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THE STRUCTURE OF A DEXTRAN PRODUCED BY *Leuconostoc mesenteroides* NRRL B-1397: THE LINKAGES AND LENGTH OF THE BRANCHES

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ABSTRACT

The structure of a dextran produced by *Leuconostoc mesenteroides* NRRL B-1397 has been investigated in relation to its immunological properties. The methylated dextran yielded on acid hydrolysis 2,3,4,6-tetra-, 2,3,4-tri-, 3,4,-di-, and 2,4-di-*O*-methyl-D-glucose, in the molar ratio of 1.0:3.1:0.7:0.2, together with a trace of 2,4,6-tri-*O*-methyl-D-glucose, indicating that the branches occur mainly at O-2 and the remainder at O-3. A carboxyl-dextran, obtained by catalytic oxidation of the dextran to convert the terminal, non-reducing D-glucose residues into D-glucuronic acid residues, was partially hydrolyzed with acid. Fractionation gave 2-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (major), 6-*O*-(α-D-glucopyranosyluronic acid)-D-glucose, and mixtures of aldotri-, aldotetra-, and aldopentaouronic acid that contain both (1 → 6)- and (1 → 2)-D-glucosidic linkages. It is concluded that the branches at O-2 are mainly single D-glucose units, whereas those occurring at O-3 may be longer than two glucose units, forming a highly branched structure having an average repeating unit of 5 sugar residues.

INTRODUCTION

Dextrans, predominantly α-(1 → 6)-linked D-glucans produced by various strains of *Leuconostoc mesenteroides* and related bacteria, are known to contain various proportions of non-(1 → 6) linkages[1,2], such as α-(1 → 3)[3–5], (1 → 4)[6], and also (1 → 2) linkages[2,7,8], most of which occur as branch points. During investigations on the relationship between structures and immunological specificities of dextrans, our attention was drawn to the particular type of dextran elaborated by the strain NRRL B-1397. Although a previous report indicated, on the basis of periodate-oxidation data[1], that this dextran contains only α-(1 → 6)- (75%) and (1 → 4) D-

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glucosidic linkages (25%), our preliminary immunochemical and acetolysis studies suggested the presence of significant amounts of α-(1 → 2) linkages. Fractionation of the acetolysis products yielded, in addition to kojibiose, three different trisaccharides containing α-(1 → 2) and (1 → 6)-D-glucosidic linkages, whose structures have already been established[9].

This paper is concerned with the detailed structural features of NRRL B-1397 dextran, particularly the nature of the branches, as revealed chiefly by methylation analysis and by characterization of the acidic oligosaccharides obtained by partial acid hydrolysis of the carboxyl-dextran.

RESULTS AND DISCUSSION

The water-soluble dextran produced by *Leuconostoc mesenteroides* NRRL B-1397 was purified by repeated precipitation from aqueous solution by 45% ethanol. It reacted strongly with concanavalin A to give a single precipitin line on an agar plate. On periodate oxidation (0.04M sodium periodate, room temperature) the dextran consumed 1.62 moles of periodate per glucose residue with production of 0.694 mole of formic acid. These results indicate 69.4% of (1 → 6)-, 23% of (1 → 4)-, and 8% of (1 → 3) glucosidic linkages, the values being comparable with those reported by Jeanes *et al.*[1]. However, when the dextran was subjected to Smith degradation[10], which involves periodate oxidation, borohydride reduction, and acid hydrolysis, only glycerol and glucose were detected. Erythritol, which should arise from (1 → 4)-linked glucose residues, could not be detected. The molar ratio of glycerol to glucose, as estimated by colorimetric determination after paper-chromatographic separation, was 14.3:1.0, indicating approximately 6.5% of (1 → 3)-linked D-glucose residues in the molecule. As both (1 → 4)- and (1 → 2)-linked D-glucose residues consumes 1 mole of periodate without production of formic acid, the lack of erythritol in the Smith-degraded dextran indicates the presence of (1 → 2) linkages rather than (1 → 4) linkages.

In order to obtain further information about the types of linkages, the dextran was methylated, the methylated dextran ($[\alpha]_D^{25}$ +223° in chloroform) was hydrolyzed with acid, and the hydrolyzate was analyzed by g.l.c. as the methyl glycosides and also as the acetates of the corresponding alditols. As shown in Fig. 1, 2, 3, 4,6-tetra-, 2,3,4-tri-, 2,4,6-tri-(minor), 3,4-di-, and 2,4-di-*O*-methyl-D-glucose were identified. These methylated glucoses were also separated by column and paper chromatography, and also by paper electrophoresis, the latter being useful for separation of 3,4-di-*O*-methyl-D-glucose (M_G 0.31) and 2,4-di-*O*-methyl-D-glucose (M_G 0.05) from the mixture. The identities and relative proportions of the cleavage fragments of the methylated dextran are summarized in Table I. The methylated sugars were identified as crystalline compounds.

Inspection of Table I reveals that NRRL B-1397 dextran possesses a very highly branched structure, with an average repeating unit of 5 glucose residues. Identification of 3,4-and 2,4-di-*O*-methyl-D-glucose provided unambiguous evidence

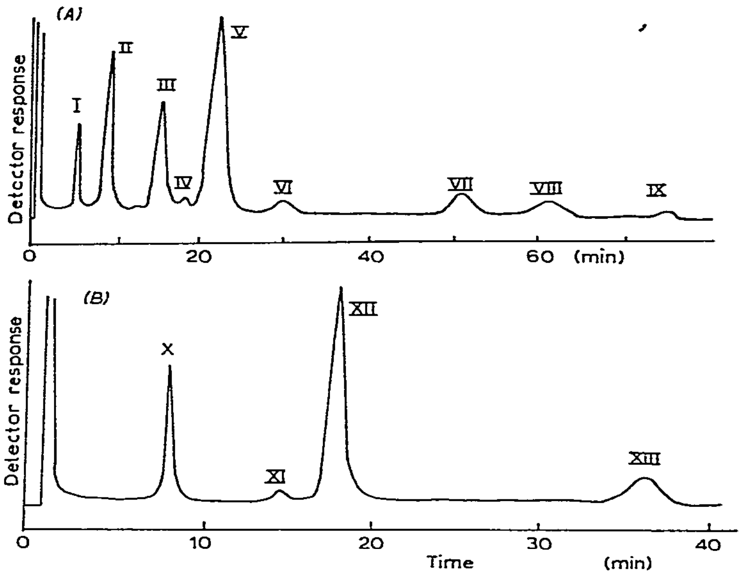

Fig. 1. G.l.c. separation of the methylated sugar fragments from methylated B-1397 dextran, (*A*) as their methyl glucosides, and (*B*) as their alditol acetates.
(I, II), 2,3,4,6-tetra-*O*-methylglucose; (III, V), 2,3,4-tri-*O*-methylglucose; (IV, VI), 2,4,6-tri-*O*-methylglucose; (VII, VIII), 3,4-di-*O*-methylglucose; (VII, IX), 2,4-di-*O*-methylglucose; (X), 2,3,4,6-tetra-*O*-methylglucose; (XI), 2,4,6-tri-*O*-methylglucose; (XII), 2,3,4-tri-*O*-methylglucose; and (XIII), mixture of 3,4- and 2,4-di-*O*-methylglucose.

TABLE I

HYDROLYSIS PRODUCTS OF THE METHYLATED DEXTRAN

O-*Methyl*-D-*glucose*	*Linkage indicated*	*Molar ratio*	
		by c.c.[a]	*by g.l.c.*[b]
2,3,4,6-Tetra-	Glc*p*-(1→	1.0	1.0
2,3,4-Tri-	→6)-Glc*p*-(1→	3.1	3.2
2,4,6-Tri-	→3)-Glc*p*-(1→	0.1	0.06
3,4-Di-	→6)-Glc*p*-(1→ 2 ↑	0.7	0.93
2,4-Di-	→6)-Glc*p*-(1→ 3 ↑	0.2	

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[a] Fractionated quantitatively by cellulose–hydrocellulose column chromatography. [b] Estimated as the alditol acetates.

that all of the (1 → 2) linkages and most of the (1 → 3) linkages represent the inter-chain linkages of (1 → 6)-linked chains. The ratio of the two di-*O*-methyl glucoses shows that approximately 75% of the branching occurs at the C-2 position and the remainder at the C-3 position of the (1 → 6)-linked D-glucose residues of the main chain. Thus, glycerol formed by Smith degradation of the dextran must have arisen, in addition to the terminal non-reducing and (1 → 6)-linked glucopyranosyl residues, from the (1 → 2)-linked branch points. The glucose that survived periodate cleavage must have arisen mainly from the (1 → 3)-linked branch points. With regard to (1 → 3)-D-glucosidic linkages, the presence of a small proportion of 2,4,6-tri-*O*-methyl-D-glucose could indicate that this dextran, like some other dextrans, may contain (1 → 3) inter-residue linkages[4,11].

Our recent immunochemical studies revealed that the precipitation reaction between NRRL B-1397 dextran and its rabbit antiserum can be inhibited to the same extent by isomaltotriose, -tetraose, and -pentaose, whereas isomaltose was less active[12]. Isomaltose showed higher inhibition than kojibiose or nigerose. However, when the (1 → 6)-linkage-specific antibody in the antiserum was absorbed by the addition of N-4 (Meito) dextran* ,which has one (1 → 3)-linked branch point per 23–24 α-(1 → 6)-linked D-glucose residues[13], kojibiose showed higher inhibitory power than isomaltose. These observations prompted us to elucidate the lengths of the side chains attached by α-(1 → 2) and also by (1 → 3) linkages. Abobtt *et al.*[6] investigated the lengths of branches in NRRL B-1416, B-1415, and B-1375 dextrans having (1 → 3)-and/or (1 → 4)-branching linkages, by isolation of aldobiouronic acids from hydrolyzates of the carboxyl-dextrans. They showed that most, if not all, of these dextrans have single-unit side chains. By applying the same technique, Lindberg and Svensson[5] suggested that most of the branches in NRRL B-512 dextran, which contains 5% of (1 → 3) branch points, are more than one D-glucose residue long. More recently, they showed by sequential-degradation techniques that about 49% of the branches in the B-512 dextran are one D-glucose residue long[14].

To study the nature of branching, NRRL B-1397 dextran was oxidized with a platinum catalyst[15], essentially as described by Abbott *et al.*[6]. The resulting carboxyl-dextran (yield, 74%), in which about 85% of the non-reducing D-glucose residues had been converted into D-glucuronic acid residues, was partially hydrolyzed with acid. Fractionation of mixture of acidic oligosaccharides gave two kinds of aldobiouronic acids (I and II), -triouronic acid, -tetraouronic acid, and -pentaouronic acid. The yields and some properties of these oligosaccharides are listed in Table II.

The structures of these acidic oligosaccharides were determined by methylation, esterification with diazomethane, reduction with lithium aluminum hydride in ether, remethylation, acid hydrolysis, and then g.l.c. analysis of the resulting methylated glucoses. The identities and molar ratios of methylated sugar components from the methylated, carboxyl-reduced oligosaccharides are shown in Table III.

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TABLE II

YIELDS AND PROPERTIES OF ACIDIC OLIGOSACCHARIDES FROM THE CARBOXYL-DEXTRAN

Acidic oligosaccharide	*Percentage*	R_{GlcA}[a]	$[\alpha]_D^{25}$ (*c 1, water*) (*degrees*)
Aldobiouronic acid I	32.3	0.56	+64
Aldobiouronic acid II	16.4	0.46	+58
Aldotriouronic acid	26.9	0.26	+103
Aldotetraouronic acid	14.7	0.11	+155
Aldopentaouronic acid	9.7	0.05	+160

[a] Paper-chromatographic mobility relative to that of D-glucuronic acid (solvent; 3:1:1 ethyl acetate–acetic acid–water).

TABLE III

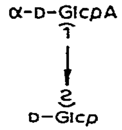
HYDROLYSIS PRODUCTS FROM THE METHYLATED, CARBOXYL-REDUCED OLIGOSACCHARIDES

O-*Methyl-glucose*	T_g[a]	*Aldo-biouronic acid I*	*Aldo-biouronic acid II*	*Aldo-triouronic acid*	*Aldo-tetraouronic acid*	*Aldo-pentaouronic acid*
2,3,4,6-Tetra-	1.0	1.0 mole	1.0 mole	1.0 mole	1.0 mole	1.0 mole
2,3,4-Tri-	2.49		0.82	0.86	1.09	1.41
3,4,6-Tri-	1.95	0.98	0.21	0.85	0.48	0.41
3,4-Di-	5.45			0.09	0.26	0.39
2,4-Di-	5.10				(trace)	(trace)

[a] The retention time of the *O*-acetylglucitol derivative relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on g.l.c.

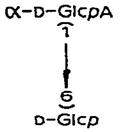
Although 3,4,6- and 2,4,6-tri-*O*-methylglucose could not be resolved by g.l.c. of their alditol acetates, they were separated partially as their methyl glycosides on g.l.c. and completely by paper electrophoresis. The structures of the aldobiouronic acid and aldotriouronic acid assigned by methylation studies, and also by examination of the products of partial acid hydrolysis (Amberlite IR-120 (H^+) resin, 18 h, 100°), are shown in Formulas **1**–**5**.

Thus, aldobiouronic acid I, which is the major component, was charcterized as 2-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (**1**), and the aldobiouronic acid II is 6-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (**2**), although the latter may have been contaminated with approximately 20% of I. The ratio of (1→2)-linked and (1→6)-linked aldobiouronic acids is 2.7:1.0. As seen in Table III, the triouronic acid fraction contains equal proportions of (1→6)- and (1→2)-linked D-glucose residues, having the structure of either **3** or **5**. On partial acid hydrolysis, the aldotriouronic acid gave aldobiouronic acid I (major), II, and isomaltose, together with D-glucuronic acid and D-glucose. These results suggest that the main component of the triouronic acid fraction is *O*-(α-D-glucopyranosyluronic acid)-(1→2)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose (**3**), although a small proportion of *O*-(α-D-glucopyarnosyluronic



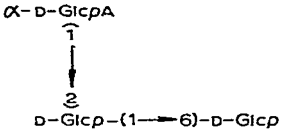
$$\begin{array}{c}\alpha\text{-D-Glc}p\text{A}\\ 1\\ \downarrow\\ 2\\ \text{D-Glc}p\end{array}$$

1



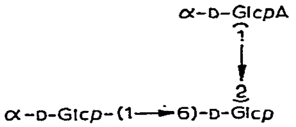
$$\begin{array}{c}\alpha\text{-D-Glc}p\text{A}\\ 1\\ \downarrow\\ 6\\ \text{D-Glc}p\end{array}$$

2



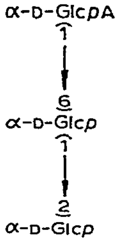
$$\begin{array}{l}\alpha\text{-D-Glc}p\text{A}\\ \quad 1\\ \quad\downarrow\\ \quad 2\\ \text{D-Glc}p\text{-}(1\rightarrow 6)\text{-D-Glc}p\end{array}$$

3



$$\begin{array}{r}\alpha\text{-D-Glc}p\text{A}\\ 1\quad\\ \downarrow\quad\\ 2\quad\\ \alpha\text{-D-Glc}p\text{-}(1\rightarrow 6)\text{-D-Glc}p\end{array}$$

4



$$\begin{array}{c}\alpha\text{-D-Glc}p\text{A}\\ 1\\ \downarrow\\ 6\\ \alpha\text{-D-Glc}p\\ 1\\ \downarrow\\ 2\\ \alpha\text{-D-Glc}p\end{array}$$

5

acid)-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 2)-D-glucose (5) may have been present. In addition, the isolation of a small proportion of 3,4-di-*O*-methyl-D-glucose in the methylated triouronic acid fraction shows that a trisaccharide branched by a (1 → 2) linkage, as shown in **4**, must be present; the proportion of 3,4-di-*O*-methylglucose corresponds to 17.5% of the branched trisaccharide in this fraction.

As the methylation study of B-1397 dextran indicated that the α-(1 → 2) linkage is located only at branch points, the isolation of a significant amount of 2-*O*-(α-D-glucopyranosyluronic acid)-D-glucose and the triouronic acid possibly having structure **3** clearly indicated that major parts of the side chains attached by α-(1 → 2) linkages to the main chain must be single D-glucose residues, although there is a possibility that a small proportion of the (1 → 2)-branched side chains are longer than a single sugar residue. The isolation of the trisaccharide, namely, *O*-(α-D-glucopyranosyl)-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 2)-D-glucose, from the acetolysis of this dextran suggests the presence of side chains containing two D-glucose residues[9].

Attempts to assign structures to the aldotetraouronic acid and -pentaouronic acid components were not considered worthwhile, because these acidic oligosaccharides were mixtures of more than two components. Methylation followed by acid hydrolysis of the carboxyl-reduced tetra- and pentasaccharides gave similar methylated sugar components, namely 2,3,4,6-tetra-, 2,3,4-tri-, 3,4,6-tri-, and 3,4-di-*O*-methyl-D-glucose, in the ratios of 1.0:1.1:0.48:0.26, and 1.0:1.41:0.41:0.39, respectively. On partial acid hydrolysis, both tetra- and pentaouronic acids yielded the α-(1 → 2)-linked aldobiouronic acid, α-(1 → 6)-linked aldobiouronic acid, and isomaltose, together with D-glucose and D-glucuronic acid. It is likely, therefore, that these oligosaccharides are mixtures of isomers containing the α-(1 → 2) inter-residue linkage or α-(1 → 2) inter-chain linkage, in addition to the α-(1 → 6) inter-residue linkage.

Interestingly, there was no appreciable amount of aldobiouronic acid or higher oligosaccharides containing the α-(1 → 3) linkage. Nevertheless, among the neutral oligosaccharides formed by partial acid hydrolysis of the carboxyl-dextran, it was found that the amount of nigerose was four times that of kojibiose, despite the fact that the content of (1 → 3) branch points in the original dextran is one third of the content of (1 → 2) branch points. In Table IV, the results of fractionation of neutral sugars by charcoal-column chromatography are listed.

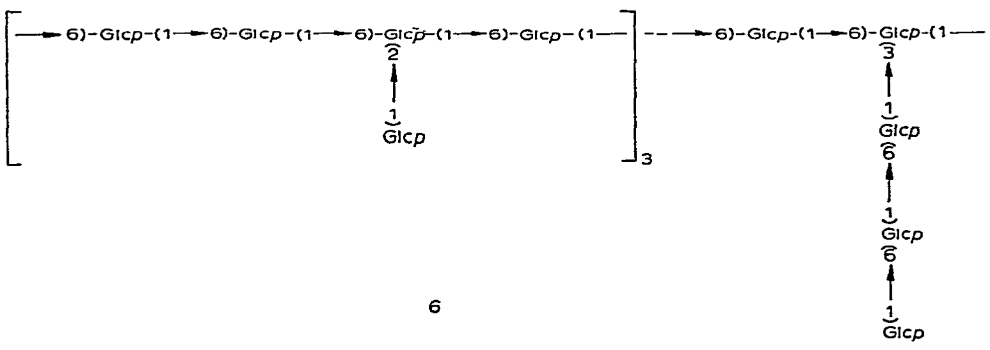
TABLE IV

FRACTIONATION BY CARBON–CELITE COLUMN CHROMATOGRAPHY OF NEUTRAL OLIGOSACCHARIDES FROM THE CARBOXYL-DEXTRAN

Fraction	*Eluted with*	*Weight (mg)*	*Sugar component*[a]
1	water	636.0	*glucose*, isomaltose
2	2.5% ethanol	39.3	*isomaltose*, kojibiose, nigerose
3	5.0% ethanol	33.8	isomaltose, *isomaltotriose*, unidentified triose
4	7.5% ethanol	17.4	isomaltotriose, *isomaltotetraose*
5	10% ethanol	12.0	isomaltotetraose, isomaltopentaose
6	20% ethanol	12.2	isomaltopentaose, higher saccharides

[a] Italics indicate the main component in each fraction.

The small but significant amount of nigerose, and the absence of (1 → 3)-linked aldobiouronic acid, suggest that most of the (1 → 3)-branched side chains have two D-glucose residues or more. The isolation of a (1 → 6)-linked aldobiouronic acid supports the foregoing suggestion. This is also consistent with recent immunochemical studies showing that isomaltotriose is at the lower limit among the α-(1 → 6)-linked D-gluco-oligosaccharides that give maximal inhibition of the precipitation reaction between B-1397 dextran and its antisera[12].



6

It is concluded that the side chains joined by α-(1 → 2)-linkages, which occupy two third of the branches are mostly (if not all) one unit long, whereas those joined by a (1 → 3)-linkage may be longer than two residues, probably three D-glucose residues, thus forming a highly ramified structure having an average chain-length of five D-glucose residues. A possible structure of B-1397 dextran is illustrated as **6**.

There is a possibility that this dextran also contains a very small amount of (1 → 3) inter-residue D-glucosidic linkages.

The occurrence of α-(1 → 2)-linked D-glucose residues in several dextrans was demonstrated by the isolation of kojibiose by acetolysis[7,8]. Recently, Lewis *et al.*[16] showed by a methylation study that the dextran C (NRRL B-1298) contains α-(1 → 2) branch points (12%) in addition to (1 → 3) and double (1 → 2) and (1 → 3) branchings. More recently, Bourne *et al.*[17] reported the structure of two types (S and L) of dextrans from B-1299; both types contained approximately 30% of (1 → 2) inter-chain linkages. These data accord with the values established by Scott *et al.*[2] Although immunochemical studies suggested that many side chains in B-1299 dextran are terminated by kojibiose residues[18], direct chemical evidence was lacking. The present study provides the first evidence concerning the α-(1 → 2)-branched side chains in B-1397 dextran. It may be noteworthy that this dextran could react strongly with rabbit antisera against bovine serum albumin-(*p*-aminophenyl β-kojibioside), and this precipitation reaction was inhibited completely by kojibiose (J. Duke and I. J. Goldstein; unpublished result). The foregoing finding supports the chemical results indicating that most of the α-(1 → 2)-branched side-chains consist of single D-glucose residues. The relationship between immunochemical specificities and the fine structure of this dextran is being further studied.

EXPERIMENTAL

General methods. — All evaporations were conducted under diminished pressure at 35–40°. Paper chromatography was usually performed on Toyo-roshi No. 51A by the descending method with the following solvent systems (*v/v*); (*A*) 4:1:5 1-butanol–ethanol–water, (*B*) 4:1:5 1-butanol–acetic acid–water, (*C*) 3:1:1 ethyl acetate–acetic acid–water, and (*D*) butanone–water azeotrope. Preparative paper-chromatography was performed with Whatman 3MM paper. Reducing sugars were detected by spraying the chromatograms with *p*-anisidine hydrochloride or *o*-amino-diphenyl reagent, and alditols were detected with alkaline silver nitrate.

G.l.c. was carried out with a Hitachi K-53 chromatograph, fitted with a flame-ionization detector and a stainless-steel column (0.4 × 2 m). Methylated sugars were converted into the corresponding methyl glycosides or alditol acetates and were separated on a column packed with (*A*) 15% butanediol succinate polyester on Neosorb NC[19] (Nishio Ind. Co., Tokyo) or (*B*) 3% ECNSS-M on Gas Chrom Q[20] (Nishio Ind. Co.). Nitrogen was used as a carrier gas, at a flow rate of 60 ml/min.

Quantitative determination of carbohydrates was generally affected with the phenol–sulfuric acid reagent[21]. Formaldehyde was determined with chromotropic acid, and alditols were determined by the procedure of Hanahan and Olley[22].

Preparation and purification of dextran. — *Leuconostoc mesenteroides* NRRL B-1397 was grown in a medium (initial pH, 7.0) containing 10% of sucrose, 0.5% of yeast extract and 0.05% of KH_2PO_4, for 5 days at 25°. The cultural broth (1 liter) was centrifuged at 4,000 *g* for 30 min, and crude dextran was precipitated from the supernatant solution by the addition of an equal volume of ethanol. The precipitated dextran was dissolved in water (approx. 2% solution), dialyzed, and the non-dialyzable solution was centrifuged at 7,000*g* for 30 min to remove insoluble material. Ethanol was gradually added to the supernatant solution; most of the dextran was precipitated when the concentration of ethanol ranged from 35 to 45%. The dextran was redissolved in water, centrifuged, and re-precipitated with 45% ethanol. After repeating this procedure two more times to remove any less-soluble dextran, the purified dextran was washed successively with ethanol, ethyl ether, and petroleum ether, and finally dried *in vacuo* at 35° (yield, 15% based on sucrose). The dextran preparation contained N, 0.15; ash, 0.11; and moisture, 6.88%. It showed $[\alpha]_D^{25}$ +198° (*c* 0.5, 0.2M sodium hydroxide), and +219° (*c* 0.5, *N,N*-dimethylformamide), and an intrinsic viscosity of 0.954 at 25°.

Methylation analysis of dextran. — The dextran of NRRL B-1397 (2.0 g) was methylated twice by the Hakomori method[23] with methylsulfinyl carbanion (prepared according to Sandford and Conrad[24]) and methyl iodide; yield, 1.98 g. The methylated dextran (5 mg) was heated for 24 h with 5% methanolic hydrogen chloride (1 ml) in a sealed tube at 100° After neutralization with silver carbonate, the methanolyzate was concentrated in an air current, and analyzed by g.l.c. (column *A*). For column-chromatographic separation, the methylated dextran (600 mg) was heated with 90% formic acid for 12 h at 100°, and then with 0.25M sulfuric acid for 2.5 h at 100°. The hydrolyzate was neutralized (barium carbonate), and evaporated to a syrup (550 mg). A portion of the hydrolyzate (10 mg) was converted into a mixture of alditol acetates[25] and analyzed by g.l.c. on a column (*B*). The remainder of the hydrolyzate (*ca.* 500 mg) was fractionated on a cellulose–hydrocellulose (1:1) column (3.5 x 60 cm) with solvent *D*, some fractions collected being further fractionated by thick-paper chromatography (solvent *D*) or paper electrophoresis (600 volts, 4 h, in 0.1M sodium borate). Each of the methylated sugars so separated was examined by g.l.c., and identified as a crystalline compound, usually as an aniline derivative.

Smith degradation. — The dextran (1.0 g) was oxidized with 0.15M periodic acid (200 ml) for 7 days at 25°, and the oxidized dextran was reduced with sodium borohydride in the usual way. The resulting polysaccharide polyalcohol was hydrolyzed by heating with 0.5M sulfuric acid for 6 h at 100°. The hydrolyzate was neutralized with saturated barium hydroxide solution, filtered, and the filtrate was evaporated to dryness, the boric acid in the residue being removed as methyl borate by evaporation of methanol from the residue. The sugar and sugar alcohols in the hydrolyzate were separated, quantitatively, on a filter-paper sheet (solvent *C*), and each component, extracted from the paper, was determined colorimetrically[26].

Preparation of carboxyl-dextran, and separation of oligosaccharides from its partial acid hydrolyzate. — To an aqueous solution (300 ml) containing B-1397 dextran (4.2 g) and sodium hydrogen carbonate (0.5 g), fresh platinum catalyst was added, and the mixture was stirred for 30 days at 70°, during which time oxygen was bubbled through[15]. The pH of the solution was kept at approx. 8.5 by the addition of sodium hydrogen carbonate. After the oxidation reaction had been completed, the catalyst was removed by centrifugation, and the oxidized dextran was precipitated by the addition of 2 volumes of ethanol. It was dissolved in water (200 ml), passed through a column of Amberlite IR-120 (H^+), and dialyzed. The carboxyl-derivative was precipitated with ethanol (2 vol.) from the non-dialyzable solution (recovery, 2.7 g).

When the supernatant solution was evaporated to dryness, an additional amount (0.42 g) of the carboxyl-dextran was recovered. The D-glucuronic acid content, determined by titration according to Whlistler *et al.*[27], indicated that more than 85% of the non-reducing terminal ends had been converted into glucuronic acid.

The carboxyl-dextran (1.1 g) was hydrolyzed with 0.5M sulfuric acid (20 ml) for 6 h at 100°. The hydrolyzate was neutralized (barium carbonate), passed through a column of Amberlite IR-120 (H^+), and concentrated to a syrup (949 mg). The separation of acidic and neutral components was effected by use of a column (2.5 × 25 cm) of Dowex-1 (formate form). The neutral compounds were eluted from the column with water, and the acidic sugars absorbed on the column were eluted with M formic acid.

The mixture of neutral sugars (738 mg) was fractionated on a column (4.0 × 25 cm) containing charcoal (Darco G-60)–Celite (1:1, mixture), sugars on the column being eluted successively with water and aqueous ethanol (5–20%). The sugars were examined by paper chromatography (solvents *B*, *C*). The disaccharide fraction (fraction 2 in Table IV) was fractionated further on thick paper, to give isomaltose, kojibiose, and nigerose. Kojibiose and nigerose were identified by methylation, acid hydrolysis, and g.l.c. of their corresponding methylated alditol acetates.

The acidic sugar fraction was evaporated to a syrup (211 mg) that was fractionated on a filter-sheet by multiple-paper chromatography (solvent *B*). This procedure yielded aldobiouronic acid I (32.0 mg), aldobiouronic acid II (16.2 mg), aldotriouronic acid (26.6 mg), aldotetraouronic acid (15.6 mg), and aldopentaouronic acid (8.3 mg) (Table II). A small proportion of glucuronic acid was also detected.

Structural analysis of the acidic oligosaccharides. — Acidic oligosaccharides (5–10 mg, each) were methylated by the Hakomori method as described previously. The methylated product was dissolved in ethyl ether and esterified with diazomethane, and the resulting methyl ester was reduced with lithium aluminium hydride (20 mg) in ethyl ether by boiling for 4 h, after which time water was added to compose the excess reagent. After filtration, the residue was washed repeatedly with acetone. Evaporation of the filtrate and washing gave a syrup, which was purified by dissolution in ether. The carboxyl-reduced, methylated oligosaccharide thus obtained, was re-methylated by the Purdie method with silver oxide and methyl iodide[28]. Completion of methylation was confirmed by i.r. spectroscopy.

The methylated, neutral oligosaccharide was subjected to methanolysis by heating with 2% methanolic hydrogen chloride for 18 h. After neutralization (silver carbonate), the methanolyzate was concentrated in an air current, and examined by g.l.c. (column *A*). A portion of the methanolyzate was hydrolyzed (0.25M sulfuric acid for 18 h at 100°.)After neutralization and concentration, the methylated sugars were reduced with sodium borohydride and converted into their acetyl derivatives, and analyzed by g.l.c. with column *B*.

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