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STRUCTURAL ANALYSIS OF LEUCONOSTOC DEXTRANS CONTAINING 3-*O*-α-D-GLUCOSYLATED α-D-GLUCOSYL RESIDUES IN BOTH LINEAR-CHAIN AND BRANCH-POINT POSITIONS, OR ONLY IN BRANCH-POINT POSITIONS, BY METHYLATION AND BY ^{13}C-N.M.R. SPECTROSCOPY*

FRED R. SEYMOUR,

Fleming Department of Rehabilitation, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

ROGER D. KNAPP,

Division of Atherosclerosis and Lipoprotein Research, Baylor College of Medicine, and Methodist Hospital, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

EDWARD C. M. CHEN,

School of Sciences and Technologies, University of Houston at Clear Lake City, Houston, Texas 77058 (U.S.A.)

STEPHEN H. BISHOP**,

Marrs McLean Department of Biochemistry, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

AND ALLENE JEANES

Northern Regional Research Center, Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, Peoria, Illinois 61604 (U.S.A.)

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ABSTRACT

It had been established by methylation-structural analysis that dextran fraction S from *Leuconostoc mesenteroides* NRRL B-1355 has two types of α-D-glucopyranosyl residues that are linked through O-3, *i.e.*, 35% of the residues carry a (1→3)-bond, and ~10% carry a (1→6)-bond in addition to a (1→3)-bond. Two similarly constituted dextrans have now been identified by methylation-structural analysis, namely, the S-type fractions from *L. mesenteroides* strains NRRL B-1498 and B-1501. The S-type fractions from *L. mesenteroides* strains B-1355, B-1498, and B-1501 are structurally differentiated from the α-D-glucans (characteristically insoluble) of certain cariogenic Streptococci which also contain both 3-*O*- and 3,6-di-*O*-substituted α-D-glucopyranosyl residues. ^{13}C-N.m.r. spectra have been recorded at 90° for both the S- and L-type fractions of strains B-1355, B-1498, and B-1501. The L-type fractions have a low degree of branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues, but no 3-mono-*O*-substituted residues. (Dextran fraction S of *Streptococcus*

*Unusual Dextrans, Part VIII. For Part VII, see ref. 1.

**Present address: Department of Zoology, Iowa State University, Ames, Iowa 50011, U.S.A.

viridans NRRL B-1351, which had been shown to have 11% of 3,6-di-*O*-substituted α-D-glucopyranosyl residues but no 3-mono-*O*-substituted residues, was used as a spectroscopic control.) These structural data for dextrans were then correlated to previously published immunochemical data. Structural correlations are more meaningful, for both ^{13}C-n.m.r. spectra and immunochemical data, when specifically substituted residues are considered, rather than relationship to specific types of saccharide linkage. The ammonia chemical-ionization mass spectra of peracetylated, aldononitrile (PAAN) derivatives of methylated D-glucoses in the hydrolyzates of methylated dextran B-1501 fraction S were recorded. The electron impact mass spectra of isotopically substituted variants of these PAAN derivatives were also examined, to confirm fragmentation pathways previously proposed.

INTRODUCTION

In recent studies, we successfully applied combined g.l.c.–m.s. analysis of peracetylated aldononitrile (PAAN) derivatives of methyl ethers of D-glucose to the determination of glucosidic linkages in a series of unusual dextrans[2,3]. These unusual dextrans were also analyzed by recording and studying their ^{13}C-n.m.r. spectra, a technique allowing cross-referencing of these data to the methylation data, and further refinement of the proposed dextran structures[4–6].

We now report the combined application of methylation analysis and ^{13}C-n.m.r.-spectroscopic analysis to an additional series of unusual dextrans, produced by strains of *Leuconostoc mesenteroides*, that were initially described by Jeanes *et al.*[7]. Essentially homogeneous dextran fractions, designated readily soluble (S) and less soluble (L), were separated by Wilham *et al.*[8] from the corresponding, polydisperse, high-molecular-weight native dextran by fractional precipitation with ethanol. The S-type dextran fractions from strains B-1355, B-1498, and B-1501 have in common a significant content of (1→3)-linked α-D-glucopyranosyl residues (that is, 3-mono-*O*-substituted residues) and a lower content of α-D-glucopyranosyl residues that branch through O-3 (that is, 3,6-di-*O*-substituted residues). The corresponding, L-type dextran fractions from these strains have no (1→3)-linked residues, but a low content of 3,6-di-*O*-substituted residues at branch points. ^{13}C-N.m.r. spectra recorded at 34° and at 90° show distinctive features that are unlike those observed for the dextrans previously studied[4–6]. The S-type fractions of dextrans B-1355, B-1498, and B-1501 will, therefore, be analyzed and discussed as specific representatives of a new class of α-D-glucans. The corresponding L-type fractions, with their low degree of branching, constitute a series that contrasts with the S series in structural, spectroscopic, and other characteristics.

We had previously shown that (*a*) linear dextran (low degree of branching) has six prominent, ^{13}C-n.m.r. resonances[4], (*b*) dextran branching contributes additional resonances to the ^{13}C-n.m.r. spectra, (*c*) the positions of these additional resonances in the anomeric (95–105-p.p.m.) region and the 80–85-p.p.m. region are diagnostic for the type of branch linkage present [*e.g.*, (1→2), (1→3), or (1→4)]

(see refs. 4 and 5), and (*d*) the intensities of these additional, ^{13}C-n.m.r. resonances resulting from branching are, in general, proportional to the percentage of non-α-D-(1→6)-linkages present in the biopolymer as established by methylation analysis[6]. It had also been established that high-temperature (90°), ^{13}C-n.m.r. spectra provide better resolution than spectra recorded at a lower temperature (ambient)[4]. This high-temperature enhancement of the spectrum is normally associated with modest changes in relative intensities of the resonances[6]. Our ^{13}C-n.m.r.-spectroscopic data are in complete accord[6] with a variety of evidence that α-anomeric linkages exclusively occur in dextrans.

While confirming the g.l.c.–m.s. analyses of PAAN derivatives of hydrolyzates of the permethylated dextrans, we had the opportunity to verify, *via* isotopic substitution, previously proposed[9] electron-impact (e.i.) mass spectral fragmentation-pathways. In addition, we were able to observe the fragment ions produced by partially methylated PAAN derivatives when subjected to ammonia chemical ionization (c.i.), and to compare these with ions from non-methylated PAAN derivatives[10]. This combination of e.i.-m.s. and ammonia c.i.-m.s. allows unambiguous identification of the peaks observed in gas–liquid chromatograms.

RESULTS AND DISCUSSION

Previous studies on the prototype of Leuconostoc dextrans containing both 3-mono- and 3,6-di-*O*-substituted α-D-glucopyranosyl residues, that is, the B-1355 fraction S, have consistently indicated the novel constitution of this biopolymer. The unusually high values of its content of (1→3)-like linkages, and its specific optical rotation[7], set it apart from other dextrans. Preliminary indications from limited acid hydrolysis of non-(1→6)-linkages in linear-chain positions[11,12] were verified by measurements of optical rotatory shifts in cuprammonium solution[13]. The absence of contiguously located 3-mono-*O*-substituted residues in this dextran fraction has been established by acetolysis[14,15] and by enzymic degradation[16,17]. Methylation-structural analysis proved the presence of 35% of 3-mono-*O*-substituted residues and ~10% of 3,6-di-*O*-substituted residues at branch points[2]. The ^{13}C-n.m.r. spectrum of this dextran was also distinctive[4]. Similar spectral features have now been found for the S-type fractions of dextrans B-1498 and B-1501, which, like the B-1355 fraction S, also have significantly high values for (1→3)-like linkages and for the specific optical rotation[7]. These dextrans were, therefore, subjected to methylation-fragmentation analysis and thorough ^{13}C-n.m.r. spectroscopic study, as reported here.

α-D-Glucans from numerous strains of cariogenic *Streptococcus mutans*, *S. salivarius*, and *S. sanguis* also contain both 3-mono-*O*-substituted and 3,6-di-*O*-substituted residues[17]. In contrast to the S-type dextrans of Leuconostoc strains reported here, however, the 3-mono-*O*-substituted residues in these streptococcal glucans occur mainly in sequential arrangement, and most of the glucans are water-insoluble[17]. A water-soluble product that may properly be designated a dextran was

TABLE I

MOLE PERCENTAGE OF METHYLATED D-GLUCOSES IN HYDROLYZATES OF METHYLATED DEXTRANS

Organism	*NRRL strain*	*Dextran fraction*	*Methyl ethers of* D-*glucose*				
			2,3,4,6	*2,3,4*	*2,4,6*	*2,4*	*3,4*
Leuconostoc mesenteroides	B-1355	S[a,b]	6.9	46.9	35.0	11.2	
	B-1498	S	9.4	51.5	29.0	10.0	
	B-1501	S	7.2	59.8	24.3	8.7	
Streptococcus viridans	B-1351	S[a]	5.8	83.3		10.5	0.3

[a]Data taken from ref. 2. [b]For comparison, the corresponding percentages[3] of methyl ethers from methylated dextran B-1355 fraction L are: 2,3,4,6-tetra-, 3.3; 2,3,4,-tri-, 91.5; 2,4,6-tri-, 1.5; and 3,4-di-, 3.7.

synthesized by a discrete component separated from the D-glucosyltransferase system of a *S. mutans* strain[17]. This dextran is constituted of approximately equimolar proportions of a D-glucosyl residue, a (1→6)-linked D-glucosyl residue that is branched at O-3, and a residue linearly (1→6)-linked; it is, therefore, analogous to dextran B-742 fraction S, which is constituted[3,5] of approximately equimolar proportions of a D-glucosyl residue and a (1→6)-linked residue that is branched at O-3.

Methylation-structural analysis. — The results of methylation-structural analysis (see Table I) show that the fraction S dextrans from strains B-1355, B-1498, and B-1501 constitute a series in which, in the hydrolyzate of the methylated dextran, the percentage of 2,3,4-trimethyl ether increases in the order named, and the percentage of 2,4,6-tri- and 2,4-di-methyl ethers decreases. For the B-1498 and B-1501 fractions, which were methylated in sonicated solutions[3], exceptional agreement was achieved in the percentages of 2,3,4,6-tetra- and 2,4-di-methyl ethers. The B-1498 fraction was completely methylated by one Hakomori[18] treatment, whereas the B-1501 fraction required three. Recovery was unusually high for branched dextrans: 80% from 30-mg samples. This difference in resistance to methylation may relate to greater interference by hydrogen bonding[3] in the B-1501 fraction S, which has fewer non-(1→6)-linked residues. The fraction S dextrans from B-1355 and B-1351 were methylated without the aid of sonication[2]; the B-1355 fraction required two Hakomori treatments followed by two Kuhn methylations, and the B-1351 fraction required four successive, Hakomori methylations. The resistance of these fractions to methylation is reflected in incomplete methylation, as evidenced by poor correspondence in the percentage of residues at nonreducing-end and branch-point positions (see Table I). Improved conditions for depolymerization also contribute to the excellent data for the B-1498 and B-1501 fractions. Formolysis[19] was extended to three hours, and was followed by the usual acetic acid–sulfuric acid hydrolysis[20], resulting in dissolution with formation of no trace of color. However, even under these conditions, the methylated B-1501 fraction S dextran showed the greatest

TABLE II

CHEMICAL SHIFTS FOR ^{13}C-N.M.R. SPECTRA AT 90° OF DEXTRANS CONTAINING BOTH 3-MONO-*O*- AND 3,6-DI-*O*-SUBSTITUTED RESIDUES OR ONLY 3,6-DI-*O*-SUBSTITUTED RESIDUES[a]

Dextran	*B-1351*	*B-1355*	*B-1498*	*B-1501*	*B-1498*	*B-1501*	*B-1416*	*B-1498*[b]
Fraction	*S*	*S*	*S*	*S*	*L*	*L*		*S*
							101.63	
		101.07	101.09	101.11				100.37
	100.88				100.88	100.88	100.88	
		99.94	99.92	99.97			100.17	
A[c]	99.48	99.75	99.47	99.51	99.50	99.48	99.51	99.48
		83.31	83.31	83.32				81.26
	82.85				82.88	82.84	82.86	
							80.19[d]	
						77.53[d]	76.47[d]	
B	75.17	75.02	75.10	75.14	75.16	75.16	75.16	74.19
	74.79				74.80(s)	74.79(s)[e]		
	73.61				73.60(s)	73.55(s)		
	73.50	73.46(*s*)	73.42(*s*)	73.50(*s*)		73.50(s)		
		73.30(*s*)	73.24(*s*)					
C	73.18		73.15	73.17	73.18	73.17	73.17	72.37
		72.37(*s*)	72.30(*s*)	72.41(*s*)				
D	71.98	71.88	71.92	71.97	71.98	71.97	71.98	71.10
	71.80							
E	71.59	71.45	71.52	71.57	71.59	71.57	71.59	70.35
F	67.78	67.67	67.72	67.77	67.78	67.77	67.79	66.38
	62.45	62.33	62.36	62.45	62.46	62.46	62.49	61.20

[a]The spectra are expressed in p.p.m. relative to tetramethylsilane. [b]This spectrum was recorded at 34°. [c]The letters A through F designate the major resonances of linear dextran (see ref. 4). [d]These resonances indicate traces of (1→4)- and (1→2)-branching. [e]The *s* symbol designates a shoulder on the major peak.

resistance to hydrolysis of any of the dextrans we have studied. The fundamental similarity between the S fractions from strains B-1355, B-1498, and B-1501 is further expressed in the properties of the dextran fractions. Thus, when precipitated from aqueous solution by the addition of alcohol, each product was a fine, dry-looking powder[7] (indicative of compact molecular-form), and the intrinsic viscosities of the fractions were low and correlated inversely with the percentage of non-(1→6)-linked residues[7]. In keeping with the usual relationship between S-type and L-type fractions of dextrans[5], the intrinsic viscosities of the L fractions were significantly greater than those of the corresponding S fractions[5].

^{13}C-N.m.r. spectroscopy. — Detailed in Table II are the chemical shifts in the ^{13}C-n.m.r. spectra of dextrans containing α-D-glucopyranosyl residues that are 3-mono-*O*- and 3,6-di-*O*-substituted (fraction S dextrans of B-1355, B-1498, and B-1501) and only 3,6-di-*O*-substituted (fraction L dextrans of B-1498 and B-1501, and

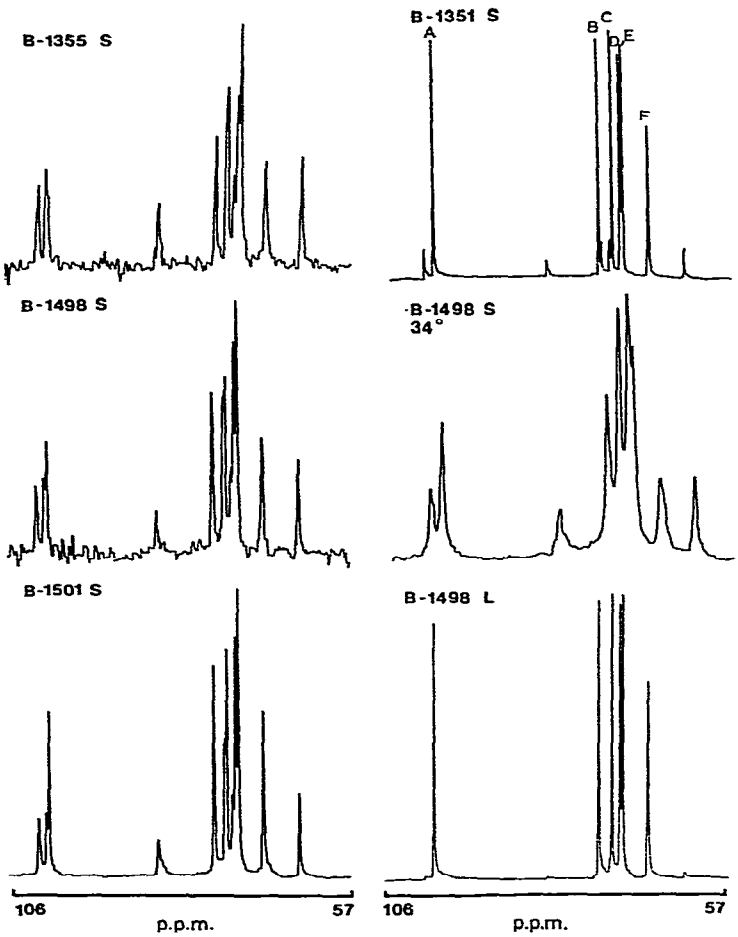


Fig. 1. ^{13}C-N.m.r. spectra at 90° for dextrans B-1355 fraction S, B-1498 fraction S, B-1501 fraction S, B-1351 fraction S, and B-1498 fraction L. The ^{13}C-n.m.r. spectrum at 34° is also shown for dextran B-1498 fraction S.

fraction S of the control, B-1351 dextran). Representative spectra are shown in Figs. 1 and 2, and the greater resolution achieved at 90°, as compared with 34°, is illustrated for dextran B-1498 fraction S. The shifts recorded at 90° are directly comparable with those previously reported for dextrans having 2,6-di-*O*-substituted[6] and 4,6-di-*O*-substituted[5] residues. We have previously analyzed ^{13}C-n.m.r. spectra by employing the hypothesis that each specifically *O*-substituted D-glucopyranosyl residue would contribute a unique set of six resonances, and that, to a first approximation, these resonances would be independent of neighboring-group effects[4]. However, that set of data included dextran B-1351 fraction S (known to contain 3,6-di-*O*-substituted α-D-glucopyranosyl residues) and dextran B-1355 fraction S (known to contain a large percentage of 3-mono-*O*-substituted α-D-glucopyranosyl residues), and the ^{13}C-n.m.r. spectra of these dextrans display similar diagnostic resonances in both the anomeric-resonance region and the 75–85-p.p.m. region.

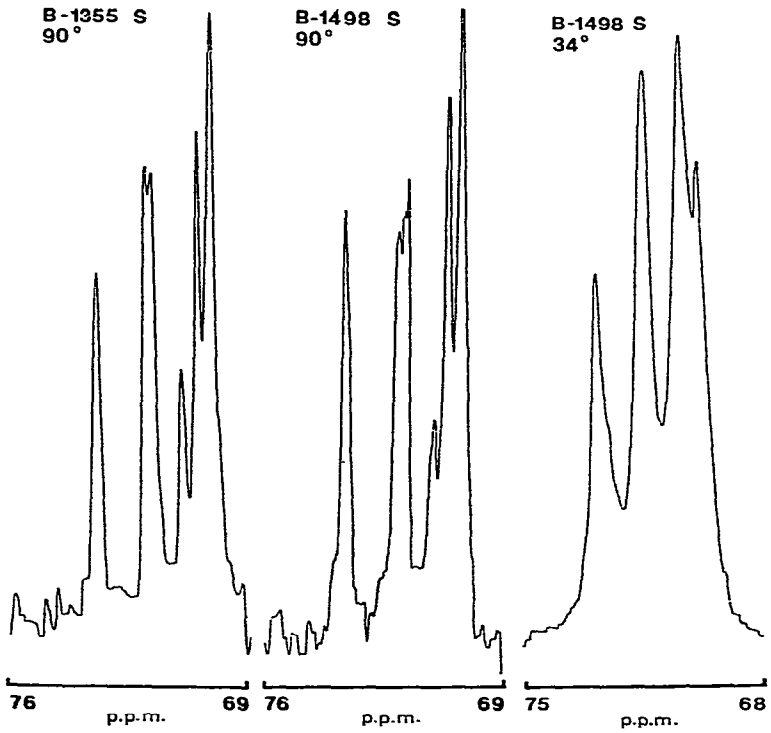


Fig. 2. The expanded, 69–76-p.p.m. region of the ^{13}C-n.m.r. spectra presented in Fig. 1 for dextrans B-1355 fraction S and B-1498 fraction S.

At that time, not being certain of the accuracy or reproducibility of the chemical shifts from polysaccharide to polysaccharide, we postulated that D-glucopyranosyl residues of greater divergence of substitution type (*e.g.*, 2,6-di-*O*-substituted and 3,6-di-*O*-substituted residues) would have greater differences of chemical shifts than residues of more similar substitution type (*e.g.*, 3-mono-*O*-substituted and 3,6-di-*O*-substituted residues) and that the latter two residues had resonances (especially with regard to the C-3 atom) that were slightly different, but not readily distinguishable, at 25 MHz. Later, we examined the ^{13}C-n.m.r. spectra of a series of dextrans, branched exclusively through the 2,6-di-*O*-substituted α-D-glucopyranosyl residue[6], that differed only in degree of linearity; the latter data[6] demonstrated that the chemical shifts diagnostic for a specific type of branching are independent of the degree of linearity of the polysaccharide, and also provide a reference for the accuracy of measurement when comparing chemical shifts from the same residue type in different polysaccharides (in general, agreement of ~0.5 p.p.m. could be obtained at 25 MHz). As the methylation data indicated that we had available biopolymers of different degrees of linearity for classes of dextrans primarily containing 3-mono-*O*-substituted or 3,6-di-*O*-substituted α-D-glucopyranosyl residues, it was of interest to determine whether distinctly different ^{13}C-n.m.r. resonances could be observed in the 25-MHz spectra for these two types of residue.

All of the anomeric resonances for the dextrans are upfield from 102 p.p.m., which, for D-glucans, indicates the presence of α linkages[22]. A resonance at 101.1 p.p.m. seems characteristic of 3-mono-*O*-substituted residues, as it only occurs for dextrans having these residues. A resonance at 100.88 p.p.m. appears to be characteristic of 3,6-di-*O*-substituted residues in the absence of 3-mono-*O*-substituted residues (see Table II). The 99.9-p.p.m. resonance appears only in spectra of dextrans having both 3-mono- and 3,6-di-*O*-substituted residues. The 99.5-p.p.m. resonance correlates with peak A of linear-type dextrans, and is assigned to linear, chain-extending residues. The slight displacement of this peak to 99.7 p.p.m. for dextran B-1355 fraction S is probably due to the difficulty of digital slope detection for this shoulder, which is shown in Fig. 1. Peak A increases in spectral prominence as the percentage of (1→6)-linked residues increases (see Table I and Fig. 1); the contrast is most striking between dextran B-1498 fraction S and the corresponding fraction L, which is essentially linear (see Fig. 1). Therefore, the ^{13}C-n.m.r. anomeric region indicates consistent, identifiable differences between the chemical shifts associated with 3-mono-*O*-substituted (99.94 p.p.m. and 101.09 p.p.m.) and 3,6-di-*O*-substituted (100.88 p.p.m.) α-D-glucopyranosyl residues, but also indicates that these differences lie near the limits of accuracy of the spectrometer. The foregoing differences in ^{13}C-n.m.r. chemical shifts contrast with the large chemical-shift differences associated with 2,6-di-*O*-substituted (97.4 p.p.m. and 98.2 p.p.m.) and 4,6-di-*O*-substituted (100.1 p.p.m. and 101.6 p.p.m.) α-D-glucopyranosyl residues. It was concluded[5] that the introduction of branch-points into the dextran chain would result in two additional, nonlinear dextran, anomeric resonances. However, for dextrans branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues, only one additional anomeric resonance was observed, and it was concluded that one of the additional resonances was not resolved from the resonance of linear dextran. The essential identity of the A resonance and one of the additional, anomeric resonances of dextrans branching through 3,6-di-*O*-substituted residues was also demonstrated by ^{13}C-n.m.r. resonance-relaxation studies[21] on the highly branched dextran B-742 fraction S.

The ^{13}C-n.m.r. resonances diagnostic for branched dextrans for the 75–85-p.p.m. region present a situation similar to the anomeric spectral-region. The 75–85-p.p.m. region contains the resonances of the O-substituted C-2, C-3, and C-4 atoms, and our preliminary investigation[5,6] indicated resonances diagnostic for dextran branching-types: ~78 p.p.m. for 2,6-di-*O*-substituted residues, ~83 p.p.m. for 3,6-di-*O*-substituted residues, and ~80 p.p.m. for 4,6-di-*O*-substituted residues. However, the resonances in the 75–85-p.p.m. region for dextrans containing 3-mono-*O*-substituted and 3,6-di-*O*-substituted α-D-glucopyranosyl residues were quite similar (~83 p.p.m.). Comparison of the spectra of dextrans of these two classes (see Table II) shows that there is a consistent difference of ~0.5 p.p.m. between the chemical shifts associated with the C-3 atom of the 3-mono-*O*-substituted α-D-glucopyranosyl residue (83.31 p.p.m.) and the chemical shift of the C-3 atom of the 3,6-di-*O*-substituted α-D-glucopyranosyl residue (82.86 p.p.m.). The relatively small, chemical-shift difference of

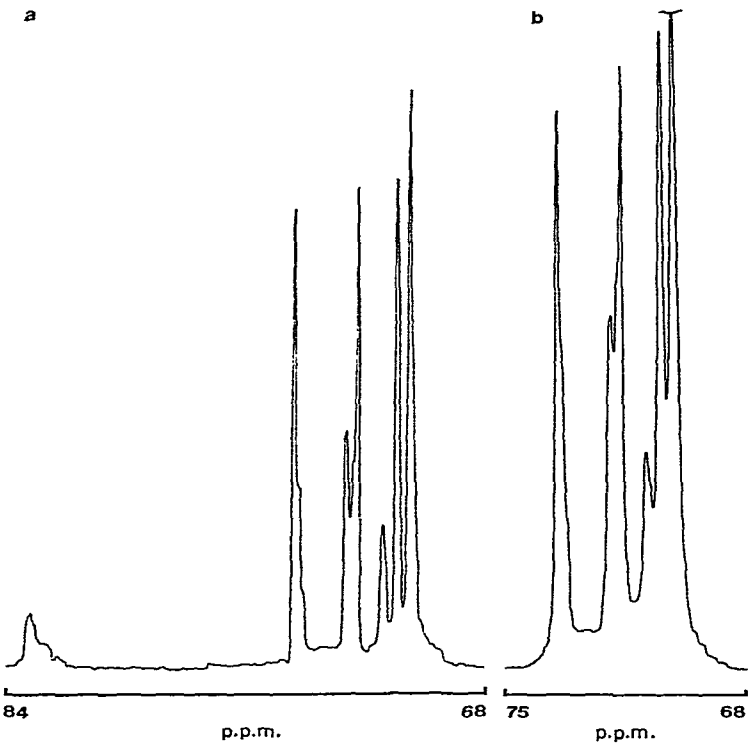


Fig. 3. The expanded, 68–84-p.p.m. region of the ^{13}C-n.m.r. spectrum at 90° of dextran B-1501 fraction S: (a) after data processing by c.d.r.e., and (b) the 68–75-p.p.m. region of the same before c.d.r.e. processing.

~0.5 p.p.m. between the resonances of these two types of C-3 atom does not allow the observation of one resonance when the other is relatively intense, and, therefore, the 82.86-p.p.m. resonance is not observed in the spectra of dextran B-1355 fraction S and dextran B-1498 fraction S. However, dextran B-1501 fraction S, a dextran containing more nearly equal percentages of 3-mono- and 3,6-di-*O*-substituted residues, displays a 75–85-p.p.m. spectral region containing an 83.3-p.p.m. resonance (see Fig. 3) and a shoulder (with a maximum at 82.9 p.p.m.) indicative of this relatively larger contribution of 3,6-di-*O*-substituted residues. It is instructive to compare the difficulty of resonance resolution for these linked C-3 atom resonances to the ease of resonance resolution when a mixture of linked C-2, C-3, and C-4 atoms is present in a polysaccharide. The high signal-to-noise spectrum of dextran B-1416 (see Fig. 4) provides an example of an essentially linear polysaccharide giving weak resonances in the 75–85-p.p.m. region; the upper trace in Fig. 4 shows a ten-fold magnification of the amplitude. The resonances in this region have been designated G, H, and I (as a continuation of the major, A through F resonances) to correspond, respectively, to the resonances of α-D-(1→3)-linked, α-D-(1→4)-linked, and α-D-(1→2)-linked residues. Resonance G (82.86 p.p.m.) and H (80.19 p.p.m.) of dextran B-1416 respectively indicate the presence of 3,6-di-*O*-substituted and 4,6-di-*O*-substituted

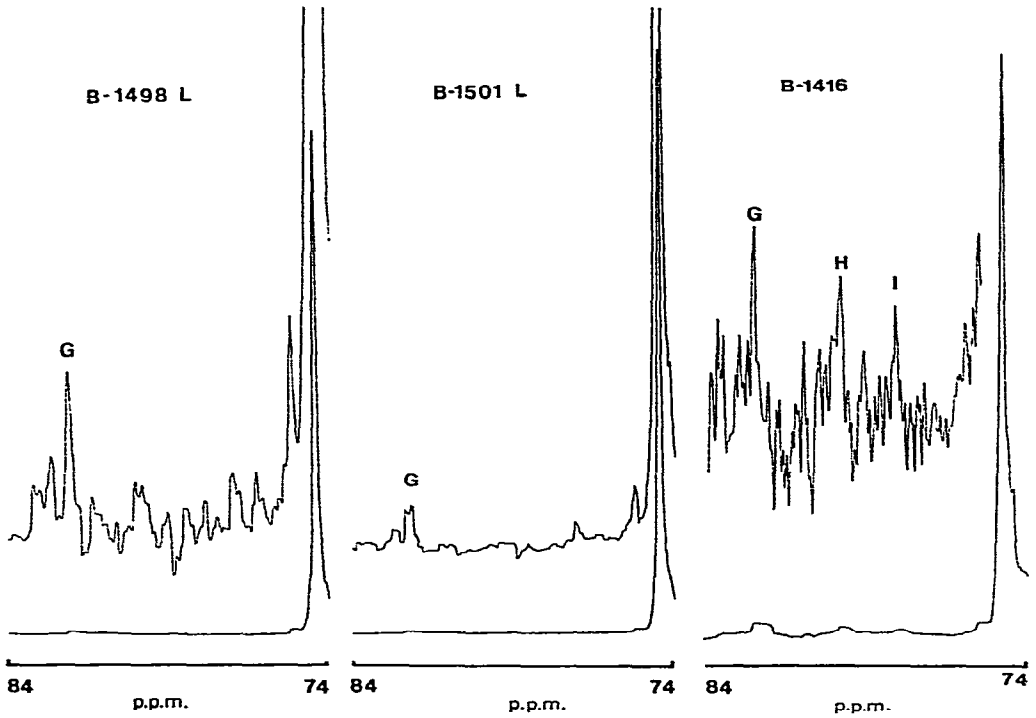


Fig. 4. The expanded, 74–84-p.p.m. region of the ^{13}C-n.m.r. spectra at 90° of dextran B-1498 fraction L, dextran B-1501 fraction L, and dextran B-1416. The symbols G, H, and I refer to diagnostic resonance-regions for dextrans respectively branching through 3,6-, 4,6-, and 2,6-di-*O*-substituted α-D-glucopyranosyl residues. The upper traces are at ten-fold magnifications of amplitude.

residues. The resonance designated I (78.34 p.p.m.) in this spectrum is actually an unknown feature, being near, but not equivalent, to the 77.5-p.p.m. resonance associated with 2,6-di-*O*-substituted residues. However, this expanded region of the dextran B-1416 spectrum clearly shows the ease of observation of the various diagnostic resonances in the presence of each other. The relatively complicated 75–85-p.p.m. region of dextran B-1416 further emphasizes the fact that all other dextrans thus far examined display only one type of branching, and not mixtures of linkages through the C-2, C-3, and C-4 atoms. On the basis of this spectrum, it is not feasible to distinguish between the possibility that dextran B-1416 represents (*a*) a class of polysaccharides having approximately equal proportions of 3,6-di-*O*-substituted and 4,6-di-*O*-substituted residues, or (*b*) a mixture of two dextrans of single branch-type.

The chemical shifts (see Table II) are shown as essentially identical for the S-type dextran fractions B-1355, B-1498, and B-1501, except for the slight displacement of the 99.75-p.p.m. resonance for the B-1355 fraction S. Expanded-scale spectra for these three S-type fractions, shown in Figs. 2 and 3, emphasize the fundamental similarities, and reveal minor idiosyncrasies that relate to the relative percentages of structural residues present (see Table I). Expansion of the 68–84-p.p.m. spectral region of dextran B-1501 fraction S (see Fig. 3), and processing of the spectrum

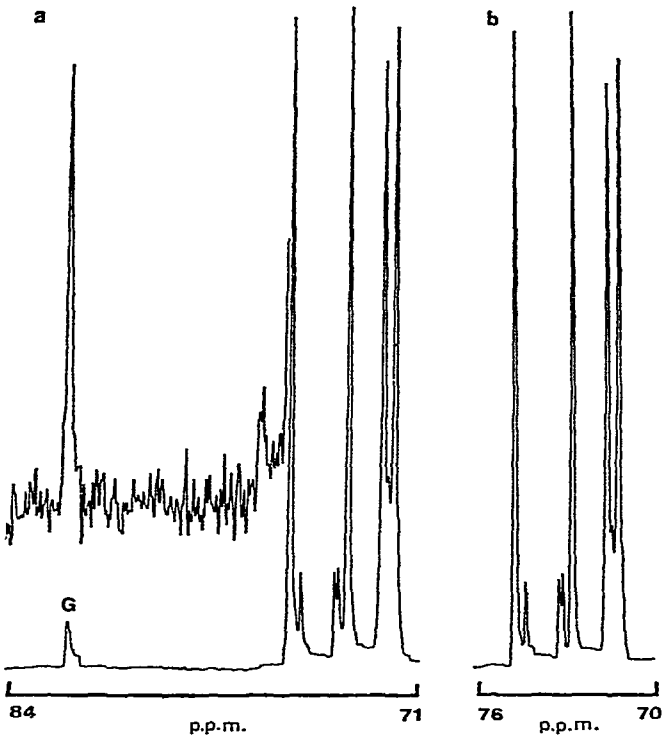


Fig. 5. The expanded, ^{13}C-n.m.r. spectrum at 90° of dextran B-1351 fraction S: (a) the 71–84-p.p.m. region before c.d.r.e. processing, and (b) the 70–76-p.p.m. region of the same after c.d.r.e. processing. The upper trace (for part a) is at a ten-fold magnification of amplitude. The symbol G, indicates the 83.8-p.p.m. resonance, diagnostic of 3,6-di-*O*-substituted, branch-point residues.

by convolution-difference resolution-enhancement (c.d.r.e.)[23], shows a strong "C" resonance at 73.17 p.p.m., accompanied by weak resonances at 72.41 and 73.50 p.p.m. The c.d.r.e. treatment also clearly shows the additional resonances at 73.32, 74.74, and 74.93 p.p.m.

The profile of the spectrum for dextran B-1498 fraction L, shown in Fig. 1, is equivalent to those[5] for the L-fractions of dextrans B-1501 and B-1355, and shows only the six peaks, A through F, of (1→6)-linked α-D-glucopyranosyl residues. However, on recording the spectra of the B-1498 and B-1501 fractions for a prolonged period (~40,000 acquisitions at 90°), high signal-to-noise spectra were obtained which allowed meaningful, vertical-scale expansion that revealed other resonances, shown in Table II. Ten-fold scale-expansion plots of the 74–84-p.p.m. region of these L fractions (see Fig. 4) reveal a weak "G" resonance at 82.9 p.p.m. for the B-1498 fraction L, indicative of less than one (1→3)-linked branch-point per 20 D-glucosyl residues; this "G" resonance is weaker still for dextran B-1501 fraction L. A high signal-to-noise spectrum (90°) and a ten-fold amplitude magnification of the 71–84-p.p.m. region of the control dextran B-1351 fraction S (see Fig. 5) also shows the resonance at 82.9 p.p.m., designated "G", which is indicative of (1→3)-branching.

No evidence appears of (1→2)-branching (77.8 p.p.m.) or (1→4)-branching (80.2 p.p.m.) for dextran B-1351 fraction S. Comparison of Figs. 3 and 4 shows that the well resolved, c.d.r.e.-processed spectra of dextran B-1351 fraction S and dextran B-1501 fraction S contain distinctly different, minor resonances. The minor resonances of dextran B-1501 fraction S (see Table II) intensify as the polysaccharide structure further deviates from that of linear dextran (*e.g.*, dextran B-1254 fraction L [$]), and ultimately dominate the spectrum of dextran B-1355 fraction S. In like manner, the minor resonances of the c.d.r.e.-processed spectrum of dextran B-1351 fraction S (see Fig. 5) are found[5] in the ^{13}C-n.m.r. spectrum of dextran B-742 fraction S (a dextran having a very high degree of branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues) and in those of the L fractions of dextrans B-1498 and B-1501 (see Table II). The spectrum of dextran B-1355 fraction L is similar to those of the L fractions of the foregoing dextrans, as it contains only resonances A through F; however, we have not recorded a high signal-to-noise spectrum for this dextran, and are not certain about the presence of small proportions of branching residues.

These spectroscopic observations accord with other structural analyses on these L-type fractions. Thus, methylation analysis[3] of dextran B-1355 fraction L shows the presence of 95% of (1→)- and (1→6)-linked residues, ~4% of C-3 branch residues, and ~1% of C-3 linearly-linked residues (see Table I). Methylation analyses have not been made for the fraction L dextrans B-1498 and B-1501. However, periodate-oxidation analysis[7] indicated 91% of (1→)- and (1→6)-linked residues in B-1498 fraction L, and 93% in B-1501 fraction L. The estimated, corrected, periodate-oxidation values[1] are 92% and 94%, respectively. Unlike the spectroscopic analysis (see Table II and Figs. 1 and 4), periodate-oxidation analyses[7] for these two dextran fractions indicated the non-(1→6)-linkages to be (1→2), or (1→4), or both; the cause of this apparent inaccuracy in periodate-oxidation data has been explained[1]. The ^{13}C-n.m.r. spectra of the two L-type fractions (see Table II and Fig. 5) do not indicate the presence of (1→2)- or (1→4)-linked residues.

TABLE III

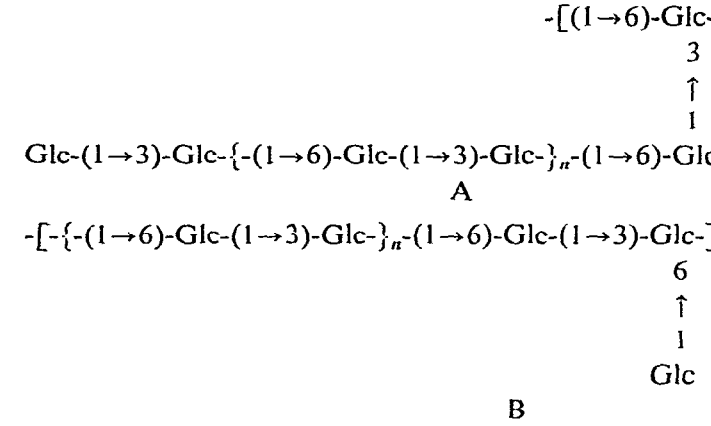
MOLAR RATIOS OF METHYLATED D-GLUCOSE DERIVATIVES IN THE HYDROLYZATES OF PERMETHYLATED DEXTRANS[a]

NRRL strain	*Dextran fraction*	*Methyl ethers of* D-*glucose*				[b](*b*−*c*)−*1*	^{13}*C-n.m.r. anomeric ratio*[c]
		2,3,4,6	*2,3,4*	*2,4,6*	*2,4*		
		a	*b*	*c*	*d*	*e*	*f*
B-1355	S	1	6.8	1.6	1.6	0.7	0.7
B-1498	S	1	5.5	3.1	1.1	1.4	1.5
B-1501	S	1	8.3	3.4	1.2	3.9	2.7

[a]Data taken from Table II. [b]The value in column *b* less the value in column *c* less unity. This value is designated *p* for structure C. [c]The height of the resonance at 99.5 p.p.m. divided by the height of the resonance at 99.9 p.p.m.

On the basis of the foregoing discussion, it is therefore concluded that the ^{13}C-n.m.r. spectra at 25 MHz of aqueous solutions of D-glucans containing 3-mono- and 3,6-di-*O*-substituted α-D-glucopyranosyl residues display distinguishable, diagnostic resonances in the 70–75-, 75–85-, and 90–105-p.p.m. spectral-regions. Moreover, as previously noted[4], the resonances in the 60–70-p.p.m. region (C-6 atoms) are insensitive to minor, structural differences.

Structural formulas of repeating units, and correlation with spectroscopic data. — The molar ratios of the various types of D-glucosyl residues present in the original dextran fractions (see Tables I and III) may be calculated from the methylation data, and suggest the nature of the general repeating units of these D-glucans. We have previously discussed dextran B-1355 fraction S with regard to the literature and to our methylation analysis[2]. On the basis of that information, two possible, average repeating-unit structures



(A and B) having all residues α-D-linked were proposed. These structures constitute an attempt to accommodate the general observations that dextran B-1355 fraction S contains high proportions of α-D-glucopyranosyl residues that have only (1→3)- or (1→6)-linkages, as well as a smaller proportion of branch-point residues that carry both (1→3)- and (1→6)-bonds. In addition, selective acetolysis[15] indicated that many of these (1→3)- and (1→6)-linked D-glucosyl residues alternate successively in the polymer chain. Therefore, a major unresolved point, which we have attempted to illustrate graphically in the proposed repeating-unit structures A and B, is whether the side chains are long or short; the structures shown represent the possible extremes of long (structure A) and short (structure B) side-chain units. At present, we know of no data for dextran B-1355 fraction S that cannot be accommodated by general structures of type A or B. However, the additional data on dextrans B-1498 fraction S and dextran B-1501 fraction S provide several new points for consideration. Firstly, the ^{13}C-n.m.r. spectra of the S fractions of dextrans B-1355, B-1498, and B-1501 contain identical resonances, unique to this series of dextrans, that differ only in intensity. Secondly, these unique resonances tend to maintain a constant relationship of intensity to one another, but the relative contribution changes for resonances

attributable to (1→6)-linked residues of linear-type dextran. On this basis, we conclude that these S-dextran fractions are representative examples of a specific, general type of α-D-glucan. A general repeating unit, C, can now be proposed that is similar

$$-[-\{-(1\rightarrow6)\text{-Glc-}(1\rightarrow3)\text{-Glc-}\}_n\text{-}(1\rightarrow6)\text{-Glc-}(1\rightarrow3)\text{-Glc-}]_x-$$
$$\begin{array}{c} 6 \\ \uparrow \\ 1 \end{array}$$
$$\underset{C}{\text{Glc-}\{-(1\rightarrow6)\text{-Glc-}\}_p\text{-}(1\rightarrow6)\text{-Glc}}$$

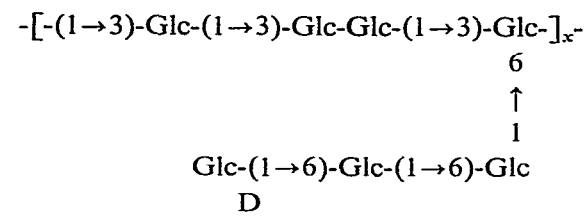
to repeating unit A. In C, all residues are α-D-linked. For dextran B-1355 fraction S, $n = 5.1$ and $p = 0.7$; for dextran B-1498 fraction S, $n = 3.1$ and $p = 1.4$; and for dextran B-1501 fraction S, $n = 3.4$ and $p = 3.9$. The schematic, repeating unit C places all (1→3)-linked α-D-glucosyl residues in the backbone chain, alternating with (1→6)-linked α-D-glucosyl residues. "Excess" (1→6)-linked α-D-glucosyl residues are then assigned to the side chains.

The data from Table III may be correlated to structure C in the following way. It is recognized that, for each polymer, the general repeating unit contains one terminal group, represented by the proportion of 2,3,4,6-tetramethyl ether, and this proportion has been normalized to unity (Table III, column a). As it is assumed that all (1→3)-linked, α-D-glucosyl residues are incorporated in the backbone chain, and that one of these residues is present in each (bracketed) backbone-chain disaccharide, the value n for structure C is expressed for each dextran fraction by the value given in column c of Table III. The extra, (1→6)-linked, α-D-glucosyl residues are then assigned to the side chain; this value p is established by first subtracting the proportion of (1→3)-linked residues (column c) from the proportion of (1→6)-linked residues (column b), which indicates the proportion of (1→6)-linked residues not incorporated into the $(\text{disaccharide})_n$. Structure C has one (1→6)-linked, backbone residue outside the $(\text{disaccharide})_n$ units, to maintain an alternation of (1→3)- and (1→6)-linked residues. Therefore, the side-chain value p will equal the value in column b less that in column c less unity (see Table III).

The foregoing, general repeating-unit accords with both the permethylation and ^{13}C-n.m.r. data. If large proportions of (1→3)-linked α-D-glucosyl residues alternate with (1→6)-linked residues, the expected result would be a non-dextran, ^{13}C-n.m.r. pattern. On elevating the recording temperature for ^{13}C-n.m.r. spectroscopy, the side chains gain additional motion relative to the backbone chain. This motion narrows the n.m.r. signals of (1→6)-linked α-D-glucopyranosyl residues in the side chains, and allows them to dominate the 90° spectra. Greater mobility of the (1→6)-linked α-D-glucosyl residues is in agreement with the larger-than-average T_1 values associated with their resonances* at 90°. The remaining two anomeric resonances,

*F. R. Seymour and R. D. Knapp, unpublished data.

at 99.9 and 101.1 p.p.m., are of major importance for the choice of structure C, because they, as well as peak A, occur for all S fractions as narrow and well-defined peaks. For the fractions S of B-1498 and B-1501 dextrans, where no acetolysis was performed (as for fraction S of dextran B-1355), this anomeric pattern excludes any major contribution from a sub-unit of structure D, in



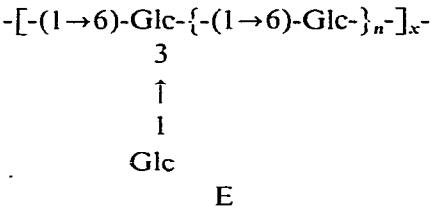
which all residues are α-D-linked. Should appropriate lengths be chosen for exclusively (1→3)-linked, α-D-glucosyl backbone residues and for exclusively (1→6)-linked α-D-glucosyl side-chain residues for each S fraction, structure D would accord with the methylation and ^{13}C-n.m.r. data in all but one respect: the ^{13}C-n.m.r. of a D-glucan composed of the foregoing repeating unit would be expected to have only two dominant, anomeric resonances, namely, a resonance resulting from the chain of (1→6)-linked α-D-glucosyl residues (peak A) and an additional, single resonance of similar intensity resulting from the chain of (1→3)-linked α-D-glucosyl residues. As this ^{13}C-n.m.r., anomeric pattern is not observed, repeating units of the type of structure D do not contribute to the spectra of the S fractions of dextrans B-1355, B-1498, and B-1501. It had been observed that pullulan has three anomeric resonances[4,24]. Two of these anomeric resonances of pullulan were attributed to the (1→4)-linked α-D-glucosyl residues which precede and follow the (1→6)-linked α-D-glucosyl residues of the linear chain of pullulan. Lack of this type of anomeric resonance splitting in the spectra of any of the dextran S-fractions further suggests that the chains contain single, (1→3)-linked residues alternating strictly with (1→6)-linked α-D-glucosyl residues.

A final point should be considered for these S fraction spectra in relation to the proposed, general repeating unit C: for the S fractions of dextrans B-1498 and B-1501, when the percentage of (1→3)-linked α-D-glucosyl residues is subtracted from the percentage of (1→6)-linked α-D-glucosyl residues, there remain more (1→6)-linked α-D-glucosyl residues than 3,6-di-*O*-substituted D-glucosyl residues, and this implies that the side chains shown in structure C are more than one D-glucose residue long. If these excess, (1→6)-linked, α-D-glucosyl residues are indeed incorporated into long-chain side-groups, the data for dextran B-1501 fraction S would indicate an average chain-length of 5 D-glucosyl residues. The accumulated ^{13}C-n.m.r. data for D-glucans indicate that the chemical shifts, especially the anomeric resonances, are quite susceptible to change when their associated D-glucosyl residues are in different environments. On this basis, it could be expected that the (1→6)-linked α-D-glucosyl residues of a side chain would exhibit a series of resonances centered around 99.5 p.p.m., an effect not observed for these D-glucans. Such an effect does not have

to occur; however, this possibility points out that additional studies will be necessary before the extra, (1→6)-linked, α-D-glucosyl residues are unambiguously assigned to side chains.

A general check on the value of *p* (see structure C and Table III) may be obtained from the ^{13}C-n.m.r. spectra by taking the ratio of the peak height of the 99.9-p.p.m. resonance to that of the 99.5-p.p.m. resonance for each S fraction dextran. Fortuitously, this ratio for dextran B-1355 fraction S is ~0.7; consequently, these ^{13}C-n.m.r. ratios, given in Table III, column f, may be directly compared to the *p* values established by methylation analysis (given in column e). This general, linear relationship between permethylation data and relative ^{13}C-n.m.r. resonance-intensity parallels a similar relationship observed for the diagnostic, branching resonances for dextrans containing 2,6-di-*O*-substituted α-D-glucosyl residues[6].

The general structure of the L-type fractions of dextrans B-1355, B-1498, and B-1501 deviates from that of a strictly linear dextran by low degree of branching through 3,6-di-*O*-substituted residues. These dextrans may be represented by the general structure E (in which all residues are α-D-linked), in a manner analogous to that previously proposed[6] for dextrans that branch through O-2. The *n* values for



E

these C-3 branched dextrans are ~20, as has been indicated in the discussion of their ^{13}C-n.m.r. spectra; in contrast, the corresponding values for the C-2 branched dextrans are almost all near 5 (or less)[6].

Correlation of ^{13}C-n.m.r. data with immunochemical data reported for dextrans. — Several observations have been made on the unusual properties of the S fractions of dextrans B-1355, B-1498, and B-1501 (especially for dextran B-1355 fraction S); these fractions have similar properties that are distinct from those of all other dextrans. The high degree of interaction of dextran B-1355 fraction S with concanavalin A has been studied by Goldstein[25], and this structure–function relationship has been discussed[2]. Immunochemical studies have consistently shown that a close relationship exists between the S fractions of dextrans B-1355, B-1498, and B-1501. Allen and Kabat[26] demonstrated that antisera $326D_3$ and $327D_3$, which are both anti-(dextran B-1355 fraction S)-sera, precipitate well with all three S dextran fractions (see Fig. 1 of ref. 26). We have previously discussed the dextran immune-response for dextrans branching through the 2,6-di-*O*-substituted α-D-glucopyranosyl residue, and concluded that the data are more readily interpretable in terms of specific residue-types than in terms of specific linkage-types[6]. For antiserum $326D_3$, large precipitates were formed with the S fractions of dextrans B-1355, B-1498, and B-1501, and much less precipitation occurred with dextrans B-512 and B-742 C3R (fraction S); the former dextrans are known

to contain large percentages of 3-mono-*O*-substituted α-D-glucopyranosyl residues, and the latter to contain 3,6-di-*O*-substituted α-D-glucopyranosyl residues. These results agree with the concept of immune response to residue-type. Such an approach assumes that compounds containing similar linkage-types could give a similar immunochemical response, but that this response would be greater between compounds having identical residue-types. For example, a similar anti-(dextran B-1355 fraction S)-serum, antiserum $326D_4$ (see Fig. 2 of ref. 26) is much more effectively inhibited by nigerose than by disaccharides that do not contain an α-D-(1→3)-linkage. However, we propose that an oligosaccharide containing an α-D-(1→3)-linked D-glucopyranosyl residue would be an even more effective inhibitor for antiserum $326D_4$. Torii *et al.*[27] performed further inhibition assays on antisera $326D_3$ and $327D_4$ with oligosaccharides containing 3,6-di-*O*-α-D-glucosyl-substituted D-glucopyranosyl residues; these gave only about the same degree of inhibition as nigerose (see Fig. 2 of ref. 27), suggesting that this substituted residue is not the favored residue.

An interesting, residue-type application of the immune response can be achieved with data published for a mouse-myeloma protein[28]. These studies were performed with NRRL dextrans, the mouse-myeloma protein (J558), and Lat_2 antiserum [an anti-(dextran B-1355 fraction S)-serum]. The J558-protein precipitation-interaction with NRRL dextrans (see Fig. 1 of ref. 28) was quite similar to that previously observed for antisera $326D_3$ and $327D_3$ (see Fig. 1 of ref. 28). We have established the structures for most of the dextrans studied with the J558 protein, and it is apparent that the latter reacts more with the S fractions of dextrans B-1355, B-1498, and B-1501 (which contain large proportions of 3- and 6-mono-*O*-substituted α-D-glucopyranosyl residues, and lesser proportions of 3,6-di-*O*-α-D-glucopyranosyl-substituted α-D-glucopyranosyl residues) than with NRRL dextrans which collectively contain large proportions of α-D-glucopyranosyl residues that are substituted by α-D-glucopyranosyl residues at O-6, O-2 and O-6, O-3 and O-6, and O-4 and O-6. Inhibition studies of the J558 protein (with dextran B-1498 fraction S) showed that nigerose is a better inhibitor than other non-α-D-linked disaccharides. However, nigero-oligosaccharides inhibited to a somewhat greater extent than nigerose (see Fig. 2 of ref. 28). Nigerose contains an α-D-(1→3)-linkage, but no α-D-(1→3)-linked D-glucopyranosyl residue or (only a 3-mono-*O*-substituted D-glucose residue). However, nigerotriose contains an α-D-(1→3)-linked D-glucopyranosyl residue, as do the higher nigero-oligosaccharides. As nigerotriose gives only 60% inhibition, and the higher nigero-oligosaccharides do little better, it is possible that the complete recognition-site is not defined. The J558 protein interacts well with such dextrans as dextran B-1355 fraction S, and the foregoing ^{13}C-n.m.r. data suggest that these dextrans are composed of alternating 3- and 6-mono-*O*-substituted α-D-glucopyranosyl residues. This structure, in turn, suggests that the recognition site of the J558 protein is not only a 3-mono-*O*-substituted α-D-glucopyranosyl residue, but such a residue bracketed on one side (or both sides) by a 6-mono-*O*-substituted α-D-glucopyranosyl residue. Significantly, the J558 protein shows little inhibition by an oligomer containing 3,6-di-*O*-substituted α-D-glucopyranosyl residues.

Inhibition with the different nigero-oligosaccharides was also studied (with dextran B1355 fraction S) in a dextran–human antidextran system, namely, Lat_2 [anti-(dextran B-1355 fraction S)-serum], by using an antiserum made more specific by removing the 6-mono-*O*-substituted α-D-glucopyranosyl activity[28]. For this system, nigerose was a poorer inhibitor than for the J558-protein study, but oligosaccharides containing 3,6-di-*O*-substituted α-D-glucopyranosyl residues were excellent inhibitors (see ref. 28, Fig. 3). This inhibition by 3,6-di-*O*-substituted α-D-glucopyranosyl residues could be expected, as dextran B-1355 fraction S contains ~10% of these residues.

All branched dextrans contain terminal α-D-glucopyranosyl groups, and, if antisera activity can be directed toward specifically linked residues, it is possible that such activity could be directed toward such nonreducing, terminal groups. However, if such effects are present, they would be difficult to detect in immunological studies that cross-reference dextrans. For every branch-point residue in a given dextran, there exists a corresponding (nonreducing) terminal D-glucopyranosyl group. Such nonreducing, terminal groups are common to all dextrans, and their extent is

TABLE IV

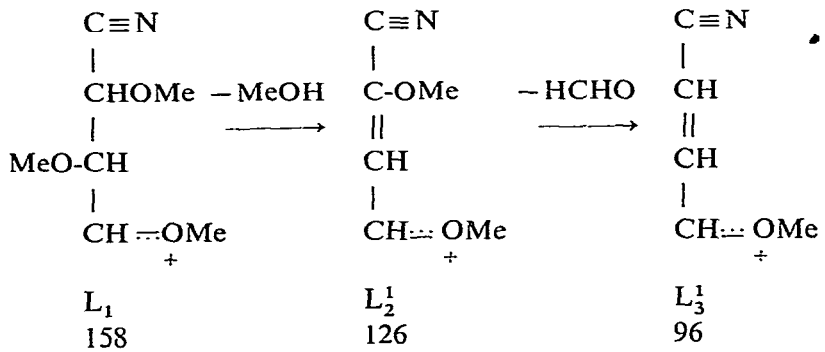
FRAGMENT IONS OBTAINED FROM ELECTRON IMPACT MASS SPECTROMETRY OF PAAN DERIVATIVES OF *O*-METHYL-D-GLUCOSES

D-*Gluconitrile methylated in positions*			
2,3,4,6[a]	*2,4,6*	*2,3,4*	*2,4*
205 (0,3) B_1	186 (1,3) G_1	233 (0,6) J_1	189 (0,6) K_1
186 (1,1)	161 (0,3) A_1	189 (0,6) K_1	186 (1,3) G_1
161 (0.3) A_1	159 (0,3) G_2^1	173 (0,3) J_2^2	159 (0,3) G_1^2
158 (1,0) L_1	154 (1,3) G_2^2	158 (1,0) L_1	154 (1,3) G_2^2
145 (0,0) B_1^2	129 (0,3) K_2^1	129 (0,3) K_2^1	129 (0,3) K_2^1
129 (0,3) A_2^3	126 (1,0) G_2^3	126 (1,0) L_2^1	126 (1,0) G_2^3
126 (1,0) L_2^1	112 (1,1) G_3^2	113 (0,0) J_3^3	112 (1,1) G_3^2
119 (0,1) A_2^2	101 (0,0) A_2^1	101 (0,0)	103 (0,3)
117 (0,3)	99 (0,0) G_3^1	99 (0,0)	99 (0,3) G_3^1
114 (1,0)	96 (1,0) G_3^3	96 (1,0) L_3^1	96 (1,0) G_3^3
113 (0,0) B_1^3	87 (0,1) A_3^3	87 (0,1)	87 (0,1) K_3^1
101 (0,0) A_2^1	74 (0,1)	73 (0,1)	74 (0,1)
96 (1,0) L_3^1	71 (0,0) A_3^1	71 (0,0) A	45 (0,0) D_1
88 (0,0) C_1	45 (0,0) D_1	45 (0,0) D_1	43 (0,3)
87 (0,1) A_3^3	43 (0,3)	43 (0,3)	
71 (0,0) A_3^1			
45 (0,0) D_1			
43 (0,3)			

[a]For each column the first number is the *m/e* value. The first number in parentheses is the increase in *m/e* value for the ^{15}N analog. The second number in parentheses is the increase in the *m/e* value for the perdeuterioacetylated analog. The letter indicates the structure previously assigned to the fragment ion (see ref. 9).

proportional to the degree of branching for a specific dextran. It should also be noted that this general approach to analysis of data in terms of specifically substituted saccharide residues, rather than in terms of specific types of linkage, can also have direct application for correlating spatial requirements of substrates to enzyme specificity (as a function of sequences of enzyme subsites)[29].

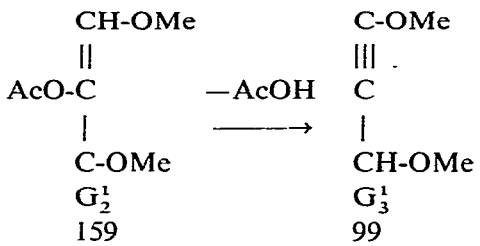
Isotopic substitution in PAAN derivatives. — The hydrolyzate of permethylated dextran B-1501 fraction S was successively derivatized with $^{15}NH_2OH$ and deuterioacetic anhydride. Results of combined g.l.c.–e.i.m.s. of these isotopic variants of the PAAN derivatives are summarized in Table IV. Relative intensities of the *m/e* values in Table IV are the same as those previously reported for the non-isotopically substituted PAAN derivatives[9]. Fragmentation pathways have previously been proposed for these compounds; the letters in Table IV refer to the specific fragment-ions assigned to these *m/e* values. The compounds studied were: 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-gluconononitrile (**1**), 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methyl-D-gluconononitrile (**2**), 5,6-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-gluconononitrile (**3**), and 3,5,6-tri-*O*-acetyl-2,4-di-*O*-methyl-D-gluconononitrile (**4**). Although the compounds reported here are D-glucose derivatives, as contrasted with the previously reported D-mannose derivatives[9], it has been shown that such analogous, specific PAAN derivatives give identical e.i. mass spectra[1]. These data confirm all fragment ions previously postulated in terms of the presence of the nitrile group (which increases the *m/e* value by unity if this group is present with ^{15}N substitution) and for the content of acetyl groups (which increase the *m/e* value by 3 for each acetyl group present on deuterioacetyl substitution). In addition, when a perdeuterioacetylated fragment-ion has lost an acetyl group as ketene, the *m/e* value of that fragment ion is also increased by one mass unit. Only the fragment ions of even-numbered *m/e* value were postulated to contain the nitrile group, and this assumption has, in general, been confirmed. For compound **1**, the presence of the nitrile group in ions *m/e* 158, 126, and 96 has been established. These ions are assigned to the L fragmentation series which arises from the nitrile end with initial cleavage between C-4 and C-5 (see Scheme 1). The weak ion *m/e* 126 is present only at high concentrations in the ionization chamber.



Scheme 1

The results of substitution with ^{15}N also indicate that *m/e* 114 contains the nitrile group and arises from simple cleavage between C-3 and C-4. Similarly, deuterioacetylation confirms that ion *m/e* 117 contains a single, intact, acetyl group, which arises from simple cleavage between C-4 and C-5, and represents the intact C-5 and C-6 portion of the original molecule.

Both compounds **2** and **4** yield ions of *m/e* 99 and 159, and ion *m/e* 159 has previously been identified as arising from the C-2, C-3, C-4 portion of the parent molecule. Based on isotopic substitution, and its presence in the mass spectra of compounds **2** and **4**, ion *m/e* 99 is identified as an additional member of the G series (see Scheme 2).



Scheme 2

Compound **3** also gives rise to the extended L series which was previously observed and identified in the foregoing discussion of compound **1**. This result was expected, as the portion of the parent molecule giving rise to the L series is identical in compounds **1** and **3**.

Ammonia chemical-ionization mass spectra (*ammonia c.i.-m.s.*). — The chromatograms of the foregoing PAAN derivatives were also examined under ammonia

TABLE V

IONS OBTAINED FROM AMMONIA CHEMICAL-IONIZATION MASS-SPECTROMETRY OF PAAN DERIVATIVES OF *O*-METHYL-D-GLUCOSES[a]

Ion identity	D-*Glucononitrile methylated in positions*		
	2,3,4,6	*2,4,6*[b]	*2,4*[c]
M + 18			349 (35)[d]
M + 1	276 (40)	304 (25)	
M + 1 − 32	244 (35)	272 (35)	
M + 1 − 60	216 (100)	244 (100)	272 (100)
M + 1 − 60 − 32			240 (10)

[a]Compounds obtained from the PAAN derivatized hydrolyzate of methylated dextran B-1501 fraction S. [b]An identical mass-spectral pattern was observed in a similar chromatogram for the 2,3,4-tri-*O*-methyl analog. [c]An identical mass-spectral pattern was observed for the 2,3-di-*O*-methyl analog in the hydrolyzate obtained from permethylated dextran B-742 fraction L. [d]The first number is the *m/e* value of the ion; that in parentheses is a representative, relative intensity for each ion-set.

c.i.-m.s. conditions, the results being tabulated in Table V. Each mass spectrum contained only a few ions. In accord with observations for non-methylated PAAN derivatives[9], the 2,4-di-*O*-methyl derivative, compound **4**, yielded ions representing the parent molecules plus the ammonium ion (M + 18). However, for the 2,3,4,6-tetra- and 2,4,6-tri-*O*-methyl derivatives, compounds **1** and **2**, the M + 1 peak was observed, and no M + 18 peak was present. It had previously been suggested that ammonia c.i.-m.s. yields an M + 18 peak for compounds containing a basic nitrogen atom, and an M + 1 peak for compounds containing no basic nitrogen group[30], and we have observed this relationship to be true for nonmethylated PAAN derivatives[9]. However, Table V indicates that such a relationship does not exist for the highly methylated PAAN derivatives. We have also observed that ammonia c.i.-m.s. is insensitive to positional isomerization; for example, the 2-deoxy and the 6-deoxy D-glucose PAAN derivatives yield identical mass spectra[9]. In like manner, PAAN derivatives having positional isomerism (*e.g.*, the 2,4- and the 2,3-di-*O*-methyl PAAN D-glucose derivatives) yield identical ammonia c.i.-mass spectra. Therefore, ammonia c.i.-m.s. is an excellent method for confirming the molecular weight of the compounds in each peak of the PAAN derivative chromatograms, provided that the change of *m/e* value from M + 18 to M + 1 is recognized. Additional *m/e* values in ammonia c.i.-mass spectra represent the M + 1 ion having undergone loss of acetic acid (60) or methanol (32). In fact, the M + 1 −60 peak dominates all ammonia c.i.-mass spectra, and allows the establishment of the largest *m/e* as representing the M + 1 ion (which is 60 mass units greater) or the M + 18 ion (which is 77 mass units greater). The displacements of the ammonia c.i.-m.s. ions, on isotopic substitution with ^{15}N or deuterioacetyl groups, were in accord with the expected nitrogen or acetyl content of each PAAN derivative. It should be noted that the relative intensity of the ammonia c.i.-m.s. ions can vary by as much as 20% on a spectrum-to-spectrum basis (~5-s intervals). Therefore, the *m/e* intensities listed in Table V are representative values.

EXPERIMENTAL

Preparation and characterization of the dextrans[7] and dextran fractions[8] have been reported. Previously described methods[2,3,31] were used for the methylation of the dextrans, and for structural analysis by combined g.l.c.–e.i.-m.s. of the per-acetylated aldononitriles. The conditions for ammonia c.i.-m.s. were as previously described; however, the tuning of the mass spectrometer for these conditions was performed as follows. First, the spectrometer was tuned to optimum e.i.-m.s. conditions by using PTBA as a standard. The spectrometer was then changed to the ammonia c.i. mode, and the pressure of the ammonia was adjusted until the non-methylated D-glucose PAAN derivative yielded a spectrum having the M + 18 and M + 1 −60 *m/e* values at equal intensity. All mass spectra were recorded with a Hewlett–Packard 5980A GC/MS integrated, g.l.c.–m.s., computer system. The g.l.c. peak-integrals reported in Table I were obtained with a Barber–Coleman Series

5000 g.l.c. instrument equipped with hydrogen-flame detectors. On-column injection of glass columns (2 mm i.d. × 1.23 m) was employed for all such chromatography.

The ^{13}C-n.m.r. conditions and methods for preparation of dextran samples have been described[4]. In general, a Varian XL-100-15 spectrometer equipped with a Nicolet TT-100 system was employed in the Fourier-transform mode. Chemical shifts are expressed in p.p.m. relative to external tetramethylsilane, but were actually calculated by reference to the lock signal.

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