When the acid obtained directly from the hydrogenation (m.p. 40–44°) was allowed to react with 0.161 g. of piperazine as described above and the crude material recrystallized 7 times from chloroform or chloroform–acetone, 0.204 g. (28.5%) of pure salt was obtained, m.p. 156.5–158.5°. The acid was regenerated and recrystallized from hexane, m.p. 51.5–53.0°, yield 145 mg. (23%).

Also, when 8 g. of the crude acid was converted directly to the amide, the 7.75 g. (m.p. 180–187°) was recrystallized 11 times from aqueous ethanol, m.p. 194–196°, yield 1.65 g. (20.8%).

cis–cis-5-Hydrindanylamine (IV).—Powdered sodium azide (100 mg., 1.5 mmoles) was added in small portions with stirring over a period of 30 minutes to a solution of 172 mg. (1.02 mmoles) of *cis–cis*-hydrindanecarboxylic acid in 5 ml. of chloroform and 2 ml. of concentrated sulfuric acid at 40°. After completion of the addition the temperature was maintained at 50° for an additional 30 minutes and then poured over ice. The chloroform layer was separated and the aqueous layer made alkaline and extracted three times with ether. The crude amine remaining after removal of the ether was benzoylated with 0.3 ml. of benzoyl chloride in 3 ml. of 1 *N* sodium hydroxide. The N-(*cis–cis*-5-hydrindanyl)-benzamide was recrystallized from aqueous ethanol, m.p. 142–143.5° (lit.[4,6] m.p. 145°), yield 63 mg. (25.7%).

Anal. Calcd. for $C_{16}H_{21}ON$: C, 78.97; H, 8.70; N, 5.76. Found: C, 79.24; H, 8.81; N, 5.67.

cis–cis-5-Hydrindanol (V).—A solution of 1.00 g. (5.95 mmoles) of *cis–cis*-5-hydrindanecarboxylic acid in 20 ml. of dry ether was added dropwise with stirring to 32 ml. of a 0.6 *M* solution of methyllithium in ether. Following the addition, the mixture was stirred for an additional 15 minutes and then poured onto ice and the ether layer separated. The ethereal layer was washed with sodium bicarbonate, dried and the ether evaporated.

The crude ketone was allowed to react with 22 ml. of a 0.36 *M* solution of perbenzoic acid in chloroform at room temperature for 7 days. After dilution with ether, the solution was washed with dilute sodium bicarbonate solution, dried and the solvent removed under reduced pressure.

The acetate ester was saponified with 10 ml. of 2 *N* sodium hydroxide in 15 ml. of methanol by refluxing for 2 hours. The reaction mixture was diluted with water, extracted with ether, the ethereal layer separated, dried and the solvent removed. The crude hydrindanol was dissolved in hexane and chromatographed on 40 g. of alumina. By elution with hexane, 20 mg. of starting ketone was obtained. After removal of the ketone, the alcohol was removed by elution of the column with ethyl ether to yield 708 mg. (85.8% based on starting acid) of *cis–cis*-5-hydrindanol. The product was characterized by allowing 0.175 g. (1.26 mmoles) to react with 0.15 ml. of phenyl isocyanate at 100° for 5 minutes. The phenylurethan was recrystallized from ligroin, yield 116 mg. (29.8% based on starting acid, 35.8% based on starting alcohol), m.p. 120.5–121.5° (lit.[4,6] 125°).

Anal. Calcd. for $C_{16}H_{21}O_2N$: C, 74.10; H, 8.16; N, 5.40. Found: C, 74.40; H, 8.34; N, 5.55.

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[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Determination of Dextran Structure by Periodate Oxidation Techniques

BY JOHN W. SLOAN, B. H. ALEXANDER, R. L. LOHMAR, I. A. WOLFF AND C. E. RIST

RECEIVED JANUARY 21, 1954

Dextrans produced by six strains of *Leuconostoc mesenteroides* were oxidized with sodium metaperiodate and the proportions of the various glucosidic linkages estimated by separation and quantitative determination of structural fragments of the oxidized dextrans. Dextran from *L. mesenteroides* NRRL B-512 was shown to contain 5% 1,3-linked anhydroglucose units in addition to the previously recognized 95% 1,6-linked units. Likewise, dextran from NRRL B-1064 yielded glucose indicating 3% 1,3-linked units where none were expected from titrimetric periodate analyses. Evaluation of the fragments obtained from NRRL B-1355 dextran disclosed no evidence of 1,4-linkage. A new technique for isolating periodate-oxidized dextrans has proved successful in producing the polymeric dialdehydes in high yields.

Bacterial dextrans are formulated as polymers of α-glucopyranosyl units linked in various ways. The kinds and proportions of these linkages may be inferred from the consumption of oxidant and the production of acid when the dextrans are subjected to periodate analysis.[2,3,4] In this analysis, by periodic acid or its salts, it is assumed that for every mole of acid produced in the oxidation, one anhydroglucose unit linked *only* in the 1- and 6-positions, or a non-reducing end group (linked at position 1) has been oxidized and its carbon 3 split out as formic acid. Because this requires the reduction of two moles of periodate, any additional periodate consumed is presumed to have been used in the opening between carbons 2 and 3 of 1,4-linked anhydroglucose rings, or in the opening between carbons 3 and 4 of 1,2-linked rings. If these possibilities do not account for all the dextran, the difference is represented by anhydroglucose units not oxidized by periodate. Such units are those linked in the 1- and 3-positions. Oxidation would also not occur in units to which a branch is linked if *both* positions 2 and 4 of a single unit were involved. However, it seems unlikely that the high proportions of unoxidized units found in some of the dextrans can be accounted for solely by the presence of units involved in multiple linkage. Generally, in dealing with periodate analysis of high molecular weight dextrans no separate allowance is made for the behavior of reducing end groups.

In order to demonstrate the presence of 1,3-linked units in dextrans and to provide more concrete evidence of other structural characteristics as well, it was desired to examine fragments of the molecule, other than formic acid, obtained from the oxidation by periodate. Methods that have been used to obtain fragments from periodate-oxidized polymers include hydrolysis and treatment with aldehyde reagents,[5] oxidation of the hydrolytic products with bromine to yield characteristic acids,[6] oxidation of the polymer with bromine water followed by hydrolysis and characterization of the acids,[7] hydrolysis and identification of

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(3) Allene Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin and C. E. Rist, Abstracts of Papers 122nd Meeting Am. Chem. Soc., Atlantic City (1952) p. 14A.

(4) R. Lohmar, THIS JOURNAL, **74**, 4974 (1952).

(5) C. G. Caldwell and R. M. Hixon, *J. Biol. Chem.*, **123**, 595 (1938).

(6) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **60**, 989 (1938).

(7) G. Jayme and S. Maris, *Ber.*, **77B**, 383 (1944).

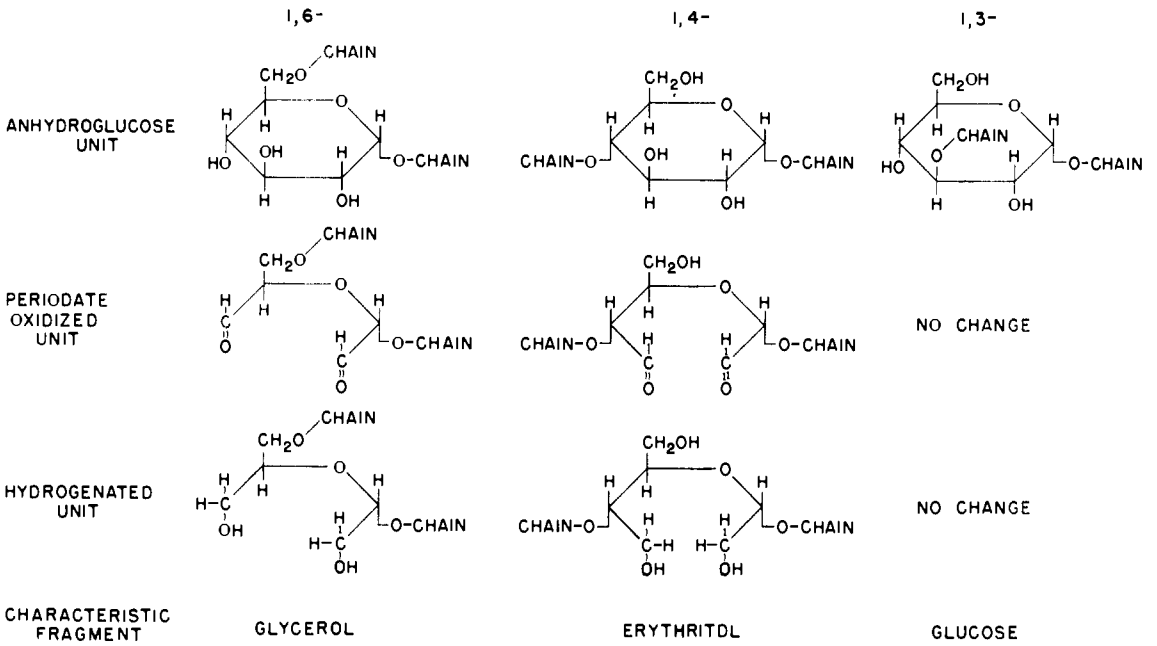

Fig. 1.—Degradation of periodate-oxidized anhydroglucose units to characteristic fragments.

aldehyde fragments as the acids formed *via* the cyanohydrin synthesis with C^{14}-labeled cyanide,[8] and hydrogenation of the polymer followed by hydrolysis and identification of the characteristic polyols.[4,7,9] We have chosen the last method and catalytically hydrogenated the aldehydic residue from dextran to the polyol which, upon hydrolysis, yields such easily identified fragments as glycerol, erythritol and glucose from 1,6-, 1,4- and 1,3-linked anhydroglucose residues in the original dextran. This separation and quantitative determination of fragments was considered a more specific characterization of the dextrans than the titrimetric method which depends solely on acid produced and periodate consumed in the oxidation.

Figure 1 traces the course of degradation of anhydroglucose units, linked in these three ways, to the final hydrolytic products. Glucose is completely characteristic for unoxidized units, as is erythritol for units linked in the 4- or 4- and 6-positions. Glycerol could result from non-reducing end groups or from units linked in the 6-, 2-, or 2- and 6-positions. In 1,2-linked units, glyceraldehyde, rather than glycolaldehyde would be the second fragment. However, an exclusive characteristic of non-reducing end groups and 1,6-linked units is that they give rise to formic acid on periodate oxidation, whereas no acid is expected when 2-linked units are oxidized.

Oxidation Conditions.—The selection of optimum oxidation conditions for this work was made from a series of oxidations using dextran from *L. mesenteroides* NRRL B-512 (hereinafter referred to as NRRL B-512 dextran) at varied periodate and carbohydrate concentrations and acidity. For preparative work it was desired to increase the dextran concentration beyond the 0.1% used in titration analyses in order to obtain larger quantities of products. Rapid over-oxidation occurred in a solution buffered at *p*H 4.2. We designate as over-oxidation the consumption of a greater number of moles of periodate than can be accounted for by the classical glycol-splitting action of periodates. An oxidation started at *p*H 1.2 (periodic acid oxidant) showed the most rapid decrease in viscosity—an indication of excessive degradation. Therefore, only sodium meta-periodate was used in subsequent oxidations because evidence of less degradation appeared at the intermediate *p*H. A series of oxidations with dextran concentrations 0.1, 0.4 and 0.81% and a ratio of 3 moles sodium metaperiodate per mole anhydroglucose unit (AGU) showed the feasibility of preparative oxidation at the higher concentration (limited by solubility in the case of some other dextrans) provided excessive over-oxidation could be avoided. A series of oxidations at 0.81% dextran concentration and varied sodium periodate ratios showed too great consumption of periodate at both 3 and 4 moles sodium periodate per mole AGU (Fig. 2) but nearly ideal consumption at 2.2 moles. The same series showed over-production of formic acid when more than a slight excess of sodium periodate was used (Fig. 3). Acidity of all three members of the series was similar and constant (*p*H 2.6–2.7) after an initial drop of approximately 0.3 *p*H unit the first 24 hours. Some difference in viscosity probably indicates less degradation with the smaller excess of periodate (Fig. 4).

Since the maxima of the formic acid curves lie at approximately 40 days and the least over-oxidation appears with about 25% excess periodate, these conditions were selected for the first preparative oxidation of this dextran. As a final precaution, subsequent oxidations were carried out at approximately 1° in order to retard over-oxidation.

(8) H. S. Isbell, Abstracts of Papers 122nd Meeting Am. Chem. Soc., Atlantic City, N. J., 1952, p. 17A.

(9) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, THIS JOURNAL, **74**, 4970 (1952).

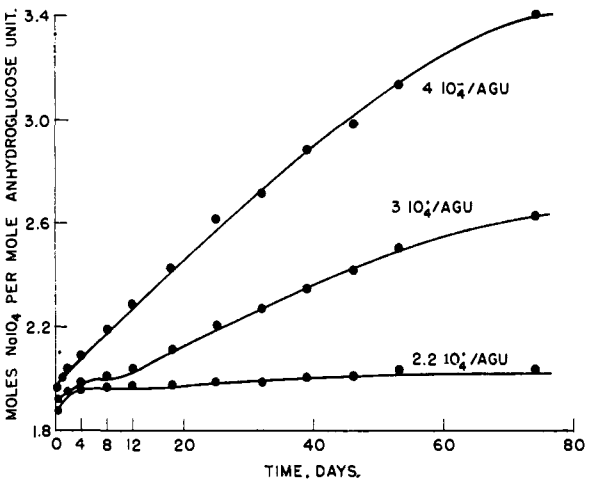

Fig. 2.—Periodate consumed by 0.81% B-512 dextran at 25°.

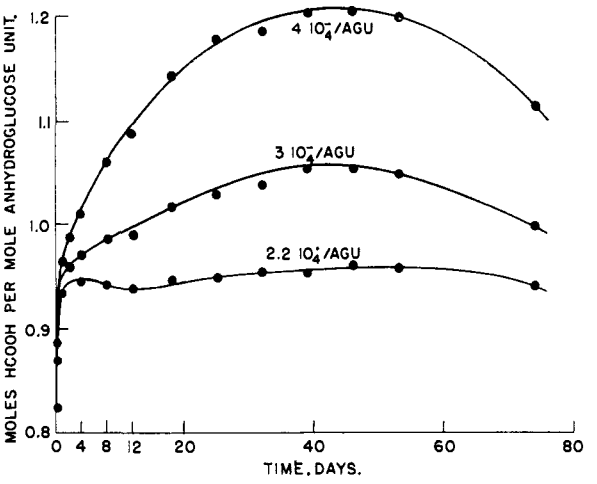

Fig. 3.—Formic acid resulting from 0.81% B-512 dextran at 25°.

Isolation and Properties of Oxidized Dextrans.—During the preliminary work to establish proper conditions for oxidation of dextran from NRRL B-512, attempts were made to recover the products from the oxidations at higher concentrations. The remaining periodate was reduced to iodate with glycol and the oxidation mixture was neutralized with barium hydroxide. The precipitated barium iodate was removed by filtration, and the filtrate was dialyzed until it gave a negative test for iodate. This gave a dialdehyde solution free of inorganic contamination but yields were low. Preliminary investigations carried out with a fraction of the dextran from NRRL B-742 gave similar results.

Isolation of Polymeric Aldehyde.—A new method was found that could be used to isolate oxidized dextran, free of all other reaction components, in much higher yields than obtained by the method that included dialysis. Sodium iodide and hydrochloric acid react with both periodate and iodate to give elemental iodine plus sodium chloride and water. These products, as well as formic acid and formaldehyde from the dextran, are all appreciably soluble in 90% (by volume) ethyl alcohol. The oxidized dextran, however, is precipitated upon addition of the aqueous mixture to absolute ethyl alcohol in the proper proportion.

In characterizing the dialdehyde (from NRRL B-512 dextran), carbonyl oxygen was determined by titration of hydrochloric acid liberated from the reaction with hydroxylamine hydrochloride[10] with glucose as a standard for comparison. Ultraviolet examination of the dialdehyde solution indicated no aldehyde absorption and infrared examination showed very little if any carbonyl group present although the presence of hydroxyl groups was indicated. Evidently the carbonyl groups are masked—probably by hydration. Rowen, Forziati and Reeves[11] in studies of the periodate oxidation product of cellulose give evidence pointing toward the existence of hydrated aldehyde groups rather than hemiacetal formation.

(10) E. K. Gladding and C. B. Purves, *Paper Trade J.*, **116**, [14], 26 (1943).

(11) J. W. Rowen, Florence H. Forziati and R. E. Reeves, THIS JOURNAL, **73**, 4484 (1951).

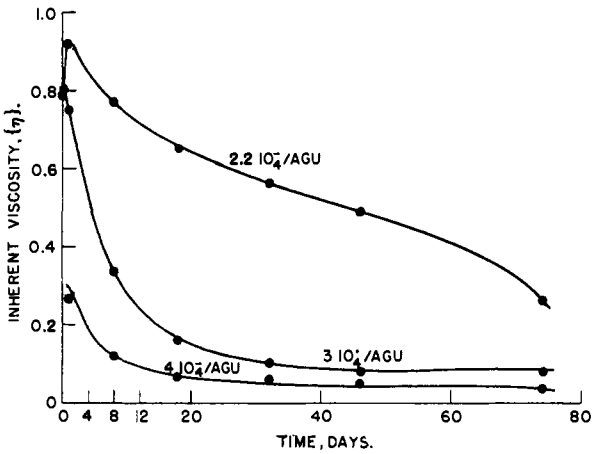

Fig. 4.—Inherent viscosity of 0.81% B-512 dextran oxidation at 25°.

The absorption spectrum of the crystalline dihydrate of the periodate oxidation product of methyl 4,6-O-benzylidene-α-D-glucoside gave no indication of free aldehyde groups. Similar observations on our product, which has no hydroxyl available for hemiacetal formation because carbon 6 is

TABLE I

DIALDEHYDE PROPERTIES[a]

	B-512		B-742-Sol.	
	Theory[b]	Found	Theory[b]	Found
Carbon, %	46.05	43.01	45.54	44.6
Hydrogen, %	4.75	5.32	5.13	5.50
Carbonyl oxygen, %[c]	23.3	21.3	17.0	14.2
Reducing power (Somogyi glucose equiv.)	2.7	0.25	...	.....
NaCl, % by conductivity	...	0.07	...	.....
$[\alpha]^{25}D$ (H_2O)	...	+171°[d]	...	+142°[e]
*p*H of 1.8% aqueous soln.	...	6.37	...	.....
Inherent viscosity	...	0.06	...	.....

[a] All analytical results on dry basis. [b] Theory calculated from structure determined by titrimetric periodate analysis. [c] Glucose standard gave 8.05% carbonyl oxygen as compared with theoretical 8.9%. [d] *c* 1.9. [e] *c* 1.0.

involved in polymeric linkage, tend to substantiate these views.

The dialdehydes were generally more difficultly soluble in water than the original dextrans; some required autoclaving. Other properties of two of the dialdehydes are listed in Table I.

The dialdehydes were reduced to polyols by hydrogenation over Raney nickel catalyst. The polyols were all liquids; some of their properties are shown in Table II. Carbon, hydrogen and acetyl analyses agreed well with theory.

TABLE II

POLYOL PROPERTIES[a]

	B-512 Theory[b]	B-512 Found	B-742-Sol. Theory[b]	B-742-Sol. Found
$[\alpha]^{25}D$ (c 0.8, H_2O)	..	− 8.1°	..	+59.4°
Inherent viscosity	..	0.06	..	.....
Acetate derivative				
Acetyl, %	39.7	40.8	41.6	41.5
Carbon, %	49.5	50.1	49.7	50.4
Hydrogen, %	6.5	6.6	6.2	6.0
$[\alpha]^{25}D$ (*sym*-tetrachloroethane)	..	+30.3°	..	+60.5°

[a] All analytical results on dry basis. [b] Theory calculated from structure determined by titrimetric periodate analysis.

Quantitative Studies.—Having selected suitable conditions for the oxidation of the two dextrans described, and for the isolation of their polymeric degradation products, five additional dextrans, from other strains of *L. mesenteroides*, were selected for study. These dextrans were selected to represent a variety of structures. Each was oxidized with 25% excess periodate and the products were isolated and hydrogenated, using conditions selected on the basis of the previous work. The hydrogenated polymers were subjected to acid hydrolysis, chromatographic separation of fragments, and their determination by anthrone[12] or chromotropic acid[13] procedure.

Comparison of the relative yields of the characteristic fragments with the values found by the titrimetric method[3,4] are given in Table III. Because the oxidation conditions for the titrimetric survey at the Northern Utilization Research Branch[1] are such as to minimize oxidation of formic acid,[14] these values were used to calculate weighted-unit molecular weights of the various dialdehydes and polyols in the compilation of the data on yields, both stepwise and over-all, shown in Table III.

Experimental

Materials.—The dextrans used in this work were prepared and purified as described by Allene Jeanes and co-workers.[3] The dextran from *Leuconostoc mesenteroides* NRRL B-742 had been further fractionated by precipitation between 41 and 90% ethyl alcohol.[4] The portion soluble in 41% ethyl alcohol will be referred to as the "soluble fraction" to distinguish it from the "insoluble fraction" which was precipitated at this alcohol concentration.

Preliminary Oxidations with NRRL B-512 Dextran.—All samples were oxidized for many days at 25° in the dark. Aliquots were withdrawn periodically for determination of periodate consumed (neutralization of remaining periodate with standard arsenite and back-titration with 0.1 N iodine solution) and formic acid produced (titration to phenolphthalein end-point with 0.01 N barium hydroxide solution). Also, acidity of the mixtures was followed by measurement of pH (glass electrode) and viscosity by the use of an 82-second outflow-time, pipet-type viscometer. This relative viscosity was converted to inherent viscosity, $[\eta]$, the ratio of the natural logarithm of relative viscosity to the concentration of the solute (we assumed the density of the solution equal to the density of the solvent, water).

Oxidation.—All oxidations were carried out in glass-stoppered flasks protected from light. Sufficient dextran to give the final concentration shown in Table I was weighed into the flask, slowly hydrated with carbon dioxide-free distilled water and made to volume including the specified amount of sodium metaperiodate. One sample of B-512 dextran was oxidized 44 days at 25° and another sample was oxidized 50 days at 1°. The preliminary work with B-742 soluble fraction at 25° showed comparable yields of fragments at 63 days and at 6 days; however, the oxidation of the insoluble fraction required 17 days at 1° in order to show production of formic acid equal to that from oxidation under conditions used for titration analysis.

Isolation of Dialdehyde.—On the basis of the final analysis of the oxidation mixture, 160% of the calculated amount of 6 N hydrochloric acid was added followed by an aqueous solution containing 150% of the calculated amount of sodium iodide. The resulting slurry was poured into 10 volumes of absolute ethyl alcohol (resulting in approximately 90% v./v. alcohol) with rapid stirring. The dextran dialdehyde precipitated and settled to the bottom, usually as a gummy mass, while the inorganic salts, acid and low molecular weight organic constituents remained in the solution and were decanted. Four to six washes with 95% ethyl alcohol (centrifuge) usually were sufficient to free the dialdehyde of substantially all extraneous ions. The residue was taken up in water and the ethyl alcohol removed by distillation at moderate temperature (30–40°) and reduced pressure. The resulting solution (or gelatinous suspension) of dextran dialdehyde was lyophilized or used directly in the next step.

The dialdehyde obtained from the 25° oxidation of NRRL B-512 dextran was used in some physical and chemical characterizations but the specific rotation, pH, viscosity and salt content results are from the cold oxidation. In order to avoid degradation, this latter sample (shown in Table I) was handled with exceptional caution throughout. It was dissolved by shaking under nitrogen and protected from high temperature and acidity.

TABLE III

CONDITIONS AND PRODUCTS IN REACTION OF DEXTRAN WITH SODIUM PERIODATE

Dextran from *L. mesenter.* NRRL B-	Prediction of structure from titration analysis[a] 1,6-	1,4-	1,3-	Oxidation conditions (1°) Concn. dextran, %	Oxidized with IO_4^-/AGU	Time, days	Yields in mole % Dialdehyde	Polyol	Total fragments	Adjusted to 100% Glyc.	Eryth.	Gluc.
512	95	5	0	1.0	2.20	50	99.5	75.6	55.8	95	0	5
742-sol.	58	18	24	0.2	3.06	6[b]	96.0	76.8	63.9	64	8	28
742-insol.	80	20	0	.4	2.26	17	71.4	61.8	46.0	95	4	Trace
523E	85	11	4	.4	2.26	6	99.8	65.0[c]	60.4	93	3	4
1064	95	5	0	.4	2.44	6	97.1	80.0	65.2	95	2	3
1355	57	8	35	.4	1.53	6	84.9	84.8	60.0[d]	53	0	47
1399	65	35	0	.4	2.06	6	0.04	...	...	..	...	...

[a] 1,6-, 1,4- and 1,3- refer to units linked as shown or equivalent structures. [b] 742-sol was oxidized at 25°. [c] Hydrogenation repeated after polyol showed slight reducing power. [d] Includes 5% hexitol.

(12) R. Dreywood, *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).

(13) Marguerite Lambert and A. C. Neish, *Can. J. Research*, **28B**, 83 (1950).

(14) P. B. Sarkar, *Nature*, **168**, 122 (1951).

Reduction, Hydrolysis and Assay.—The dextran dialdehydes were hydrogenated in a stainless-steel bomb at 100° and 1800–2000 p.s.i. of hydrogen over Raney nickel catalyst[15] for 1 hour. The catalyst was filtered off and washed with boiling water. The filtrate and washings were made to volume. Evaporation of an aliquot to dryness (viscous liquid) enabled calculation of the yield of polyol.

For purposes of characterization, a portion of the lyophilized polyol was dried over phosphorus pentoxide, dissolved in pyridine, and acetylated with acetic anhydride on a steam-bath for two hours. The acetate was also liquid and it was washed and recovered in chloroform solution.

The polymeric polyols were chromatographed and faint spots appeared in the glycerol position for 523 E and 1064 only—there were no other spots. All polyols were shown to have no reducing power (qualitative with Fehling solution) before proceeding.

Another aliquot of polyol solution was made 1 *N* with sulfuric acid and heated under reflux for 12–14 hours on a steam-bath. In all except very dilute solutions (0.3% polyol), brown color developed in 3–4 hours and deepened as the hydrolysis progressed. The hydrolysate was freed of acid by precipitation with barium hydroxide (to *p*H 6.5–6.7) and digestion at 100° for 1–2 hours. The filtrate was concentrated *in vacuo*, cooled, refiltered and made to volume.

The hydrolysates were spotted quantitatively in alternate guide and quantitative strips with an ultramicroburet on Whatman No. 1 filter paper and constituents separated by descending irrigation with butanol–pyridine–water mixture (6:4:3). Guide strips were sprayed with ammoniacal silver nitrate reagent to locate spots corresponding to glucose, erythritol and glycerol on the quantitative strips. The respective regions were then cut out of the quantitative strips and, with appropriate blanks, were eluted with water, following the procedure in use at this Laboratory.[16] Glucose was determined by the anthrone method[16] and glycerol and erythritol by the method of Lambert and Neish.[13]

In order to obtain quantities of fragments sufficient to allow the preparation of derivatives, the hydrolysate of the polyol from dextran B-742-sol. was first decolorized with Darco G-60, then streaked from a 1-ml. pipet on Whatman No. 4 paper, after the method of Zamecnik, *et al.*[17] Development with butanol–pyridine–water showed a glucose band and a single zone containing erythritol and glycerol. In order to free the erythritol from the overlapping glycerol, the erythritol zone was cut out, eluted and respotted for better resolution (this method was also used to determine erythritol in the hydrolysates from B-523E and B-1064). The fragments were obtained in sufficient amounts to prepare derivatives.

Glucose: derivative, benzimidazole hydrochloride, recrystallized product gave m.p. and mixed m.p. 179–180°. Also characterized previously as sorbitol hexaacetate[4]

Glycerol: derivative, tribenzoate; recrystallized from ethanol–water m.p. and mixed m.p. 72–73°

Erythritol: derivative, tetrabenzoate; recrystallized from pyridine–water m.p. 186–187°, mixed m.p. 186–188°

Discussion

Generally the yield of oxidized dextran obtained from each dextran type has been good. In two specific instances, lower yields are believed to have resulted from the use of oxidation conditions not well suited to the particular dextran or possibly from a characteristically greater solubility of the dialdehyde in ethanol. Some considerable losses resulted from the hydrogenation, possibly through adsorption on the catalyst so strongly as to resist removal by hot water washing. Further losses are presumed to have occurred during hydrolysis because caramelization was apparent from the color and odor of the hydrolysates.

(15) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(16) R. J. Dimler, W. C. Schaefer, C. S. Wise and C. E. Rist, *Anal. Chem.*, **24**, 1411 (1952).

(17) P. C. Zamecnik, R. B. Loftfield, M. L. Stephenson and J. M. Steele, *Cancer Research*, **11**, 592 (1951).

Comparison with Titrimetric Method.—The proportion of erythritol found was in all cases below that calculated from the titrimetric analysis. With the B-512 and B-1064 dextrans, it is apparent that the agreement between the titrimetric results and those reported here is within experimental error of the periodate consumed. For the other dextrans this may not be the case. Control experiments showed that added erythritol is not lost during hydrogenation of the dialdehydes or during hydrolysis of the polyol.

As the titrimetric analyses cannot distinguish between 1,2- and 1,4-linked units, the possibility that some of these dextrans also contain 1,2-linked units must be considered. These would yield glycerol when degraded by our scheme and would not be distinguishable from 1,6-linked units, without determination of the second fragment, D-glyceraldehyde. Only traces of material having the chromatographic mobility of glyceraldehyde could be detected in our hydrolysates. Furthermore, the dextran showing the greatest departure from predicted structure, B-742-insoluble, showed only a trace of unoxidized units. Thus if it contains 1,6-, 1,4- and 1,2-linked units, there are no branch points involving both positions 2 and 4.

The lowering of the viscosity of the dextrans during oxidation probably indicates hydrolysis of the glucosidic bonds. Such hydrolysis uncovers new sites for oxidation by periodate, resulting in the formation of more formic acid and the destruction of the moiety characteristic of the particular linkages involved. Under our conditions the over-oxidation, as judged by formic acid production,[18] does not appear to have been extensive enough to vitiate our results. Furthermore, such destruction would not be selective for the erythrose moiety unless most of the 1,4-linkages were contiguous. Analogy with other polysaccharides renders such structure unlikely. During the isolation of the dialdehyde, oxidation of the erythrose moiety by free iodine in acid solution, analogous to the well known bromine oxidation of aldoses, would prevent its conversion to erythritol. Here again it is unlikely that the oxidation would be so selective that the glycerose portion of other units would not be attacked also.

Two possible errors in interpretation of the results of titrimetric periodate analysis may lead us to expect more 1,4-linkage than is actually present. One would result from the lag in the production of formic acid behind consumption of periodate,[19] although this should be quite small by the end of 4 days (oxidation time used in the titrimetric determination). The other would result from the oxidation of formic acid by periodate, indicating a lesser percentage of 1,6-linkages, hence more 1,4-linkages. However, no such effect would be expected under the analytical conditions used.[3,14] One or both of these considerations may account for the prediction of 5% 1,4-linkage in B-512 where none was found (also 5% 1,4-linkage predicted in B-1064 but only 2% found). Smith and co-workers,[9]

(18) G. Neumüller and E. Vasseur, *Arkiv Kemi*, **5**, 235 (1953).

(19) G. Hughes and T. P. Nevell, *Trans. Faraday Soc.*, **44**, 941 (1948).

using a method similar to the one described here but a different sample of dextran from *L. mesenteroides* NRRL B-512, found approximately 95.6% 1,6-linkage with the remainder equally divided between 1,4- and 1,3-linkage.

It should be noted that Schardinger dextrins, which contain no end groups, can consume more periodate than required by theory.[20] No formic acid is produced and hydrolysis is less likely than when dextrans are oxidized. Hence there is a possibility that periodate oxidation, even under mild conditions, is sufficiently non-selective or that the analytical determination of periodate lacks the accuracy to permit precise calculation of the relative proportions of the various linkages. Barker, *et al.*,[21] have determined, by methylation analysis, that a dextran from *Betacoccus arabinosaceous* contains 57% 1,6-linked units and 17% end groups. This agreed well with the formic acid produced on periodate oxidation, but the dextran consumed 1.67 moles periodate per AGU, whereas the methylation data would lead one to expect only 1.48 moles.

Atypical Behavior of Some Dextrans.—Dextran from B-1399 was chosen for study because of the large proportion of 1,4-linkage, and this may be responsible for the highly anomalous behavior of this type. Table III shows that the attempt to recover an oxidized product from B-1399 dextran failed: The product did not precipitate in 90% ethyl alcohol in the isolation step and the alcohol (plus elemental iodine) was removed in a circulating evaporator. Only a very small amount of product was recovered after dialysis of this solution. The dextran appears to have been highly degraded, probably by oxidation conditions not suited to this type of dextran. This result indicates the desirability of preliminary investigations for each dextran.

A hexitol having the chromatographic characteristics of sorbitol was found in hydrolysates from B-1355 dextran. It was isolated as the pyridine complex and characterized by conversion to sorbitol hexaacetate.[4] The sorbitol is presumed to have been produced from glucosidic end groups resulting from hydrolysis during hydrogenation. Hydrolysis of full acetals has been noted during hydrogenation over Raney nickel.[22] Quantitative analysis, by the same method used for glycerol and erythritol, showed the sorbitol to be present in about one-sixth the quantity of glucose. In Table III, the sum of the molar percentages of glucose and sorbitol has been used to calculate the proportion of 1,3-linkages. Here again anomalous behavior was noted in a dextran that was chosen for study because of a structural characteristic—the very high proportion of 1,3-linkage.

Interpretation of Results.—A word of caution is in order regarding the interpretation of the data presented in this paper, or of any results based on periodate oxidation. Increased proportions of 1,4- or 1,3-linked units should not be accepted as sole indications of higher degrees of branching in dextran molecules. It is known that at least two different linkages may occur at intervals in a straight chain.[23] Furthermore, a high proportion of 1,6-linked units as indicated by periodate oxidation methods does not necessarily represent an approach to linearity of the molecule. A highly branched molecule would have many non-reducing end groups, which are indistinguishable from 1,6-linked units on periodate oxidation.

Acknowledgment.—The authors are indebted to C. H. Van Etten and Mrs. Clara E. McGrew of the Analytical, Physical-Chemical, and Physics Section of this Laboratory for the carbonyl oxygen, carbon and hydrogen analyses; to E. H. Melvin for the infrared and ultraviolet examinations; and to Mrs. Phyllis L. Patrick and R. W. Jones of the Starch and Dextrose Section of this Laboratory for viscosity, reducing power and glucose (by anthrone) determinations. Also we are grateful for the supply of dextran types made available by Allene Jeanes. The use of trade names in this paper should not be construed as an endorsement of these products or of the manufacturers thereof.

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(20) D. French and R. L. McIntire, THIS JOURNAL, **72**, 5148 (1950).

(21) S. A. Barker, E. J. Bourne, G. T. Bruce and M. Stacey, *Chemistry and Industry*, 1156 (1952).

(22) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **75**, 4503 (1953).

(23) R. J. Bayly and E. J. Bourne, *Nature*, **171**, 385 (1953).