[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY[1]]

# Periodate Oxidation of Dextran

BY ALLENE JEANES AND C. A. WILHAM

Periodate oxidation is a well-known method for obtaining structural information on polysaccharides. This paper reports the application of this method to the dextran produced from sucrose by the action of *Leuconostoc mesenteroides*. Methylation studies on a few dextran preparations[2,3,4,5] have established these as polymers of α-D-glucopyranose having a predominance of 1,6-linkages and a small, apparently variable amount of 1,4-linkages at points of branching. Previous indications[6,7,8] that dextrans vary in structure with the strain of the organism employed in their preparation have been confirmed in the present study.

Six different dextrans from four different strains of *Leuconostoc mesenteroides* have been oxidized with sodium metaperiodate at 25°. The formic acid produced and the periodate reduced reached definite values which were independent of the periodate concentrations used, were not changed by extended reaction time, and were the same when either sodium or potassium[9,10] metaperiodate was employed.

The results obtained when these dextrans and a fraction of acid-hydrolyzed dextran were treated with three moles of sodium metaperiodate per mole of anhydroglucose unit are shown in Table I. These results show that the dextrans vary in the proportion of anhydroglucose units present which produce formic acid. The data show further that the amount of sodium metaperiodate reduced agrees closely with that calculated to produce the formic acid and to oxidize the units which did not produce formic acid. In high molecular weight dextrans, which have not been shown to have reducing end groups,[8] one mole of formic acid is expected to be produced with the resultant reduction of two moles of periodate by each anhydroglucopyranose unit which is linked into the molecule through the 1- or the 6-positions, or both. Units which are linked through both the 1- and 4- or the 1- and 2-positions would not be expected to produce formic acid but to reduce one mole of periodate. Since analysis of completely methylated dextrans has indicated only 1,4-linkages in addition to 1,6-[4,5] we assume this to be true also of the dextrans used in this study.

TABLE I

SODIUM METAPERIODATE OXIDATION DATA, 25°

| Substance | NRRL strain of *Leuconostoc mesenteroides* used in preparation of dextran | Moles formic acid obtained per mole of anhydroglucose[a] | Calculated number of 1,6-linked units per 1,4-linked unit[b] | Moles sodium metaperiodate reduced per mole of anhydroglucose: Observed[a] | Calculated[c] |
|---|---|---|---|---|---|
| Water-soluble dextrans | | | | | |
| "Autolyzed" | B-512 | 0.96 | | 1.96 | 1.96 |
| High viscosity | B-512 | .95 | 20 ± 4 | 1.97 | 1.95 |
| Calcium carbonate-buffered | B-512 | .94 | | 1.94 | 1.94 |
| Hassid and Barker's | | .91 | 10 | 1.94 | 1.91 |
| "Hibbert's" | B-742 | .76 | 3 | 1.81 | 1.76 |
| Water-insoluble dextran[d] | B-523 | .77 | 3 | 1.72 | 1.77 |
| Fraction of acid-hydrolyzed dextran[e] | B-512 | 1.12 | | 2.06 | |
| Levoglucosan | | 0.99 | | 1.99 | 2.00 |

[a] These values are averages from analyses on four to eight separate samples. The maximum variation in formic acid measurements was ±1%, and in the periodate measurements ±2%. [b] Calculated as follows: formic acid data for "autolyzed" dextran show the ratio of 1,6- to 1,4-linkages to be 96 to 4, or 24 to 1. [c] Calculated for "autolyzed" dextran as follows: 2 (0.96) + 0.04. [d] This dextran was dissolved in 1 *N* sodium hydroxide, the solution was neutralized with hydrochloric acid without the reappearance of insoluble material, and then sodium metaperiodate solution was added. [e] This fraction has an average of 15 anhydroglucose units per reducing group.

The data for the dextran from the strain NRRL B-742 indicate that oxidation with sodium metaperiodate gives information on the ratio of 1,4- to 1,6-glycosidically linked anhydropyranose units in good agreement with methylation data. This dextran was obtained from the same strain of organism as was the dextran used by Levi, Hawkins and Hibbert for structural study by the methylation procedure.[4] Their results indicate a ratio of 1,4- to 1,6-linked units of 1 to 4 as compared with our value of 1 to 3. This difference might be due to unavoidable variations between

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) Peat, Schlüchterer and Stacey, *J. Chem. Soc.*, 581 (1939).
(3) Hassid and Barker, *J. Biol. Chem.*, **134**, 163 (1940).
(4) Levi, Hawkins and Hibbert, THIS JOURNAL, **64**, 1959 (1942).
(5) Stacey and Swift, *J. Chem. Soc.*, 1555 (1948).
(6) Sugg and Hehre, *J. Immunology*, **43**, 119 (1942).
(7) Evans and Hibbert, "Advances in Carbohydrate Chemistry," Academic Press, Inc., New York, N. Y., 1946, Vol. II, p. 203.
(8) Jeanes, Wilham and Miers, *J. Biol. Chem.*, **176**, 603 (1948).
(9) Brown, Dunstan, Halsall, Hirst and Jones, *Nature*, **156**, 785 (1945).
(10) Halsall, Hirst and Jones, *J. Chem Soc.*, 1427 (1947).

culturing conditions for our preparation and for that of Tarr and Hibbert.[11] The high frequency of occurrence of two different linkages in this dextran correlates with certain physical observations. Under conditions which caused all the other water-soluble dextrans used in this study to produce an X-ray diffraction line pattern,[12] this dextran remained amorphous. Likewise, under conditions which resulted in the formation of filaments from dextrans from NRRL B-512,[12] this dextran produced none.

The three dextrans obtained from the strain B-512 under different cultural conditions are not definitely distinguishable by periodate oxidation, although the formic acid values (Table I) suggest they may not be identical. However, a small but definite difference is indicated between these dextrans and that of Hassid and Barker.[3] This same conclusion follows from the observation that the dextran of Hassid and Barker activates both the potato and muscle phosphorylase systems, whereas no activation effect has been obtained from any of the dextrans from B-512.[13,14] This dissimilarity in activating ability appears to indicate a difference in the occurrence or the length[14] of side chains. Substantiation of this possibility, however, cannot be obtained from periodate oxidation data since they give only the ratio of 1,4- to 1,6-linked units.

Although the dextrans from the strains B-742 and B-523 differ distinctly in solubility (Table I), periodate oxidation does not differentiate them. The water-insolubility of the dextran from B-523 undoubtedly arises from an as yet unresolved structural peculiarity. This dextran, in contrast to all the water-soluble dextrans studied, is incompletely solubilized and hydrolyzed by treatment with 7.0 *N* hydrochloric acid at 50°.

The stability of the primary product of oxidation of dextran and of acid-hydrolyzed dextran to the secondary action of the oxidant is in sharp constrast to the behavior of the corresponding products from amylaceous carbohydrates. When treated at 25° with 1.5 moles of sodium metaperiodate per mole of anhydroglucose unit, corn amylose and waxy corn starch reached no definite end values, and after a greatly extended reaction time, iodine crystallized from the reaction solutions containing these oxidized products. The corresponding solutions containing oxidized dextran and acid-hydrolyzed dextran developed no color even after standing four times as long as the amylaceous substances. This stability of dextran and acid-hydrolyzed dextran to over-oxidation by sodium metaperiodate obviates the application to these substances of specialized procedures recently recommended for amylaceous substances.[15]

(11) Tarr and Hibbert, *Can. J. Research*, **5**, 414 (1931).
(12) Jeanes, Schieltz and Wilham, *J. Biol. Chem.*, **176**, 617 (1948).
(13) Swanson and Cori, *ibid.*, **172**, 815 (1946).
(14) Hestrin, *ibid.*, **179**, 943 (1949).
(15) Meyer and Rathgeb, *Helv. Chim. Acta*, **32**, 1102 (1949).

A difference in stability of the primary products from periodate action on 1,4- and 1,6-linked disaccharides comparable with the difference between amylaceous substances and dextran has been observed by Ahlborg.[16] Our observations are also in agreement with the proposed mechanism of the over-oxidation of amylaceous substances by periodates.[9,10,15,17] This theory indicates that before over-oxidation can start at a reducing end group, the pyranose ring structure in that group must be eliminated to permit removal of $C_6$ by oxidation. Oxidation then continues on down the carbohydrate chain. In dextran this point of attack of secondary oxidation is blocked by either or both a non-reducing end group or the absence of a primary hydroxyl group on one of the first few hexose units of the chain. The effectiveness of this blockage even when reducing groups are present is indicated by the complete stability to over-oxidation of the fraction of acid-hydrolyzed dextran used in this study.

## Experimental

**Materials.**—The preparation and characterization have been reported previously for the dextrans from *Leuconostoc mesenteroides* NRRL B-512 and NRRL B-523[8] and for fractions of acid-hydrolyzed dextran.[12]

To obtain the dextran of Hassid and Barker[3,18] in a finely divided state comparable to that of the other dextrans used, it was reprecipitated from 2% aqueous solution by addition to ethanol.[8]

The dextran from *Leuconostoc mesenteroides* NRRL B-742 was prepared under conditions closely comparable with those described by Tarr and Hibbert.[11,19] Six ml. of inoculum was added to one liter of medium (at *p*H 6.2) contained in a 2-liter erlenmeyer flask. Incubation was for fourteen days at 25°. Bacterial cells were removed by supercentrifugation. The polysaccharide which precipitated from the supercentrifugate upon addition of ethanol to make 50% by volume was removed, then purified and isolated in the usual manner.[8] The dextran, isolated in 4% yield instead of the 25 to 30% reported,[11] dissolved very slowly in water and showed a specific rotation of +200° (*c*, 1, 1 *N* sodium hydroxide). The accuracy of this determination was diminished by the turbidity of the solution. Fowler, Buckland, Brauns and Hibbert[20] reported $[\alpha]^{25}D$ +198.3° (*c*, 1.86, 1.12 *N* sodium hydroxide).

**Analytical Methods.**—Periodate was determined by the method of Fleury and Lange as described by Jackson.[21]

Formic acid was determined by a modification of the method described by Halsall, Hirst and Jones.[10] An aliquot of the sodium metaperiodate oxidation solution was treated with purified ethylene glycol[10] and allowed to stand for about an hour in the dark to reduce the excess perio-

(16) Ahlborg, *Svensk. Kem. Tid.*, **54**, 205 (1942).
(17) Potter and Hassid, THIS JOURNAL, **70**, 3488 (1948).
(18) We are indebted to Prof. W. Z. Hassid of the University of California, Berkeley, for this sample.
(19) Tarr and Hibbert obtained the organism which they used and designated as "Culture 4" from the New York Agricultural Experiment Station, Geneva. We are greatly indebted to Dr. C. S. Pederson of that Experiment Station for providing us a culture of this same strain of the organism. This strain is now designated NRRL B-742 in the Culture Collection of this Laboratory. We wish to express our gratitude to Dr. Wm. C. Haynes of the Fermentation Division of this Laboratory for his assistance in obtaining and culturing this organism.
(20) Fowler, Buckland, Brauns and Hibbert, *Can. J. Research*, **15B**, 486 (1937).
(21) Jackson, "Organic Reactions," John Wiley and Sons, Inc. New York, N. Y., 1944, Vol. II, p. 361.

date. Formic acid was then titrated with 0.01 *N* sodium hydroxide, with phenolphthalein as indicator. Flasks in which the determinations were made were flushed with carbon dioxide-free nitrogen before adding the test portion and titrations were made in a carbon dioxide-free nitrogen atmosphere. Corrections were applied for acidity found in blanks in which sodium metaperiodate had been reduced with ethylene glycol. These corrections were equivalent to 0.5 to 1.0% of the total formic acid titration.

Heretofore it has been the custom to use methyl red indicator in titrating formic acid in reaction solutions from which excess periodate had[10,17] or had not[22] been removed by ethylene glycol. Our observations, in agreement with statements of Meyer and Rathgeb,[23] showed that in the absence of periodate there is no reason for using methyl red indicator and that its use resulted in low values for formic acid.

The accuracy of the measurements of periodate reduced and of formic acid liberated by the polysaccharides was established by using D-glucosan < 1,5 > $\beta$ < 1,6 > (levoglucosan)[24] as a reference standard. Prior to use, the levoglucosan was dried over phosphorus pentoxide *in vacuo* at 78°. The results obtained by treating one mole of levoglucosan with 2.2 moles of sodium metaperiodate (Table I) are in close agreement with theory and with previous observations.[25]

**Periodate Oxidation Procedure.**—Air-dried polysaccharides, of known moisture content,[8] were used.

To prevent oxidation of formic acid, all oxidations with sodium metaperiodate were carried out with concentrations of reactants comparable with those used by Halsall, Hirst and Jones.[10] Tests showed formic acid was not destroyed under these conditions. Duplicate samples of carbohydrate, in amount sufficient to produce about 10 mg. of formic acid per 100 ml., were dissolved or dispersed in freshly boiled distilled water in volumetric flasks. The desired amount of approximately 0.3 *M* sodium metaperiodate was added and the contents were made to volume with freshly boiled distilled water. Since a difference in rate only was observed in preliminary work when dextran was treated with 2.2 or 3.0 moles of sodium metaperiodate per mole of anhydroglucose unit, 3.0 moles was used in all subsequent oxidations of dextran and acid-hydrolyzed dextran. The flasks were placed in a constant temperature room at 25° and aliquots of the solutions were with drawn at intervals for the analytical determinations. Aliquots from dextran solutions were taken usually after seventy-two and ninety-six hours reaction time, when the reaction was found to be complete for the most slowly oxidized dextrans.

The procedure used for oxidation with potassium metaperiodate was that of Halsall, Hirst and Jones,[10] carried out at 25°.

Unlike oxidized waxy corn starch, which is insoluble in the sodium metaperiodate reaction mixture, oxidized dextran is soluble.

(22) Jackson and Hudson, THIS JOURNAL, **61**, 1530 (1939).

(23) Meyer and Rathgeb, *Helv. Chim. Acta*, **31**, 1540 (1948).

(24) We are indebted to Dr. Ivan A. Wolff for this highly purified sample.

(25) Jackson and Hudson, THIS JOURNAL, **62**, 958 (1940).

## Summary

1. Conditions have been described for the oxidation of dextran with sodium metaperiodate at 25°, and for measurement of the formic acid produced.

2. The results obtained give a measure of the ratio of 1,4- to 1,6-glycosidically linked anhydropyranose units present and appear to be in good agreement with those from the methylation procedure.

3. Ratios of 1,4- to 1,6-glycosidically linked anhydropyranose units have been observed to vary from 1 to 3 to 1 to 24 for dextrans from different strains of *Leuconostoc mesenteroides*.

4. The primary products of periodate oxidation of dextran and of acid-hydrolyzed dextran are stable to further oxidation by the reagent.

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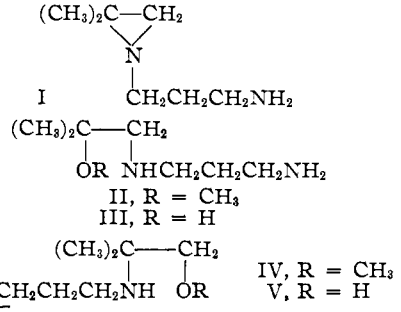
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

# Acid-catalyzed Ring-opening Reactions of Some Unsymmetrical Ethyleneimine Derivatives

BY D. STANLEY TARBELL AND PAUL NOBLE, JR.

In some studies of products obtained from 2,2-dimethylethyleneimine and acrylonitrile, which were initiated with the object of examining the resolvability of the trivalent nitrogen in ethyleneimine, the compound I was obtained by catalytic reduction of the primary addition product.[1]




(1) Tarbell and Fukushima, THIS JOURNAL, **68**, 2499 (1946).

This compound formed an unstable dipicrate, which was converted, by crystallization from methanol, to a new compound, which had the composition expected for the dipicrate of the methanolysis product II or IV. The present paper describes a study of the acid-catalyzed methanolysis and hydrolysis of I, and demonstrates that the product in each case has the structure II (or III); the isomeric compounds IV and V do not appear to be formed.

It was found that the action of acids on the imine I in methanol solution usually led to polymeric materials; this was observed with dry hydrogen chloride, formic acid, ammonium chloride, boron fluoride or acetic acid. The action of picric acid on I in methanol usually led to the contamination of the main product, the dipicrate of the *methanolysis* product II, m. p. 199–200°, with the dipicrate of the *hydrolysis* product III, m. p. 184–187°. This