstoffe*, **5**, 128 (1948).

not DL-2,4-dihydroxybutyric, acid and so explain the excess of the former acid.

The summary of yields in Table I suggests that some products are not accounted for. This is partly due to small losses of the resinous acidic products and of the supposed Cannizzaro rearrangement products during isolation by paper chromatography. The yields of glycolic and DL-2,4-dihydroxybutyric acids were approximately corrected for non-quantitative removal from paper chromatograms through determination of the loss suffered when known amounts of glycolic acid are separated on paper and subjected to quantitative analysis. Also, in lime-water degradations a sediment was obtained which is not included in the table. In the short-term lime-water reaction this sediment included a small amount of undissolved oxystarch and in both long- and short-term reactions considerable amounts of calcium carbonate were present in addition to the small amount of insoluble Cannizzaro rearrangement product. It seems probable, therefore, that some of the deficiency in the recoveries shown in Table I is due to reactions producing carbon dioxide.

No interpretation is offered at present of the source of the formic acid, which is also an important product of alkaline degradation of periodate oxycellulose.[7]

Experimental

The following solvents and sprays were used for paper chromatography with Whatman No. 1 paper at 25°: solvent A, ethyl acetate–acetic acid–water (10:1.3:1.0 v./v.); solvent B, ethyl acetate–pyridine–water (40:11:6 v./v.); sprays A, brom thymol blue indicator[11]; B, hydroxylamine–ferric chloride[12]; C, ammoniacal silver nitrate.[13] R_g is the rate of movement relative to D-glucose; R_l is the rate of movement relative to lactic acid.

Preparation of Periodate-oxidized Starch.—Defatted corn starch was oxidized with 1.5 moles of sodium metaperiodate per mole of D-glucose unit, as described by Sloan, Hofreiter, Mellies and Wolff.[14] The product was recovered by filtration, washed with water and ethanol, dried over calcium chloride and kept in an air-tight bottle. The moisture content of the bulk product was 8.4%.

Alkaline Degradation of Periodate-oxidized Starch.—There were four different treatments in each of which an aqueous slurry of oxystarch was added to the relevant oxygen-free alkali at room temperature: (1) *N* sodium hydroxide for 30 minutes, (2) *N* sodium hydroxide for about five months, (3) *N* saturated lime-water for 30 minutes, (4) saturated lime-water for 40 days. Solution temperature always rose to 30–34° upon addition of the oxystarch. In the reaction 4, mechanical stirring was used for the first three hours, after which the oxystarch appeared to be completely dissolved. The concentration of oxystarch was 4% in sodium hydroxide solutions and 0.2% in lime-water solutions. A small amount of fine sediment formed during the long-term sodium hydroxide degradation and a much larger amount of sediment was observed in both short-term and long-term lime-water degradations. Since the oxystarch did not completely dissolve during the short-term lime-water treatment, this sediment contained undissolved oxystarch.

Rate of Degradation.—Development of acidity was determined by acidification of aliquot portions of the reaction mixtures and back titration with standard alkali. Results are shown in Fig. 1. The early stages were not reproducible due to the heterogeneous and exothermic nature of the reaction at this stage.

(11) F. Cramer, "Papier chromatographie," Verlag Chemie, G.m.b.H., Weinheim/Bergstrasse, Germany, 1958, p. 154.

(12) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 5859 (1951).

(13) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(14) J. W. Sloan, B. T. Hofreiter, R. L. Mellies and I. A. Wolff, *Ind. Eng. Chem.*, **48**, 1165 (1956).

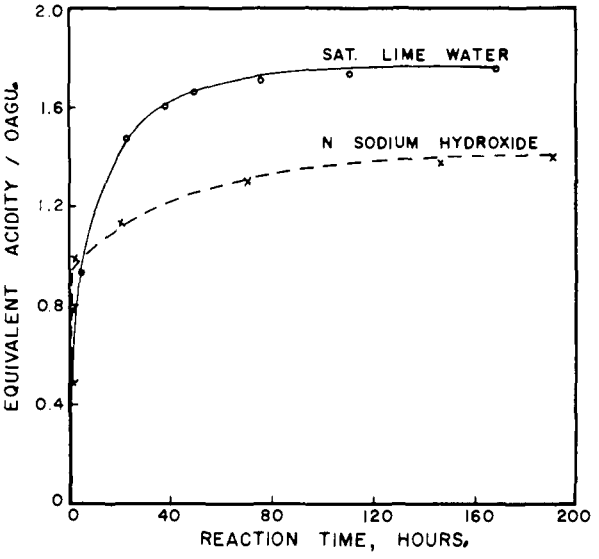


Fig. 1.—Development of total acidity with reaction time in alkaline degradation of periodate-oxidized starch in *N* sodium hydroxide and in satd. lime-water; OAGU = oxidized D-glucose unit.

Isolation and Preliminary Examination of Degradation Products.—The degradations in *N* sodium hydroxide were stopped by passing the alkaline solutions through excess Amberlite resin[15] IR-120(H). For lime-water treatments, the reaction mixtures were first neutralized with carbon dioxide and then concentrated to an optimum volume before this resin treatment. The effluents were next stirred overnight at room temperature with an excess of Amberlite resin IR-401 (carbonate form). The filtrates and washings from the resin were combined and evaporated to dryness to yield the neutral degradation products. The acidic fractions were then eluted with an excess of *N* sulfuric acid; the effluents were neutralized with barium carbonate, filtered and concentrated. These barium salt solutions were used in the following determinations.

(A) Neutral Components.—The neutral fractions were obtained as glassy solids which dissolved in cold water to give turbid solutions. Chromatographic investigation of these fractions from the four different treatments was performed with solvents A and B and with spray C. Each gave a long streak from the origin. A trace component corresponding to glucose was detected in each instance, but the major component, giving a diffuse spot of approximately R_g 7.0 (solvent A) and R_g 1.7 (solvent B), was not identified.

(B) Acidic Components.—Samples of barium salt solutions were treated with Amberlite resin IR-120(H). In each case, paper chromatography in solvent A, spray A, B and C showed glycolic acid (R_l 0.75–0.76), DL-2,4-dihydroxybutyric acid (R_l 0.55) and its lactone (R_l 1.05), an acidic streak (R_l 0–0.25), an elongated acidic zone (R_l 0.29) subsequently shown to be due to a Cannizzaro rearrangement product, a trace of unidentified acid (R_l 0.63) and a trace of unidentified acid (R_l 0.95). The latter two were obtained in greatest amount from the long-term lime-water treatment.

(C) Sedimentation Products.—The sedimentation product formed in the long-term sodium hydroxide treatment was less than 10 mg. per g. of oxystarch and was mixed with the solution passing through the resin column. Both the short-term and long-term degradations with saturated lime-water produced much more sediment which was separated by filtration before treating the solution with carbon dioxide. The yield of this sediment was 0.225 g. per g. of oxystarch for the long-term treatment and 0.338 g. per g. of oxystarch for the short-term treatment.

These sediments were completely soluble in dilute hydrochloric acid with evolution of carbon dioxide. In dilute sulfuric acid, they also dissolved with the evolution of carbon dioxide and the formation of a large amount of white

(15) Product of Rohm and Haas Co., Philadelphia 5, Pa.

precipitate which was mainly calcium sulfate. One-half gram of the sediment from the long-term degradation (calcium content 23.5%; carbon dioxide evoluted, 19.9%) was heated with 5 ml. of *N* sulfuric acid at 100° for 3 hours, neutralized with barium carbonate and filtered. The filtrate was treated with Amberlite resin IR-120(H). The resulting solution when examined by paper chromatography with solvents A and B and spray C, gave spots corresponding to erythronic acid and its lactone, glyoxylic acid and erythritol. The solution was stirred with 10 g. of Amberlite resin IR-400(OH) at room temperature for 20 hours, filtered and evaporated to dryness. The residue (10 mg.) crystallized slowly and gave an X-ray diffraction pattern identical with that of authentic erythritol.

Identification of Major Acidic Products.—The stock barium salt solution from the short-term sodium hydroxide degradation was stirred with an excess of Amberlite resin IR-120(H) at room temperature for 30 minutes. The solution was transferred uniformly to sheets of Whatman 3MM paper which had been washed with water and dried. Approximately 5 meq. of the acid fraction were applied to each 46 × 56 cm. paper without heating and the paper was irrigated with solvent A for 6 hours. Guide strips were sprayed with A and B and the relevant zones were eluted with water. The following components were identified:

DL-2,4-Dihydroxybutyric acid was identified as its anilide, m.p. 115–116° alone or in admixture with an authentic sample kindly supplied by Dr. J. W. Green, Institute of Paper Chemistry, Appleton, Wis. It was also characterized as its brucine salt,[16] m.p. 172–174°.

Anal. Calcd. for $C_{27}H_{36}O_8N_2$: N, 5.5. Found: N, 5.5.

Glycolic acid was identified as its amide, m.p. and mixed m.p. 119–120°, and also as its 4-bromophenacyl ester, m.p. and mixed m.p. 137–138°.

DL-2,4-Dihydroxybutyrolactone was identified as the anilide, m.p. and mixed m.p. 115–116°.

Quantitative Determination of Degradation Products.—Total acidity was determined by treating an aliquot portion of the barium salt stock solution with an appropriate amount of newly washed Amberlite resin IR-120(H). The filtrate was back titrated after treatment at room temperature for 30 minutes with a fourfold excess of 0.025 *N* sodium hydroxide.

Volatile acidity and formic acid were determined as described by Richards and Sephton.[17]

Quantitative determinations of the neutral fraction of the four alkali-degraded oxystarch samples were made by direct weighing of the dried solids after deionizing with ion exchange resins.

Five of the acidic degradation products were determined semi-quantitatively for non-volatile acids by paper chromatography. A portion of the barium salt stock solution was treated with an excess of Amberlite resin IR-120(H) at room temperature for 30 minutes and filtered. The exact acid concentration of the filtrate was obtained by back titration after addition of an excess of alkali. An aliquot portion of the acid sample containing 0.2–0.5 meq. of acid was applied to the center 12 cm. section of a piece of water-washed Whatman 3 MM paper (24 × 57 cm.) and an equal loading was applied to the outer 3-cm. guide strips. After developing the papers with solvent A for 6 hours, the guide strips were sprayed with sprays A and B and the relevant zones from the center section eluted with water (25 ml. per zone). The resinous acidic substance (R_1 0–0.25) and the supposed Cannizzaro rearrangement products (R_1 0.28–0.30) were dried and weighed directly after combining the eluates from several such papers. Separation of these two components was incomplete due to extensive streaking on the chromatograms. The yields recorded are therefore very approximate.

For glycolic acid and DL-2,4-dihydroxybutyric acid and its lactone, the concentrations of the acids in the respective eluates were determined spectrophotometrically. Calkins' method[18] was found to be applicable to both acids after separation. Glycolic acid gave a pink color with an absorption maximum at 5400 Å. while DL-2,4-dihydroxybutyric acid gave a greenish-yellow color which showed an absorption maximum at 4550 Å. Similar amounts of authentic samples of glycolic acid when subjected to the same process gave a recovery of 67 ± 5%. In the absence of a sufficient supply of authentic DL-2,4-dihydroxybutyric acid for a similar calibration, the same correction factor was applied to both acids. The results of all of the above determinations are summarized in Table I. The recovery of acid from this type of determination is, however, dependent on the loading of the paper and the acid yields are approximate and possibly subject to error of the order of ±10%.

Acknowledgment.—The authors wish to express their thanks to the American Maize Products Co. for partial support of this work.

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LAFAYETTE, IND.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

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

BY ROY L. WHISTLER AND RICHARD SCHWEIGER

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Amylopectin is rapidly attacked by hydrogen peroxide over the *p*H range 7 to 12.5. The initial effect is depolymerization which is followed by a rapid and extensive oxidation presumably of end units, to produce mainly carbon dioxide and formic acid in 1:6 ratio and lesser amounts of methylglyoxal, D-arabinose and D-erythronic, glyoxylic and glycolic acids. Recovery of large quantities of D-glucose from the hydrolyzed oxidation products obtained by use of large amounts of hydrogen peroxide tends to confirm the theory that the attack occurs principally on reducing end units and other oxidized sites.

Sodium or hydrogen peroxide often has been used for bleaching ground wood and semi-chemical pulp and, at times, has been used as a final stage in the bleaching of sulfite and sulfate pulps. In addition to its action on lignin and color bodies, peroxide reacts with cellulose,[3,4] although in commercial operations the effect may be minor because of the low oxidant levels used. While the initial effect of hydrogen peroxide on cellulose is depolymerization, Haskins and Hogsed[5] detected in the products from oxidized cotton linters, carbon di-

(1) Journal Paper No. 1363 of the Purdue University Agricultural Experiment Station.

(2) This is paper number 5 in a series concerning Action of Oxidants on Carbohydrates. Previous papers in the series are: R. L. Whistler and S. J. Kazeniac, *J. Org. Chem.*, **21**, 468 (1956); R. L. Whistler, E. G. Linke and S. J. Kazeniac, THIS JOURNAL, **78**, 4704 (1956); R. L. Whistler and R. Schweiger, *ibid.*, **79**, 6460 (1957); R. L. Whistler and R. Schweiger, *ibid.*, **80**, 5701 (1958).

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(5) J. F. Haskins and M. J. Hogsed, *J. Org. Chem.*, **15**, 1264 (1950).