was covered directly with 1000 ml. of 6 *N* sulfuric acid and boiled under reflux 3 hours. After cooling, the acid solution was decanted from the resinous solid, and the solid washed with water by decantation. The solid was dissolved in benzene, washed first with sodium bicarbonate solution and then with water, and finally dried by boiling under a water-separatory head. The dry benzene solution was concentrated to dryness under reduced pressure to yield 46 g. of IV as a very viscous light yellow oil which would not solidify and which was probably a mixture of *cis* and *trans* isomers. Paper chromatography indicated only one compound with R_f 0.80 (R_f 0.80 in 10:3:3 butanol–pyridine–water). All attempts at recrystallization failed.

Acetylation of the above non-crystalline IV with acetic anhydride in pyridine gave an almost quantitative yield of its diacetate IX as a crystalline solid, which upon recrystallization from methanol yielded fine white needles of IX melting at 194–195° and fluorescing strongly under ultraviolet light. The ultraviolet absorption spectrum showed a maximum λ_{max} 280 mμ, ϵ 7860.

Anal. Calcd. for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found: C, 69.89; H, 6.88.

Attempted reductions of VII with sodium in boiling ethanol yielded only the starting VII.

Propiovanillone Hydrazone.—A mixture of 40 g. of V, 14 g. of 85% hydrazine hydrate and 50 ml. of isopropyl alcohol was boiled under reflux for 8 hours and cooled. The clear solution was concentrated to dryness under reduced pressure and stirred with benzene. The granular precipitate was filtered and recrystallized from benzene to yield 24 g. of propiovanillone hydrazone melting at 109–110° and having the maxima in its ultraviolet absorption spectrum: λ_{max} 272 mμ, ϵ 11900; λ_{max} 214 mμ, ϵ 17250.

Anal. Calcd. for $C_{10}H_{14}O_2N_2$: N, 14.42. Found: N, 14.54.

Oxidation of this hydrazone to the corresponding diazo compound and decomposition by sulfur dioxide in accordance with the procedure of Gyar[11] failed to produce IV.

Acknowledgment.—The author wishes to thank Mr. Harold Willemsen for the analyses and spectra reported in this paper.

(11) R. G. V. Gyar, British Patent 526,927 (Sept. 27, 1940).

APPLETON, WISCONSIN

[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

The Structure of NRRL B-512 Dextran. Methylation Studies[2]

BY J. W. VAN CLEVE, W. C. SCHAEFER AND C. E. RIST

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Fully methylated NRRL B-512 dextran was completely hydrolyzed by heating in an acetic acid solution containing aqueous hydrochloric acid. The resulting mixture of reducing sugars, on passage through a cellulose column, afforded 2,4-di-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose in a molecular ratio of 1:21:1. The proportion of 1,6-linked anhydroglucose units and non-reducing end-groups thus indicated agrees closely with the results obtained through periodate oxidation. The isolation and identification of 2,4-di-*O*-methyl-D-glucose in the products of hydrolysis of the methylated polysaccharide proved that NRRL B-512 dextran contains the unusual 1,3-glucosidic linkage and that this linkage occurs at the branch points.

The dextran produced from sucrose by *Leuconostoc mesenteroides* NRRL B-512[3] has recently gained considerable importance through its use in the manufacture of a synthetic blood-volume expander. Related to its effectiveness for such use is the manner in which the clinical material is metabolized in the human body. As a consequence the chemical structure of the native dextran has been of considerable interest.

Early structural information was provided by periodate oxidation studies[4] which showed that 95% of the anhydroglucose units of this polyglucosan are 1,6-linked residues or non-reducing end-groups. The remaining linkages, the nature of which was not defined, have been assumed to be 1,4[4a] although some evidence has indicated the presence of other linkage types[5] in this dextran.

A more definitive approach to the problem of structure is afforded through methylation studies such as are described in the present paper. We have hydrolyzed a fully methylated sample of NRRL B-512 dextran and have resolved the resulting mixture of reducing sugars into its components by passage through a cellulose column. The methylated sugars so isolated were identified through the preparation of crystalline derivatives. Also, from the molar ratio of the isolated reducing sugars, the average length of the unit chain and the limits of its structural possibilities could be ascertained.

Preliminary examination by paper chromatography of the products of hydrolysis of the trimethyldextran revealed, in addition to the methylated sugars, a number of hydrolytic artifacts. A detailed investigation of the nature of these artifacts and the elaboration of quantitative methods for dealing with them were necessary prerequisites for obtaining structurally accurate results from the separation of the reducing sugars on the cellulose column.

Experimental

All melting points are uncorrected and were determined with a Fisher–Johns melting point apparatus.[6] All evaporations were performed at reduced pressure unless otherwise stated.

Materials and Apparatus. (a) **Chromatography.**—For qualitative chromatography Whatman No. 1 filter paper was used, development being performed in the descending manner. Sugars were detected on developed chromato-

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture.

(2) Presented at the 125th meeting of the American Chemical Society, Kansas City, Missouri, March 30, 1954.

(3) The dextran used for the present studies was produced by *Leuconostoc mesenteroides* NRRL B-512F as described by A. Jeanes, C. A. Wilham and J. C. Miers, *J. Biol. Chem.*, **176**, 603 (1948). NRRL B-512F is a substrain which in 1950 supplanted the original strain, NRRL B-512, at the Northern Utilization Research Branch. This substrain is the organism which is used industrially. Since 1950 it has been commonly designated (though inexactly) as NRRL B-512 in numerous publications. This common designation is used throughout the present article. *Cf.* THIS JOURNAL, **76**, 5045 (footnote 27) (1954).

(4) (a) A. Jeanes and C. A. Wilham, *ibid.*, **72**, 2655 (1950); (b) J. C. Rankin and A. Jeanes, *ibid.*, **76**, 4435 (1954).

(5) (a) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, *ibid.*, **74**, 4970 (1952); (b) J. W. Sloan, B. H. Alexander, R. L. Lohmar, I. A. Wolff and C. E. Rist, *ibid.*, **76**, 4429 (1954); (c) S. C. Burket and E. H. Melvin, *Science*, **115**, 516 (1952).

(6) The mention in this article of firm names or commercial products does not constitute an endorsement of such firms or products by the U. S. Department of Agriculture.

grams with *p*-dimethylaminoaniline spray reagent.[7] For quantitative chromatography a cellulose column[8] and an automatic fraction collector[9] were used.

The partitioning solvent used for both paper and cellulose column chromatography was methyl ethyl ketone–water azeotrope.[7,8] This solvent was purified before use as follows: To 2 liters of methyl ethyl ketone (tech.) saturated with water or to 2 liters of the azeotrope was added 0.5 g. of magnesium sulfate followed by a few small crystals of potassium permanganate. While the mixture was repeatedly agitated, the small additions of permanganate were continued until the color was no longer rapidly discharged. After standing overnight the treated solvent was decanted from the manganese dioxide and was distilled at atmospheric pressure with a 2-ft. stillhead. The fraction distilling at 73–74° was collected and stored in the dark to avoid light-catalyzed autoxidation.

(b) **Reference Compounds.**—Compounds 4 and 5, the preparations of which are given below, are useful characterizing derivatives of 2,3,4-tri-*O*-methyl-D-glucose which are described here for the first time. They have the advantage of ease of preparation in good yields and are readily obtained in analytically pure condition.

(1) **2,4-Di-*O*-methyl-D-glucose.**—This substance[10] had m.p. 128–130°; $[\alpha]^{25}D$ (equil.) +76.5° (*c* 1.6, water). It was chromatographically uniform and completely free of 2,3- and 3,4-di-*O*-methyl-D-glucose, the most likely impurities in this preparation.[10]

(2) **N-*p*-Bromophenyl-2,4-di-*O*-methyl-D-glucosylamine.**—This compound[10] had m.p. 243–244°; $[\alpha]^{25}D$ −147° (*c* 0.5, pyridine).

(3) **2,3,4-Tri-*O*-methyl-D-glucose.**—This trimethylglucose[11] had $[\alpha]^{25}D$ (equil.) +71.9° (*c* 3.4, acetone). Irvine and Oldham reported $[\alpha]^{20}D$ +70.5° (acetone) for this substance.

Anal. Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: OCH_3, 41.9, 41.7.

The preparation contained trace amounts of dimethylglucose and acid reversion products. These impurities were removed on passage of the trimethylglucose through the cellulose column.

(4) **N-*p*-Nitrophenyl-2,3,4-tri-*O*-methyl-D-glucosylamine.**—To a solution of 2,3,4-tri-*O*-methyl-D-glucose (0.111 g.) in absolute ethanol (3 ml.) was added *p*-nitroaniline (0.115 g.) and 1 drop of glacial acetic acid. The mixture was refluxed for 2 hours then allowed to cool. The product, N-*p*-nitrophenyl-2,3,4-tri-*O*-methyl-D-glucosylamine, which started to crystallize during the refluxing, was washed with a few ml. of ethanol; yield 0.104 g. (61%). The dark crystals were dissolved in absolute ethanol; the solution was treated with activated charcoal and filtered. Concentration gave the pure product (light yellow crystals), m.p. 224–225°; $[\alpha]^{25}D$ −251° (*c* 0.5, pyridine).

Anal. Calcd. for $C_{15}H_{22}O_7N_2$: C, 52.6; H, 6.5; N, 8.2; OCH_3, 27.2. Found: C, 52.7; H, 6.4; N, 8.2; OCH_3, 27.5.

(5) **N-*p*-Bromophenyl-2,3,4-tri-*O*-methyl-D-glucosylamine.**—To a solution of 2,3,4-tri-*O*-methyl-D-glucose (0.133 g.) in absolute ethanol (3 ml.) was added *p*-bromoaniline (0.132 g.) and 1 drop of glacial acetic acid. The mixture was refluxed for 2 hours and allowed to cool. The crystalline N-*p*-bromophenyl-2,3,4-tri-*O*-methyl-D-glucosylamine, which separated during the refluxing, was washed with a few ml. of absolute ethanol; yield 0.152 g. (79%). After two recrystallizations from absolute ethanol the product had m.p. 198°; $[\alpha]^{25}D$ −146° (*c* 0.5, pyridine).

Anal. Calcd. for $C_{15}H_{22}O_5NBr$: C, 47.9; H, 5.9; N, 3.7; Br, 21.2; OCH_3, 24.7. Found: C, 47.8; H, 5.8; N, 3.8; Br, 21.2; OCH_3, 24.4.

(6) **2,3,4,6-Tetra-*O*-methyl-D-glucose.**—This substance[12] had m.p. 89–93°; $[\alpha]^{25}D$ +79.7° (*c* 5, water). West and Holden gave $[\alpha]^{20}D$ +81.3° (*c* 4, water); the melting point of the pure product according to them varies with the ratio of α- and β-anomeric forms present. The preparation was recrystallized repeatedly from petroleum ether (b.p. 40–60°) until free of all traces of lesser methylated glucoses as detected by paper chromatography.

Methylation of NRRL B-512 Dextran.[13]—A dried sample of finely divided, fluffy NRRL B-512 dextran[3] (9.4 g.) having an intrinsic viscosity of 1.09 (water, 25°) and a molecular weight of 30,000,000[14] was methylated in liquid ammonia with sodium and methyl iodide.[15] After 4 methylations the methoxyl content[16] was 42%; after 9 methylations, 44.5%; after 13 methylations, 45.4% (theoretical, 45.6% OCH_3); yield 6.8 g. (58%).

A sample (1.13 g.) of the fully methylated product ($[\alpha]^{25}D$ +215° (*c* 0.53, *sym*-tetrachloroethane)) having an intrinsic viscosity of 1.01 (*sym*-tetrachloroethane, 25°) was fractionated by dissolving in chloroform (28 ml.) and adding ligroin (b.p. 90–100°) in measured amounts. The results of this fractionation (Table I), indicated only slight differences in intrinsic viscosity among the fractions separated. Thus the methylated dextran was essentially homogeneous.

TABLE I

FRACTIONATION OF METHYLATED NRRL B-512 DEXTRAN

Fraction	Ligroin, %	Intrinsic viscosity (*sym*-tetrachloroethane) $[\alpha]^{25}D$ (*sym*-tetrachloroethane)	Intrinsic viscosity (*sym*-tetrachloroethane) $[\eta]^{25}D$	Wt., g.	% of sample
1	67	....	..	0.01	1
2	79	+215°	0.91	.52	46
3	80	+215°	.89	.28	25
4	81 or >81	+218°	.95	.24[a]	21
			Total recovery	1.05	93

[a] Approximately 88% of fraction 4 was precipitated at a solvent composition of 81% ligroin; the remainder was obtained by evaporation of the solvent.

Hydrolysis of the Trimethyldextran.—Fully methylated dextran (0.2 g.) (unfractionated whole sample) was dissolved in glacial acetic acid (10 ml.) by warming (steam-bath). An equal volume of 1 *N* hydrochloric acid was added and the resulting solution was heated at 95° (bath) to constant optical rotation (14 hours). Chloride was precipitated from the hydrolyzate with basic lead carbonate; distillation of the filtrate (bath, 40–45°) removed the aqueous acetic acid. The residue was dissolved in water, and the solution saturated with hydrogen sulfide. Evaporation to dryness (bath, 40–45°) gave a residue which was extracted with methanol. The methanolic solution was filtered and evaporated to give a sirupy mixture of methylated glucoses.

Preliminary Chromatographic Examination of the Hydrolyzate.—Paper chromatography of the above mixture indicated the presence of four principal substances. Three of these were identified tentatively as (1) 2,4-di-*O*-methyl-D-glucose, (2) 2,3,4-tri-*O*-methyl-D-glucose and (3) 2,3,4,6-tetra-*O*-methyl-D-glucose by chromatographic comparison with authentic specimens of various methylated glucoses. The fourth substance ($R_g > 1$), as shown below, proved to be acetylated 2,3,4-tri-*O*-methyl-D-glucose.

A careful search was made for other methylated glucoses in the trimethyldextran hydrolyzate. Chromatograms of the hydrolyzate, whether "spotted" lightly or heavily on the starting line, failed to reveal either 2,3-di- or 2,3,6-tri-*O*-methyl-D-glucose. Likewise no tetramethylfructofuranose could be detected in these chromatograms when the specific spray reagent for methyl ketoses, N-(1-naphthyl)-ethylenediamine, was used.[7]

Close visual examination revealed that each of the three spots on the paper chromatogram corresponding to 2,4-di-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose were contaminated with

(7) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

(8) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949); L. Boggs, L. S. Cuendet, M. Dubois and F. Smith, *Anal. Chem.*, **24**, 1148 (1952).

(9) R. J. Dimler, J. W. Van Cleve, Edna M. Montgomery, L. R. Bair, F. J. Castle and J. A. Whitehead, *ibid.*, **25**, 1428 (1953).

(10) J. W. Van Cleve and W. C. Schaefer, THIS JOURNAL, **77**, 5341 (1955).

(11) J. C. Irvine and J. W. H. Oldham, *J. Chem. Soc.*, 1754 (1921).

(12) E. S. West and R. F. Holden, *Org. Syntheses*, **20**, 97 (1940).

(13) Performed by J. E. Hodge.

(14) Weight average molecular weight (M_w): N. N. Hellman and F. R. Senti, unpublished work, this Laboratory.

(15) J. E. Hodge, S. A. Karjala and G. E. Hilbert, THIS JOURNAL, **73**, 3312 (1951).

(16) E. P. Clark, *J. Assoc. Offic. Agr. Chemists*, **25**, 136 (1932).

TABLE II

COLUMN SEPARATION OF METHYLATED GLUCOSES FROM TRIMETHYL NRRL B-512 DEXTRAN HYDROLYZATE

	Dimethylglucose Wt., g. (cor.)	Mmoles	Trimethylglucose Wt., g. (cor.)	Mmoles	Tetramethylglucose Wt., g. (cor.)	Mmoles	Molar ratio[a] Di-	Tri-	Tetra-
Duplicate 1	0.039	0.186	0.862	3.88	0.027	0.116	1.0	20.9	0.6
Duplicate 2	.028	.135	.625	2.81	.029	.122	1.0	20.8	.9
Control expt. (recovered sugars)	.024	.114	.514	2.31	.013	.053	1.0	20.2	.5

[a] In an independent experiment quantitative paper chromatography[18] of trimethyl NRRL B-512 dextran hydrolyzate gave a molar ratio of 1 (di-):20 ± 1 (tri-):1 (tetra-).

small amounts of other substances. These all proved, as shown below, to be hydrolytic artifacts produced by the action of the acidic hydrolyzing medium on 2,3,4-tri-*O*-methyl-D-glucose.

Hydrolytic Artifacts of the Methylated Glucoses.—Chromatographically homogeneous and analytically pure samples of each of the three methylated sugars produced from trimethyl NRRL B-512 dextran were, in separate experiments, dissolved in 1 *N* hydrochloric–glacial acetic acid (1:1, v./v.) mixture and heated for 14 hours at 95° (bath). The product of each experiment, isolated from the acidic mixture in the manner previously described, was examined by paper chromatography.

From 2,3,4-tri-*O*-methyl-D-glucose there were produced a mixture of the original sugar plus three additional products. One of these products ($R_g > 1$) was quantitatively transformed to 2,3,4-tri-*O*-methyl-D-glucose by deacetylation with barium methoxide at 0°,[17] and consequently must consist of acetylated 2,3,4-tri-*O*-methyl-D-glucose. The other two, which were not affected by the deacetylation, consisted of a small amount of dimethylglucose and a substance which appeared on the chromatogram as a long streak extending from the leading edge of the 2,3,4-tri-*O*-methyl-D-glucose spot into the region of the solvent front. The dimethylglucose, which must have been produced from 2,3,4-tri-*O*-methyl-D-glucose by demethylation, was shown by quantitative paper chromatography[18] to correspond, in amount, to 0.5% of the original weight of 2,3,4-tri-*O*-methyl-D-glucose. The substance represented by the long streak on the chromatogram was investigated separately.

It had been observed that a sample of 2,3,4-tri-*O*-methyl-D-glucose prepared by hydrolysis of 2,3,4-tri-*O*-methyl-D-levoglucosan with hydrochloric acid gave a long streak on a paper chromatogram similar to that described above. These streaks were assumed in both instances to represent the same material. A quantity of this material was isolated by passing 2,3,4-tri-*O*-methyl-D-glucose[11] (2.0 g.) through the cellulose column. A sample of the streaking material (0.02 g.) thus separated from the trimethylglucose preparation was subjected to the hydrolysis and deacetylation procedure described above for the trimethylglucose. The sole product of this treatment was shown by paper chromatography to be 2,3,4-tri-*O*-methyl-D-glucose. Thus, the long streaks on the paper chromatograms proved to be reversion products of 2,3,4-tri-*O*-methyl-D-glucose.

From the tetramethylglucose experiment were recovered the original sugar plus a small amount of trimethylglucose. The latter was evidently a demethylation artifact. As determined by quantitative paper chromatography,[18] it corresponded to 1.1% of the starting weight of 2,3,4,6-tetra-*O*-methyl-D-glucose. No other artifacts could be detected.

From 2,4-di-*O*-methyl-D-glucose was recovered only the original product, no artifacts having been produced which could be detected chromatographically.

Column Separation of the Mixture of Methylated Sugars Produced from Trimethyl NRRL B-512 Dextran.—A sample (1.842 g.) of the fully methylated NRRL B-512 dextran (not fractionated) was hydrolyzed in the hydrochloric acid–glacial acetic acid mixture (200 ml.) as previously described and the hydrolyzate deacetylated in the usual manner. The product (1.859 g., yield 93%) was added to the product of two previous hydrolyses (total, 2.311 g.), and the combined sirup was divided into two equal parts for duplicate separations on the cellulose column.

Each duplicate separation yielded three fractions corresponding to di-, tri- and tetramethylglucose. The tetramethylglucose fraction (*ca.* 0.1 g.) thus obtained was an oil which would not crystallize. It was dissolved in the hydrochloric acid–glacial acetic acid hydrolyzing mixture (40 ml.) and the contaminating reversion products were hydrolyzed completely by heating at 95° (bath) for 14 hours. The product was deacetylated in the usual manner and was then passed through the cellulose column. In this way there was obtained chromatographically pure 2,3,4,6-tetra-*O*-methyl-D-glucose which crystallized spontaneously. In addition a small supplemental amount of 2,3,4-tri-*O*-methyl-D-glucose, corresponding to the hydrolyzed reversion products, was also obtained. This small supplement was added to the previously separated trimethylglucose fraction. Thus three fractions consisting of dimethylglucose, trimethylglucose, and tetramethylglucose were obtained for each duplicate separation of the mixture of methylated D-glucoses from trimethyl NRRL B-512 dextran. To each fraction corrections were applied for demethylation of 2,3,4-tri-*O*-methyl-D-glucose (0.5%) and 2,3,4,6-tetra-*O*-methyl-D-glucose (1.1%). Each fraction in addition to the methylated sugar contained some extraneous material from the column or the solvent; therefore it was assayed for purity by means of quantitative paper chromatography,[18] and the appropriate corrections were applied.

Table II gives results of the two duplicate separations. The foregoing corrections for purity and for demethylation changes were applied to give the corrected weights of the isolated sugars.

In a control experiment a mixture of analytically and chromatographically pure samples of 2,4-di-*O*-methyl-D-glucose (0.028 g.), 2,3,4-tri-*O*-methyl-D-glucose (0.626 g.) and 2,3,4,6-tetra-*O*-methyl-D-glucose (0.032 g.) (molar ratio, 1:21:1) was subjected to exactly the same procedure of hydrolysis, deacetylation and separation on the cellulose column as was used above for the trimethyldextran. The results, corrected for purity and demethylation changes, also are given in Table II.

Identification of the Methylated Glucoses.—The combined dimethylglucose fractions from the two duplicate column separations were dissolved in water and heated on the steam-bath with decolorizing charcoal. Filtration followed by evaporation gave sirupy 2,4-di-*O*-methyl-D-glucose (0.069 g.) which was identified by conversion to N-*p*-bromophenyl-2,4-di-*O*-methyl-D-glucosylamine,[10] m.p. and mixed m.p. 242°.

The 2,3,4-tri-*O*-methyl-D-glucose from the column separations was identified through the preparation of the two derivatives previously described, N-*p*-nitrophenyl- and N-*p*-bromophenyl-2,3,4-tri-*O*-methyl-D-glucosylamines. The former had m.p. and mixed m.p. 225°; the latter gave m.p. and mixed m.p. 195°.

The tetramethylglucose fractions from the two column separations were combined and treated with decolorizing charcoal in warm methanol (200 ml.). Evaporation of the filtered solution gave a sirup which on seeding crystallized completely. Two recrystallizations from petroleum ether (2–3 ml.) at 2° gave 2,3,4,6-tetra-*O*-methyl-D-glucose (0.027 g.), m.p. and mixed m.p. 85–87°.

Results and Discussion

Hydrolysis of trimethyl NRRL B-512 dextran gave approximately 1 mole of 2,4-di-*O*-methyl-D-glucose, 21 moles of 2,3,4-tri-*O*-methyl-D-glucose and 1 mole of 2,3,4,6-tetra-*O*-methyl-D-glucose. From these results it is apparent that NRRL B-512 dextran is a branched polysaccharide composed entirely of anhydroglucopyranose residues approximately 91% of which are mutually joined by 1,6-

(17) H. S. Isbell, *J. Research Natl. Bur. Standards*, **5**, 1185 (1930).

(18) W. C. Schaefer and J. W. Van Cleve, *Abs. Papers, Am. Chem. Soc.*, **124**, 12D (1953).

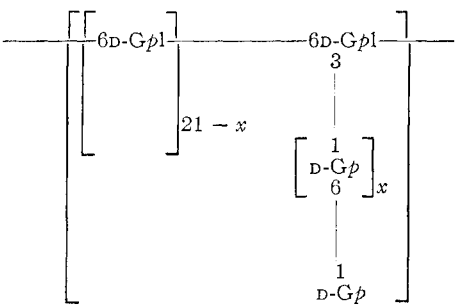
linkages. In addition about 4% of the residues consist of non-reducing end-groups. Thus, on oxidation of the dextran by periodate, formic acid should be produced by 95% of the anhydroglucose residues. This result, as previously stated, is in close agreement with that of Jeanes and co-workers.[4] The remaining residues must consist of 1,3,6-linked units representing branch points.

Due to a prior assumption of the presence of 1,4-linkage in this dextran[4a] it was of interest to examine closely the products of hydrolysis of the trimethyldextran for evidence of this type of linkage. Methylation of 1,4- and 1,4,6-linked anhydroglucose residues should give rise to 2,3-di- and 2,3,6-tri-*O*-methyl-D-glucose, respectively. No 2,3-di-*O*-methyl-D-glucose could be detected in the trimethyldextran hydrolyzate although in control experiments admixtures of 2,3- and 2,4-di-*O*-methyl-D-glucoses were effectively resolved, on paper chromatograms, into their components. Unfortunately we were unable to ascertain the presence or absence of small amounts of 2,3,6-tri-*O*-methyl-D-glucose in the hydrolyzate mixture through paper chromatography inasmuch as the partitioning solvent, methyl ethyl ketone–water azeotrope, failed to resolve small admixtures of this sugar with 2,3,4-tri-*O*-methyl-D-glucose. However, it would appear that little if any 2,3,6-tri-*O*-methyl-D-glucose was present in the hydrolyzate from the following considerations. Periodate oxidation results[4] have indicated that 95% of the anhydroglucose units of this dextran are either 1,6-linked or are non-reducing end-groups. Also, according to the present methylation study, about 4% of the anhydroglucose units must be 1,3,6-linked. Thus, after allowances for experimental errors, not more than 1% of the linkages remain unaccounted for and could conceivably be composed of 1,4,6-linked residues.

The acid reversion products of 2,3,4-tri-*O*-methyl-D-glucose interfered with the column separation of 2,3,4,6-tetra-*O*-methyl-D-glucose from the trimethyldextran hydrolyzate. These reversion products constituted about 70% of the weight of the isolated tetramethylglucose fraction, which consequently failed to crystallize. A similar difficulty had been encountered by others during methylation studies on amylose[19] and on certain inulins.[20] This difficulty in our case was resolved by a secondary hydrolysis, in dilute solution, of the impure tetramethylglucose fraction and repassage of the resulting mixture of trimethyl- and tetramethylglucose through the cellulose column. In this way crystalline tetramethylglucose of chromatographic purity was obtained.

Unfortunately the excessive manipulations required by the above method of isolation of tetramethylglucose made losses difficult to avoid. As a result recoveries tended to be low and erratic. Therefore the length of the unit chain could not be satisfactorily calculated in the customary manner from the recovered tetramethylglucose. Theoretically, it should be possible, however, to calculate it from the dimethylglucose produced by fully methylated dextran if one assumes that an equimolar relationship exists between branched points and end-groups. In view of the fact that the dextran sample used in the present study was methylated to the theoretical methoxyl content it appeared that hydrolysis should yield dimethylglucose corresponding accurately in amount, after allowance for demethylation artifact, to the branch points present. Hence calculation of the average unit chain length is based on the recovered dimethylglucose.

The results indicate a structure for NRRL B-512 dextran in which the average unit chain consists of 23 anhydroglucose residues. Of this number 21 are 1,6-linked residues, one is a 1,3,6-linked residue (branch point), and one is a non-reducing end-group. The following formula shows the range of structural possibilities for the unit chain as defined by the methylation results



Further elucidation of the structure of the repeating unit of this polysaccharide must await the outcome of other investigations such as partial hydrolysis by enzymes or acid. Recent studies of this nature[21] favor side chains consisting, predominantly, of only one glucose residue ($x = 0$).

Acknowledgment.—The authors are indebted to C. H. Van Etten and Clara E. McGrew for the microanalyses.

PEORIA, ILLINOIS

(19) E. J. Bourne, K. H. Fantes and S. Peat, *J. Chem. Soc.*, 1109 (1949).

(20) D. J. Bell and Anne Palmer, *ibid.*, 3763 (1952).

(21) R. W. Jones, R. J. Dimler, Allene Jeanes, C. A. Wilham and C. E. Rist, *Abs. Papers, Am. Chem. Soc.*, **126**, 13D (1954).