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SIX UNUSUAL DEXTRANS: METHYLATION STRUCTURAL ANALYSIS BY COMBINED G.L.C.–M.S. OF PER-*O*-ACETYL-ALDONONITRILES

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ABSTRACT

Six bacterial dextrans from NRRL strains *Leuconostoc mesenteroides* B-1299, B-1308, B-1355, and B-1399; *Streptobacterium dextranicum* B-1254; and *Streptococcus viridans* B-1351 were examined by methylation structural analysis. Methyl ethers of D-glucose that were present in hydrolyzates of permethylated dextrans were analyzed by combined g.l.c.–m.s. as peracetylated aldononitriles (PAAN). Fundamental differences were found with respect to ease of methylation and subsequent hydrolysis of the methylated dextrans. The various dextrans differed significantly in frequency and type of chain branching.

A g.l.c. procedure permitted, for the first time, separation of the 2,3,4- from the 2,3,6-tri-*O*-methyl derivative of D-glucose. Deuteriomethylation of B-1254 fraction L dextran permitted confirmation of a previously proposed fragmentation pathway for the m.s. of the PAAN derivatives of 2,3-di-*O*-methylaldohexoses.

INTRODUCTION

In a recent study[1], we successfully applied combined g.l.c.–m.s. analyses of peracetylated aldononitrile (PAAN) derivatives of methyl ethers of D-mannose[2] to the determination of glycosidic linkages in a series of permethylated α-D-linked D-mannans. We now report the application of this procedure to the methylation–fragmentation analysis of a series of dextrans. These dextrans have been shown, through chemical[3–9], physical[3,10], immunological[11], and immunochemical[12] procedures, to possess a variety of α-D-glucosidic linkages and sequences. Sidebotham[13] has most recently reviewed the current status of this knowledge.

The dextrans studied were selected because of the need for precise structural detail to correlate with their unusual immunochemical and other biochemical properties, properties known to depend primarily on the rare linkages present and the mode of branching. The dextrans were produced by the following bacteria, designated by the strain number in the ARS Culture Collection at the Northern Regional

*Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Research Center: *Leuconostoc mesenteroides* NRRL B-1299, B-1308, B-1355, and B-1399; *Streptobacterium dextranicum* NRRL B-1254; and *Streptococcus viridans* NRRL B-1351. A number of the dextrans are essentially homogeneous fractions separated from the corresponding, polydisperse, high-molecular-weight, native dextran; these are: B-1299 dextran fraction S, B-1355 dextran fraction S, B-1399 dextran fraction L, B-1254 dextran fraction S, and B-1351 dextran fraction S. As fractionation was not repeated exhaustively, however, traces of the other major fraction could introduce spurious indications of extraneous linkages.

Except for dextrans B-1308 and B-1351, which display certain similarities in structure, all of the dextrans were found to differ significantly from one another on the basis of frequency and position of chain branching.

The methylation analyses were facilitated by the development of a g.l.c. procedure for separating the PAAN derivatives of 2,3,4- and 2,3,6-tri-*O*-methyl-D-glucose. It was thus possible to determine, quantitatively and unambiguously, the presence or absence of unbranched, (1→4)-linked, D-glucosyl residues previously inferred from periodate-oxidation data. In addition, deuteriomethylation of B-1254 dextran permitted re-examination of the previously proposed[2] mass-spectral fragmentation-pathway for the PAAN derivative of a 2,3-di-*O*-methyl-aldohexose.

RESULTS AND DISCUSSION

Separation and identification of methylated D-*glucose PAAN derivatives.* — In an earlier study, combined g.l.c.–m.s. of PAAN derivatives in the methylation–fragmentation analysis of extracellular, yeast D-mannans[1] was facilitated by (*1*) having available all possible tri- and di-*O*-methyl PAAN derivatives of D-mannose that could arise from methylation–fragmentation of a D-mannan, and (*2*) development of a programmed, g.l.c. method for separating them[2]. A comparison of the mass spectra of methylated D-mannose PAAN derivatives with those for the corresponding tetra- and tri-*O*-methyl-D-glucose derivatives published by Dmitriev *et al.*[14] confirmed that the fragmentation patterns are dependent on the location of the methoxyl groups and are independent of the sugar stereochemistry[15]. Distinctive mass spectra were also recorded for the di-*O*-methyl-D-mannose derivatives. These spectra can be employed as reference standards for corresponding PAAN derivatives of aldohexoses in general.

Through the kindness of Dr. John W. Van Cleve of this laboratory, authentic samples of 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3,4-tri-*O*-methyl-D-glucose were available for conversion into PAAN derivatives and for use as g.l.c. standards. Although the fragmentation patterns for the m.s. of these compounds are the same as for the corresponding PAAN derivatives of D-mannose, the g.l.c. retention-times are different. Table I compares the g.l.c. retention-times for corresponding D-glucose and D-mannose PAAN derivatives. The retention times listed for 2,4,6-tri- and 2,4- and 3,4-di-*O*-methyl-D-glucose PAAN derivatives are taken from chromatograms of hydrolyzates of permethylated dextrans. Also listed is the retention time of the

TABLE I

RELATIVE G.L.C. RETENTION-TIMES OF PERACETYLATED ALDONONITRILES OF METHYL ETHERS OF D-GLUCOSE AND D-MANNOSE

Methyl ether	*Column B*[a]			*Column C*[b]
	D-*Glucose*	D-*Glucose*[c]	D-*Mannose*[d]	D-*Glucose*
2,3,4,6-Tetra-	1.00	0.94	1.00	1.00
2,4,6-Tri-	1.54	1.45	1.59	1.33
3,4,6-Tri-	1.97	1.85	1.89	
2,3,6-Tri-	2.13	2.00	1.65	1.42
2,3,4-Tri-	2.13	2.00	2.03	1.67
2,4-Di-	3.03	2.84	3.16	2.60
2,3-Di-	3.72	3.50	2.55	2.12
3,4-Di-	3.82	3.58	3.68	2.06

[a]Column B: 5% of butanediol succinate [b]Column C: 5% of Apiezon L. [c]Relative to 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannononitrile. [d]Data from ref. 2.

3,4,6-tri-*O*-methyl-D-glucose PAAN which was prepared from the hydrolyzate of partially methylated *L. mesenteroides* B-1299 fraction S dextran. These additional D-glucose derivatives were identified by their mass spectra, which were identical to those of the corresponding D-mannose reference compounds.

The separation of 2,3,4- from 2,3,6-tri-*O*-methyl-D-glucose as the PAAN derivatives on Apiezon L is noteworthy. Until now, these methyl ethers of D-glucose could not be separated, either as PAAN derivatives or as peracetylated alditols[16]. Amylosaccharides and pullulans, for example, can now be analyzed by the procedure used here.

Reference standards for the 2,6-, 3,6-, and 4,6-di-*O*-methyl-D-glucose PAAN derivatives were not available. No evidence was found for the presence of these compounds in any of the hydrolyzates of methylated dextrans. Had these dimethyl ethers been unresolved from those present, they would have been detected by their contributions to the mass spectra. We have confidence in this result, because PAAN derivatives emerge from the gas chromatograph in the order of their decreasing degree of methylation, and neither these particular dimethyl ethers nor mono-methyl ethers were observed when column A, used to detect all classes of PAAN derivatives, was operated under the conditions employed by Dmitriev *et al.*[14] to detect the derivative of unmethylated D-glucose. Our results, therefore, do not confirm those of other investigators[9] with regard to the presence of mono-*O*-methyl-D-glucose.

Methylation analyses. — In contrast to the ease of methyation displayed by extra-cellular D-mannans from yeast, only two dextrans, B-1254 fraction L and B-1299 fraction S, were methylated by a single Hakomori[17] methylation. The other dextrans required multiple Hakomori methylations. For B-1355 fraction S dextran, additional Kuhn methylations[18] gave an improved ratio of tetra- to di-*O*-methyl PAAN derivatives. Like many dextrans, the majority of D-mannans contain α-D-(1→6)-linked, backbone, sugar chains. The permethylated D-mannans were all satisfactorily

hydrolyzed by the procedure of Stellner *et al.*[19]. Only dextran B-1254 fraction L, however, behaved like the D-mannans; the other dextrans resisted hydrolysis by the Stellner procedure until rendered soluble by formolysis[20].

Completeness of methylation was judged by the correspondence between the proportions of tetra- and di-*O*-methyl sugars, which represent nonreducing end-groups and branching units, respectively. Where only small amounts of material are available for methylation, this correspondence is the best criterion of complete methylation, provided that the hydrolysis procedure employed does not bring about demethylation[20].

Table II lists the results of methylation–fragmentation analyses of six dextrans. These D-glucans display wide variation in their degree of branching. Dextran B-1254 fraction L differs from the others in having branched residues linked through O-1, O-4, and O-6, in addition to a small percentage of unbranched, (1→4)-linked D-glucopyranosyl residues. Dextran B-1355 fraction S differs, in that it contains a high proportion of non-(1→6)-linked D-glucosyl residues that are not involved in branching. These residues are (1→3)-linked. For the other dextrans, non-(1→6)-linkages occur exclusively on D-glucosyl residues involved in chain branching.

TABLE II

MOLE PERCENTAGE OF METHYLATED D-GLUCOSE COMPONENTS IN HYDROLYZATES OF METHYLATED DEXTRANS

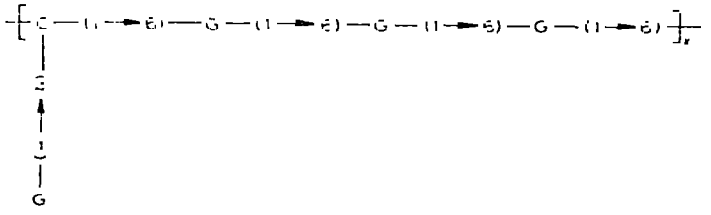
Organism	*NRRL strain*	*Dextran fraction*	*Methyl ethers of* D-*glucose*						
			2,3,4,6	*2,3,4*	*2,3,6*	*2,4,6*	*2,3*	*2,4*	*3,4*
Streptobacterium dextranicum	B-1254	L	22.1	55.0	3.4		19.5		
Leuconostoc mesenteroides	B-1299	S	39.1	26.0					34.9
Leuconostoc mesenteroides	B-1308		3.7	92.6				3.7	
Streptococcus viridans	B-1351	S	5.8	83.3				10.5	0..
Leuconostoc mesenteroides	B-1355	S	6.9	46.9		35.0		11.2	
Leuconostoc mesenteroides	B-1399	L	12.8	74.5				5.9	6.8

It bears emphasis, at this point, that methylation–fragmentation analysis provides information relating solely to the proportions and kinds of linkages present. Frequency of branching is revealed, and average repeat-unit structures can be proposed. In order to establish lengths of side chains, however, other methods are necessary. Where side chains are largely non-(1→6)-linked and of heterogeneous length, as in yeast mannans, acetolysis, and separation by high-pressure liquid chromatography, of the oligosaccharides formed can rapidly provide this infor

mation[1]. Where side chains consist mainly of (1→6)-linked residues, the variety of techniques used is illustrated for the dextran from *L. mesenteroides* NRRL B-512(F), which has 95% of α-(1→6)- and 5% of α-(1→3)-linkages[21]. Although one or two residues constitute 85% of the branches[22], physicochemical studies indicate that a few branches may consist of 50 or more residues[23–25]. Results of enzymic degradation indicate possible chain-lengths as great as 33 residues, and non-uniform distribution of branches[26]. Details on branching in several, more highly branched dextrans have been reviewed[13].

Complete methylation was apparently not achieved for two dextrans, B-1351 fraction S and B-1355 fraction S, which yielded an excess of di-*O*-methyl PAAN derivatives.

Dextran B-1254 fraction L. — This dextran is notable in having only 1,4,6 tri-*O*-substituted sugar residues at the branch points. The methylation data (see Table II) indicate one D-glucosyl group, three (1→6)-linked D-glucosyl residues, and one 1,4,6-tri-*O*-substituted sugar residue per average repeating-unit. Assuming an exclusively (1→6)-linked backbone chain, an average repeating-unit structure can be drawn as shown (where G is a D-glucopyranosyl group or residue, or a D-glucopyranose residue).



Suzuki and Hehre[7] analyzed disaccharide products from the acetolysis of B-1254 fraction L dextran, and obtained evidence for the presence of both (1→3)- and (1→4)-linkages. We found no evidence of (1→3)-linked D-glucosyl residues in our polysaccharide, which apparently was completely methylated.

Dextran B-1299 fraction S. — The series of research developments correlating the immunological behavior that initially distinguished this dextran as "unusual" with the presence of α-(1→2)-linkages in high proportion has been reviewed[7,11]. According to the data in Table II, the (1→2)-linkages occur exclusively in 1,2,6-tri-*O*-substituted D-glucosyl residues at points of branching. The data are compatible with a structure consisting of a (1→6)-linked D-glucosyl backbone that bears D-glucopyranosyl groups in α-(1→2)-linkage on alternate residues. Our results are similar to those of Bourne *et al.*[8], except that we could not detect the presence of unbranched, (1→3)-linked, D-glucopyranosyl residues. According to methylation and Smith-degradation studies, Bourne *et al.*[8] reported that 7% of the unbranched D-glucosyl residues are (1→3)-linked; they also detected nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) in an acetolyzate of B-1299 fraction S dextran (see also, ref. 7). This result

may, however, have originated either through undermethylation or through trace contamination with fraction L of B-1299 dextran. Jeanes *et al.*[3] found by periodate oxidation that fraction S contained no (1→3)-linkages, whereas fraction L appeared to have a small proportion. Because of the limitations of dependability of the periodate-oxidation analysis, however, these observations did not prove the presence or absence of (1→3)-linkages. Significantly, Bourne *et al.*[8] found 15% of (1→3)-linkages in fraction L.

The results of Kobayashi *et al.*[9,27] from methylation analysis of five fractions, taken from native B-1299 dextran and shown to be homogeneous, also differ from ours, mainly in that (1→3)-linkages were found. These investigators reported (1→3)-linked residues in linear-chain positions, as had Bourne *et al.*[8]; they also reported some branching through both O-2 and O-3 in all fractions. These disagreements among results from three groups of investigators, if not traceable to analytical and preparative procedures, appear to indicate differences in the enzymic constitution of the dextransucrase complex[28,29] formed under the fermentation conditions employed.

Bourne *et al.*[30] later analyzed a series of oligosaccharides obtained by the action of *Penicillium lilacinum* dextranase on acid-degraded B-1299 fraction S dextran. They concluded[30] that the unusual structure of this dextran "consists, principally, of segments of isomaltose (6-*O*-α-D-glucopyranosyl-D-glucose) homologues which are mutually linked through positions 1 and 2 of their terminal D-glucose residues. The average repeating-unit, containing a total of fifteen D-glucose residues, possesses five branches which occur at each position 6 of such segments and at positions 2. Branches consist mainly of α-D-glucopyranosyl groups and some appear to be terminated by α-nigerosyl groups". Except for the absence of α-nigerosyl groups, our data are compatible with these conclusions.

Dextran B-1308. — Of the dextrans listed in Table II, that of B-1308 has the lowest degree of branching. Methylation data indicate in this dextran a molar ratio of 1:23:1 for nonreducing, 1,6-di-, and 1,3,6-tri-*O*-substituted sugar residues, respectively. Because solutions of this dextran display behavior characteristic of highly branched polysaccharides, it is likely that the low degree of branching reflects the presence of long side-chains composed of (1→6)-linked D-glucosyl residues.

Dextran B-1351 fraction S. — According to the results given in Table II, this dextran has the same type of linkages as dextran B-1308. Assuming undermethylation of 3-hydroxyl groups, we estimate ~15 (1→6)-linked D-glucosyl residues for every branch point. The exact number of (1→6)-linked D-glucosyl residues cannot be determined, as, according to the excess amount of di-*O*-methyl- compared to tetra-*O*-methyl-D-glucopyranoside, we were unable to methylate the polymer completely; we have no explanation for the resistance of B-1351 dextran to permethylation. The absence of additional methyl ethers in the g.l.c. chromatogram suggests specific undermethylation at O-3 of D-glucosyl residues. There are previous reports[31,32] of resistance to complete methylation of 3-hydroxyl groups in dextrans.

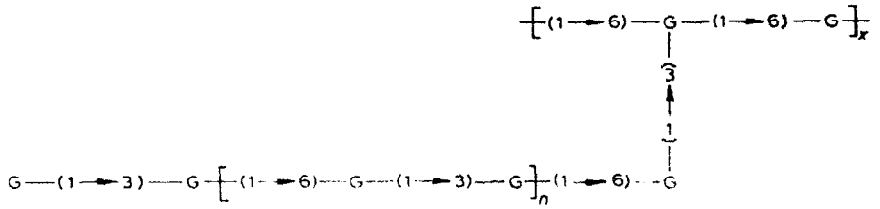
Hehre[33] suggested that the dextransucrase of *S. viridans* B-1351 has a higher affinity for sucrose as an acceptor substrate than dextransucrases from some

Leuconostoc strains. This property accounts for the relatively low-molecular-weight distribution of the dextran, which makes it a potentially economical candidate for clinical use, as well as for its relatively high content of D-fructose, *i.e.*, 0.27–0.37% in certain fractions[33]. We would probably not have detected D-fructose, however, as only 2,3,4,6-tetra-*O*-methyl-D-glucose PAAN could be detected from a derivatized hydrolyzate of permethylated sucrose. Failure to detect, in methylated dextran B-1351, a tri-*O*-methyl-D-glucose other than 2,3,4-tri-*O*-methyl-D-glucose further implies that the 6-hydroxyl group belonging to the D-glucosyl moiety of sucrose serves as the initial D-glucosyl acceptor for B-1351 dextransucrase.

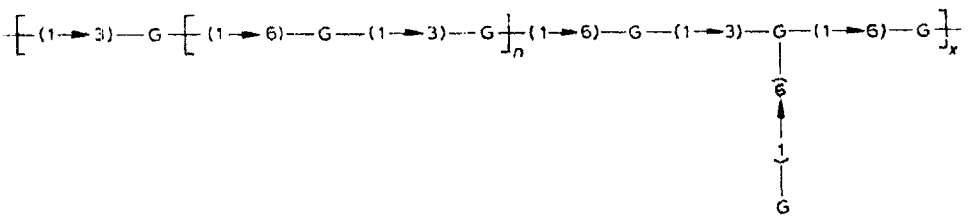
Dextran B-1355 fraction S. — Fraction S of the dextran synthesized by *L. mesenteroides* B-1355 differs from the other dextrans in Table II, in that it has two major types of unbranched, D-glucopyranosyl residues, *viz.*, (1→3)- and (1→6)-linked. It is unfortunate that the methylation data again indicate a certain degree of undermethylation, as precise data are needed in order to permit distinguishing between several possible structures.

From a partial acetolyzate of B-1355 fraction S dextran, Goldstein and Whelan[6] isolated glucose, nigerose, and lesser amounts of isomaltose and the trisaccharide *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose. No trace of nigerotriose in the acetolyzate, and failure to detect nigerose as a product of Smith degradation, suggested that B-1355 fraction S dextran contains linear sequences composed of alternating (1→3)- and (1→6)-linked D-glucopyranosyl residues. The recent finding by Torii and Sakakibara[34] that acetolysis of B-1355 fraction S dextran yields only glucose and nigerose further confirms the structure suggested.

If it is assumed that 2,3,4,6-tetra-, 2,4,6-tri-, 2,3,4-tri-, and 2,4-di-*O*-methyl-D-glucoses would be obtained in respective 9, 36, 46, and 9% yields from the fully methylated dextran, the corresponding molar ratios would be 1:4:5:1. These molar ratios would accord with a value of $n = 3$ in the following repeat-unit structures:



A



The importance of correspondence between the proportions of tetra- and di-*O*-methyl compounds can be seen from the fact that, if the value of *n* for the repeating nigerosyl unit were 2 (rather than 3), the respective, calculated percentages of tetra-, 2,4,6-tri-, 2,3,4-tri-, and di-methyl ethers would be 11, 33, 44, and 11. A value of $n = 4$ changes the respective percentages to 8, 38, 46, and 8.

Our methylation data are in general agreement with periodate-oxidation studies[3] that indicated 35% of (1→3)-linkages, but do not confirm the periodate value of 8% of (1→4)-like linkages, which probably was due to overoxidation. It is difficult completely to reconcile the foregoing structures with the proportions of nigerose and D-glucose obtained through acetolysis[34]. These structures, where $n = 3$–4, require a total nigerose content of ~90%. Whereas structure A assumes a (1→6)-linked poly-D-glucose backbone for B-1355 fraction S dextran, structure B depicts a linear chain of alternating (1→3)- and (1→6)-linked D-glucopyranosyl residues to which side-chain D-glucopyranosyl groups are attached at 6-hydroxyl groups. Again, it should be emphasized that the size distribution of side chains cannot be determined from methylation data.

Inferences from immunochemical studies as to the structure of dextrans are usually based on the concepts that the bonding of dextran antigen with antidextran antibody is most strongly effected through nonreducing end-groups on the polysaccharide, and that the specificity is determined either by the glycosidic linkage of the terminal D-glucosyl group or of one closely adjacent to it[12]. Thus, B-1355 fraction S dextran would appear to have nonreducing ends terminated with both isomaltosyl groups and nigerosyl groups, as it reacts strongly with human antidextrans of α-D-(1→6) specificity[35] and of α-D-(1→3) specificity[36], respectively.

Structures A and B both accord with immunochemical evidence relating the proportion of (1→6)-linkages in dextrans with their capacity to precipitate Types II and XX antipneumococcal sera[37]. Oligosaccharides of the isomaltose series are the most potent inhibitors, and it was deduced that terminal, nonreducing chains of α-D-(1→6)-linked D-glucose residues are involved in these cross-reactions. A similar conclusion regarding the presence of side chains terminated by nigerosyl groups could be drawn from the observation that nigerose was found the most effective disaccharide inhibitor of the cross-reaction between B-1355 fraction S dextran and Type IX antipneumococcal serum[38]. Type IX antisera gave stronger cross-reactions with dextrans that contained high proportions of non-(1→6)-linkages [*i.e.*, α-D-(1→2) and α-D-(1→3)] than with dextrans that were mainly α-D-(1→6)-linked. Significantly, nigerose and *both* isomeric trisaccharides, *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose and *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→3)-D-glucose, isolated from a partial hydrolyzate of nigeran, were all equally effective inhibitors of the cross-reaction between B-1355 fraction S dextran and Type IX antipneumococcal serum which had been absorbed with B-1299 fraction S dextran[38]. Unlike A, structure B provides a high proportion of nonreducing ends terminated by isomaltosyl groups, and, therefore, could account for the strong cross-reactions with antibodies of α-D-(1→6) specificity. The high proportion of multiple,

repetitive, interchain sequences of alternating α-D-(1→3)- and α-D-(1→6)-linkages in B-1355 fraction S dextran could well account for the observations regarding cross-reactions with sera that contain antibodies of α-D-(1→3) specificity. Recent work[39] has shown that α-D-(1→6)-specific, human antidextrans are mixtures of molecules having terminal and nonterminal specificity. Perhaps α-D-(1→3)-specific, antidextran sera also contain antibodies having nonterminal specificity.

Dextran B-1399. — The dextran described here is not the same as that reported earlier[3,10] and subsequently investigated chemically[7] and immunochemically[7,38] by others. The earlier product, obtained from a culture of the newly isolated strain, appeared homogeneous, and required no fractionation. According to periodate oxidation[3], the percentages of (1→6)-, (1→4)- or (1→2)-, and (1→3)-like linkages present were 65, 35, and 0, respectively. However, all subsequent preparations from lyophilized subcultures of the original strain required fractionation, and showed some physical properties different from those of the original product. The best preparation gave two fractions. Periodate oxidation indicated that the larger fraction (85%), L, contained 81 and 19%, respectively, of (1→6)-like and (1→4)- or (1→2)-like linkages. Fraction S (15%) gave corresponding values of 55 and 45%. As an insufficient quantity of the original B-1399 dextran was available, methylation analysis was performed on Fraction L of the later preparation.

Two types of branching, involving 1,2,6- and 1,3,6-tri-*O*-substituted residues present in nearly equal amounts, make fraction S of B-1399 dextran unique. As in B-1299 fraction S dextran, the (1→2)-linked D-glucosyl residues occur exclusively as branching residues. Methylation analysis (see Table II) indicates that B-1399 dextran contains D-glucosyl groups, (1→6)-linked D-glucosyl residues, and 1,2,6- and 1,3,6-tri-*O*-substituted D-glucosyl residues in the respective molar ratios of 2:11:1:1.

Recent ^{13}C-n.m.r. studies have failed to detect the presence of D-glucopyranosyl units linked to 3-hydroxyl groups in B-1399 dextran fraction L (see ref. 55). The presence of 2,4-di-*O*-methyl-D-glucopyranose among the products of methylation–hydrolysis could be due to resistance of 3-hydroxyl groups to methylation[31,32]. In this regard, it is perhaps significant that the products of fewer Hakomori methylations contained higher percentages of 2,4-di-*O*-methyl-D-glucose.

Unusual aspects of the dextrans. — Precise definition of the identity and proportion of the D-glucosidic linkages of the dextrans reported here will permit further clarification and extension of the principles of the biochemical systems in which these dextrans have shown specific, structurally determined behavior. In all of these systems, the interaction is that of the dextran with proteins or related substances, such as the homologous or heterologous antibodies of mammalian sera, nonspecific participants in serological reactions such as complement and conglutinin, certain macroglobulins of myeloma sera, and plant-hemagglutinins such as concanavalin A.

Dextrans B-1299 fraction S and B-1355 fraction S constituted the first water-soluble antigens available having high proportions of the α-D-(1→2)- and α-D-(1→3)-linkages, respectively, which were the secondary linkages, virtually exclusive of any

others. Immunochemical studies of the resultant antisera, which had specificity directed to these linkages in the homologous and other dextrans, were conducted in conjunction with the inhibitory action of oligosaccharides derived in part from these dextrans. These studies resulted in establishment of the dimensions of the combining sites on the antibody, and in their correlation with corresponding structural features of the dextran[12].

Establishment of the presence of kojibiosyl (2-*O*-α-D-glucopyranosyl-D-glucopyranosyl) units in dextran B-1299 fraction S[5] and also in Type XII pneumococcal polysaccharide[40] provided the structural basis for the strong cross-reaction of this dextran with Type XII antisera, the behavior which initially focused attention on this dextran[7,11].

In a screening program for myeloma proteins having antibody activity, the unique reactivity of dextran B-1355 fraction S resulted[41] in discovery of a new type of macroglobulin (IgM) from the murine plasmacytoma MOPC 104E. Subsequently, this dextran has been used extensively in immunospecific, purification procedures for[42] the IgM, and in a variety of immunochemical[43,44], genetic[45], and medical researches.

Dextran B-1355 fraction S is the standard for assaying concanavalin A activity[46] and has been used extensively by Goldstein and his co-workers in research on this lectin[13,47]. The usefulness of this dextran fraction is based on its high reactivity and the low solubility of its complex with this plant agglutinin[47]. (Its activity is, however, less[48] than that of dextran B-1299 fraction S and dextran B-1399.) These characteristics contrast strongly with those of the dextran from NRRL B-512(F), even though its 5% of nonreducing end-groups[21] is near to that (7%) of the B-1355 fraction S dextran. It appears, therefore, that some factor(s)[49] other than the commonly mentioned availability of branch ends[13,47] determines the formation and properties of the complex of B-1355 fraction S dextran with concanavalin A.

Dextran B-1355 fraction S has also been used as a water-soluble prototype and standard for comparison in research on the insoluble α-D-glucans produced by cariogenic streptococci. These D-glucans generally have a high content of α-D-(1→3)-linkages[50].

The solubility and high reactivity of dextran B-1254 fraction L have made it valuable in studies on the kinetics and mechanism of action of properdin[51], conglutinin[52], and complement[51,52], all of which play incompletely understood roles in immunological systems. Its reactivity with IgM MOPC-104E, and the ease of dissociation of the resultant complex[41], are the bases for its value in isolating this macroglobulin of plasmacytoma[53].

In its reaction with bovine conglutinin[52], dextran B-1308 (as well as dextran B-1355 fraction S) more resembles dextrans having a high content of non-(1→6)-links (presumably, more branched) than it does those of more evident linearity. This observation, as well as the gum properties of dextran B-1308, which are atypical of a dextran having only 4% of nonreducing end-groups[3], directs attention to an apparently unusual manner of branching.

Deuteriomethylated B-1254 dextran. — The ease of methylation of B-1254

dextran made it an ideal source of 4,5,6-tri-*O*-acetyl-2,3-di-*O*-deuteriomethyl-D-gluconononitrile, which could be obtained (as a derivative) from the hydrolyzate of the perdeuteriomethylated polysaccharide. We have proposed a mass-spectral fragmentation-pathway for the corresponding di-*O*-methyl-D-mannose derivative[2]. In that fragmentation pathway, fragment ions of series N^2 (*m/e* 85, 127, 187, and 229) and P (*m/e* 115, 157, and 217) were assumed to represent fragments that contained neither 2- nor 3-methoxyl groups. The correctness of these assumptions is now confirmed by the finding that all these fragment ions are retained in the mass spectrum of the di-*O*-deuteriomethyl compound. Fragments belonging to series N^1 (*m/e* 99, 159, 201, and 261) were assumed to contain the 3-methoxyl group. In the mass spectrum of the di-*O*-deuteriomethyl compound, each of these fragment ions is displaced by +3 mass units. Series M ions (*m/e* 87 and 114) were assumed to contain both 2- and 3-methoxyl groups. In the di-*O*-deuteriomethyl spectrum, however, we observed fragments of *m/e* 87, 90, 93, and 120; this result indicates that alternative fragmentation-pathways also contribute to these ions.

EXPERIMENTAL

Materials. — The preparation and characterization of the dextrans[3] and dextran fractions[3,54] have been reported.

Methylation. — Permethylation of dextrans was conducted by the procedure of Hakomori[17] as previously described[1]. Dextran samples (15–30 mg) were dried *in vacuo* over phosphorus pentaoxide for 12 h at room temperature, and then for an additional 4 h at 50°. The dried dextrans were dissolved in dimethyl sulfoxide (4 ml; dried over 4A molecular sieve) with the aid of magnetic stirring under dry nitrogen. Dimethylsulfinylsodium reagent (0.4 ml) was added and, after stirring for 30 min, methyl iodide (0.4 ml). (For deuteriomethylation of *Streptobacterium dextranicum* NRRL B-1254 fraction L dextran, trideuteriomethyl iodide was substituted for methyl iodide.) After stirring for an additional 30 min, the reaction mixtures were dialyzed, initially for 12 h against running tap water, and then for 4 h against distilled water. The suspended, methylated products were recovered by lyophilization prior to either remethylation or hydrolysis.

Incompletely methylated dextrans were subjected to additional Hakomori, and Kuhn[18], methylations until either the amount of dimethyl ether equaled the amount of 2,3,4,6-tetra-*O*-methyl-D-glucose, or further methylation failed to change the ratios of these components. Dextrans B-1254 fraction L and B-1299 fraction S required only single Hakomori methylations, whereas dextrans B-1351 fraction S, B-1399 fraction L, and B-1308 each required four, successive, Hakomori methylations. Dextran B-1355 fraction S was subjected to two Hakomori methylations followed by two Kuhn methylations.

Hydrolysis and derivatization. — The most successful procedure for hydrolyzing the methylated dextrans was brief formolysis[20] followed by hydrolysis with sulfuric acid in acetic acid, according to Stellner *et al.*[19]. Permethylated dextran B-1254

fraction L was hydrolyzed directly, without formolysis. Hydrolysis of methylated dextran B-1299 fraction S with 72% sulfuric acid as described by Bourne *et al.*[8] led to extensive charring; methanolysis in 7.2% methanolic hydrogen chloride[8] gave rise to two, additional, major components which could not be identified by g.l.c.–m.s. No trace of 2,4,6-tri-*O*-methyl-D-glucose could be detected as the PAAN derivative following hydrolysis of the methanolyzate of methylated dextran B-1299 fraction S.

Sulfate ion was removed from hydrolyzates by passage through columns (0.5 × 5 cm) of Dowex 2-8X (acetate) (200–400 mesh) anion-exchange resin. The columns were successively eluted with 4-ml portions of water and methanol. These eluates were evaporated to dryness at 40° in a rotary, vacuum evaporator, and then re-evaporated with absolute ethanol (2 × 5 ml).

The prepation of peracetylated aldononitrile (PAAN) derivatives has been described[2].

Gas–liquid chromatography. — A gas chromatograph equipped with a hydrogen-flame ionization detector, helium carrier, and stainless-steel columns (1.83 m × 3.18 mm) was used. Column A consisted of 3% of neopentyl glycol succinate on Supelcoport (an acid-washed, silane-treated Chromosorb W), and was temperature-programmed from 165 to 200° at 2°/min, and then held at 200° with a carrier flow-rate of 50 ml/min. Column B consisted of 5% of butanediol succinate on Supelcoport, temperature-programmed from 185 to 210° at 1°/min and then held at 210° with a carrier flow-rate of 50 ml/min. Column C consisted of 5% of Apiezon L on Gas Chrom W, temperature-programmed from 150° at 1°/min with a carrier flow-rate of 35 ml/min. All g.l.c. supports were 60–80 mesh. Column A was used for rapid detection of all classes of PAAN derivatives, ranging from D-glucose to its 2,3,4,6-tetramethyl ether. Column B was used for general resolution of di-, tri-, and tetra-*O*-methyl PAAN derivatives. Column C resolved the 2,3,4-and 2,3,6-tri-*O*-methyl-D-glucose PAAN derivatives.

Mass spectrometry. — The conditions employed were essentially those previously described[2], except that the emergent gas-streams containing PAAN derivatives separated on columns B and C were introduced into the mass spectrometer and automatically scanned at 8-sec intervals. This refinement permitted the recording and storage of spectra in a computer for (*1*) later reconstruction of the chromatogram by the computer as a plot of total ionization *versus* retention time, (*2*) identification of each g.l.c. peak, and (*3*) confirmation of peak homogeneity.

The identity of each g.l.c. peak was confirmed by comparing the mass spectrum to those of either authentic PAAN derivatives of methylated D-glucose or corresponding derivatives of D-mannose. As fragmentation patterns of PAAN derivatives depend on the location of the methoxyl groups, and are independent of the sugar configuration[14,15], mass spectra of D-mannose reference compounds are usable in the methylation analysis of dextrans. The same ratios of methylated components were obtained by measuring the integral of the hydrogen-flame ionization chromatography curve as by computer integration of total fragment-ion output in the mass spectrometer. Based on previous experience with methylated D-mannose PAAN derivatives[2], the response of the hydrogen-flame ionization detector is also considered

to be the same for the various D-glucose compounds derived from methylated dextrans.

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