[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY[1]]

Gluconic Acid from Hypoiodite-oxidized Hydrocellulose[2]

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Hypoiodite-oxidized hydrocellulose was subjected to methanolysis; the residual hydrochloric acid was neutralized by diazomethane; and most of the glucosides were removed by direct crystallization. The oxidized residue thus concentrated was isolated as D-gluconic phenylhydrazide and as D-gluconic amide and the methylamide. Twenty-one per cent. of the carboxyl indicated by analytical methods to be in the original oxycellulose was accounted for by isolation from the alcoholysate; most of the remainder was found in the insoluble residue from the methanolysis where it existed in an unesterified form, either as free acid or as lactone. D-Gluconic methylamide was prepared in 95% yield by the reaction of the γ-lactone in a cold methanolic solution of methylamine. Analyses and the higher melting point indicate the preparation to have greater purity than that previously described by van Wijk.

Different oxidizing agents are known to attack preferentially different positions of the glucose units of cellulose. For example, carbon six is believed to be the locale of the preferential attack by nitrogen dioxide; carbons two and three, by periodate; and carbon one of reducing chain-ends, by hypoiodite. Partial isolations after hydrolysis of the suitably treated oxycelluloses support the mechanisms proposed for the oxidation of cellulose by periodate[3] and by nitrogen dioxide[4]; but no isolations of oxidized fragments from hypoiodite oxycelluloses have been reported.

The preferential action of hypoiodite upon cellulose is believed to be the oxidation to carboxyl of aldehydic chain ends. Such action would result in the conversion of terminal D-glucose units to terminal D-gluconic acid.[4a] The acceptance of this mechanism is based upon empirical correlations between chain length and consumption of oxidant, upon the correspondence between oxidant consumed and number of acidic (non-uronic) groups introduced, upon the decrease of reducing power with extent of oxidation, and upon analogy with the known behavior of this reagent toward simple sugars.[5,6] Since no isolation of D-gluconic acid as a cleavage product of hypoiodite-oxidized cellulose, nor indeed of any oxidized cellulose, has been reported in the literature, this investigation was undertaken to devise a technique by which such isolation might be achieved.

The oxycelluloses studied in the investigation were prepared from hydrocelluloses because of their relatively large number of end-groups (one aldehyde group for approximately 140 glucose units) as compared to purified cotton cellulose. The hydrocelluloses were subjected to oxidation by hypoiodite under controlled conditions similar to those recommended for the reducing end-group estimation[5] but differing as to concentrations in order to facilitate larger scale operation. The oxidized hydrocelluloses, containing significant amounts of carboxyl groups, were subjected to alcoholysis with methanolic hydrogen chloride. This treatment, which brought about solution of only 3% of the cellulosic material, removed about one-third of the carboxyl. Hence, a relatively large proportion of the dissolved material was derived from the acid end-groups. These could readily be isolated and identified as known derivatives of D-gluconic acid, for the small residual amount of catalyst could be removed with diazomethane leaving as the major contaminants easily crystallizable methyl glucosides.

Isolations were effected as the phenylhydrazide, the amide, and the methylamide. The first was the most satisfactory, for it is easily purified and identified whereas the melting points and solubilities of the amides do not differ greatly from those of mixtures of the anomeric glucosides. New constants are reported for D-gluconic methylamide, the reference compound prepared in connection with this investigation having greater purity than that previously described in the literature.[7] The quantity of D-gluconic acid actually isolated as crystalline derivatives amounts to 60% of the carboxyl removed from the oxycellulose during alcoholysis or to 21% of the total carboxyl content.

Experimental

Preparation of Oxycellulose.—Hydrocellulose was prepared by autoclaving purified Empire cotton[8] for 2 hr. at 120° in 0.5 *N* hydrochloric acid, this process resulting in about 6% loss of weight. The analytical method of Martin, Smith, Whistler and Harris[5] was adapted for preparative oxidation of hydrocellulose by diminishing the volume of reagents and increasing the iodine concentration accordingly.

Buffer solution (containing 35.8 g. of sodium carbonate, 1.8 g. of sodium bicarbonate and 1500 ml. of water; *p*H 10.6) and iodine solution (750 ml. of 0.25 *N* iodine containing 40 g. of potassium iodide per liter) were precooled to 0° and were mixed immediately before the addition of 150 g. of hydrocellulose. The mixture was stirred in a closed container for 4 hr. with the temperature maintained at 0°. The reaction was stopped by the addition of 5 *N* hydrochloric acid until evolution of carbon dioxide ceased. The product was washed with 0.2 *N* hydrochloric acid until almost colorless, with 0.02 *N* sodium thiosulfate until free of iodine, with 0.2 *N* hydrochloric acid for removal of metallic ions, with ethanol until washings were neutral and chloride-free, and with ether. The recovered residue containing 5% moisture weighed 149 g. Its copper number[9] was 0.71 as against 7.0 prior to oxidation.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Report of a study made under the Research and Marketing Act of 1946.

(3) D. H. Grangaard, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **115**, No. 7, 41 (1942); E. L. Jackson and C. S. Hudson, THIS JOURNAL, **60**, 989 (1938); G. Jayme and S. Maris, *Ber.*, **77B**, 383 (1944).

(4) F. S. Head, *J. Chem. Soc.*, 1135 (1948).

(4a) Similar products have been designated "gluconic acid oxycelluloses" by A. Meller, *TAPPI* **34**, 171 (1951).

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(6) H. A. Rutherford, F. W. Minor, A. R. Martin and M. Harris, *ibid.*, **29**, 131 (1942).

(7) W. E. van Wijk, *Rec. trav. chim.*, **40**, 221 (1921).

(8) J. H. Kettering and C. M. Conrad, *Ind. Eng. Chem., Anal. Ed.*, **14**, 432 (1942).

(9) T. F. Heyes, *J. Soc. Chem. Ind.*, **47**, 90T (1928).

The hypoiodite consumption of a one-gram sample of the hydrocelluloses under conditions analogous to the preparative method was equivalent to 4.47 ml. of 0.02 *N* thiosulfate or 0.045 mM/g. The consumption of methanolyzed cellulose under the same conditions was 0.003 mM/g., this figure being indicative of the amount of secondary oxidation to be expected. Increasing the time of oxidation from 4 to 6 hours caused no additional consumption of hypoiodite in excess of that consumed by the blank. From these figures the number-average degree of polymerization of the oxycellulose is calculated to be 140.

The oxidized cellulose contained 0.049 mM carboxyl per gram as measured by the back-titration method of Neale and Stringfellow[10] and 0.045 mM/g. by methylene blue absorption.[11] The methylene blue absorption figure for the hydrocellulose prior to oxidation was 0.002 mM/g.

Isolation of D-Gluconic Acid.—Methanolysis of 125 g. of oxycellulose in methanolic hydrogen chloride (initially, 0.5 *N*; finally, less than 0.01 *N*) for 2 hr. at 120° solubilized about 3% of the material. The insoluble residue was washed with ethanol until acid-free and with ether. Weight of residue, 121 g.; carboxyl by back-titration 0.028 mM/g., carboxyl by methylene blue consumption, 0.029 mM/g.; ester methoxyl, 0.003 mM/g. calculated from a colorimetric determination of methanol[12] after alkaline saponification.

The soluble portion of the methanolyzate with washings was neutralized with ethereal diazomethane and was concentrated under reduced pressure. The concentrate yielded 1.87 g. of crystalline methyl glucosides in three crops from absolute alcohol. The mother liquor, containing 1.30 g. of solids, was diluted to 25 ml. and aliquots were used for further investigations.

A 5-ml. aliquot was concentrated in a stream of air to about 1/2 ml. and to this solution was added 100 mg. of phenylhydrazine in 1/2 ml. of absolute ethanol. After a few hours at 5° D-gluconic phenylhydrazide began to precipitate, the yield after 2 days being 0.066 g., m.p. 200–202°.[13] One recrystallization from hot water increased the melting point to 204–205°, which value was not changed by admixture with the phenylhydrazide prepared from D-gluconic γ-lactone. The specific rotation of the isolated material was $[\alpha]^{25}D$ +12° (*c* 1, water, 1-dm.), identical with the literature value.[14] On the basis of this yield, the entire methanolysate would contain 0.33 g. of phenylhydrazide, equivalent to 0.23 g. of D-gluconic acid; the recovery amounts to 60% based on the difference in carboxyl analyses before and after methanolysis.

Aliquots treated with alcoholic ammonia and with alcoholic methylamine, respectively, produced in lower yields crystalline materials with melting points not changed by admixture with authentic specimens of the respective amides but depressed by either the α- or β-form of methyl D-glucopyranoside. Second crops were contaminated by glucosides.

Preparation of D-Gluconic Methylamide.—A solution of 100 mg. of D-gluconic γ-lactone in 1 ml. of methanol saturated with methylamine deposited during 24 hr. at 5° the crystalline amide; weight, 0.111 g. (95% of the theoretical yield), m.p. 150.5–151.5°. Constants determined after one recrystallization from 95% ethanol were m.p. 152–153°, $[\alpha]^{26}D$ +39.9° (*c* 4, water, 2-dm.) unchanged by a second recrystallization. van Wijk[7] reports m.p. 127°; $[\alpha]^{12}D$ +33.8°; N, 6.49.

Anal. Calcd. for $C_7H_{15}O_6$: C, 40.19; H, 7.22; N, 6.69. Found: C, 40.13; H, 6.92; N, 6.65.

(10) S. M. Neale and W. A. Stringfellow, *Trans. Faraday Soc.*, **33**, 881 (1937).

(11) G. F. Davidson, *Shirley Institute Memoirs*, **21**, 47 (1947).

(12) C. L. Hoffpauir and R. E. Reeves, *Anal. Chem.*, **21**, 815 (1949).

(13) All melting points were determined with a Fisher-Johns micro-melting-point apparatus.

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NEW ORLEANS, LOUISIANA

[CONTRIBUTION FROM THE FRUIT PROCESSING DIVISION, WESTERN REGIONAL RESEARCH LABORATORY]

Enzymatic Browning of Fruits. III. Kinetics of the Reaction Inactivation of Polyphenoloxidase

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The inactivation of polyphenoloxidase which accompanies the aerobic oxidation of the substrate, catechol, has been studied at various enzyme concentrations by the usual method of following the concentration of a secondary reductant, ascorbic acid. The amount of ascorbic acid oxidized at any chosen reaction time and the maximum amount of ascorbic acid oxidized are both proportional to the initial enzyme concentration when the latter is varied over a 5-fold range. Also, the time for half the maximum amount of ascorbic acid to be oxidized is independent of the initial enzyme concentration. These data are consistent with first order kinetics with respect to enzyme concentration rather than a three-halves order kinetics previously proposed.

Introduction

Dawson and co-workers[1] developed a valuable method for measuring the activity of polyphenoloxidase. It is based on the secondary oxidation of ascorbic acid by the quinone formed in the enzymatic oxidation of the catechol. Since the enzyme is inactivated during the course of the reaction, the rate of the reaction decreases with time. This inactivation has been termed "reaction inactivation."[2] These authors found that the data could be represented by the "chronometric equation"

$$Q = at/(b + t) \qquad (1)$$

where Q is the amount of quinone produced, t is the time and a and b are experimentally determined constants. This equation represented the data so well over 80% of the reaction that Asimov and Dawson[3] sought to find its theoretical significance. They showed that the equation could be derived by assuming that the inactivation was proportional to the three-halves power of the enzyme concentration. They also considered the possibility of a first order enzyme dependence which leads to the equation

$$Q = a'(1 - e^{-t/b'}) \qquad (2)$$

This equation was rejected on the basis that Equation 1 fitted the experimental data (then available) better during the latter part of the reaction than Equation 2, when values of the parameters, $a' = 8.06$ mg. ascorbic acid and $b' = 100.7$ sec. were chosen. These values were determined over the more precise portion of the Q–t curve, that portion under two minutes.

(1) W. H. Miller, M. F. Mallette, L. J. Roth and C. R. Dawson, THIS JOURNAL, **66**, 514 (1944).

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(3) I. Asimov and C. R. Dawson, *ibid.*, **72**, 820 (1950).