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STRUCTURAL ANALYSIS OF DEXTRANS CONTAINING 4-*O*-α-D-GLUCOSYLATED α-D-GLUCOPYRANOSYL RESIDUES AT THE BRANCH POINTS, BY USE OF ^{13}C-NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY*

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.)

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

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

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ABSTRACT

Dextran fractions from NRRL strain *Streptococcus* sp. B-1526 and the native, structurally homogeneous dextrans from *Acetobacter capsulatum* B-1225, *Leuconostoc mesenteroides* B-1307, and *L. dextranicum* B-1420 were examined by ^{13}C-n.m.r. spectroscopy at 90°. Dextran B-1526 fraction I and dextran B-1420 were also examined by g.l.c.–m.s., methylation-structural analysis. All of these dextrans and dextran fractions branch, either primarily or exclusively, through α-D-(1→4)-glucopyranosyl linkages; however, their degrees of branching differ. Several ^{13}C-n.m.r. resonances that are diagnostic for 4,6-di-*O*-substituted α-D-glucopyranosyl residues have been identified. Comparison was made with dextrans from *L. mesenteroides* B-742 fraction L and *Streptobacterium dextranicum* B-1254 fraction S[L], for which previously published, methylation-structural analyses had established the presence of 4,6-di-*O*-substituted α-D-glucopyranosyl residues at the branch points. These

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*Unusual Dextrans, Part XI. For Part X, see ref. 1.
**Present address: Department of Zoology, Iowa State University, Ames, Iowa 50011, U.S.A.

methylations also indicate small proportions of 3-mono-*O*-substituted residues in dextran B-742 fraction L, and of 4-mono-*O*-substituted residues in dextran B-1254 fraction S[Ł]. The authenticity of regarding these residues as integral parts of the respective dextrans remains to be proved. The occurrence of α-D-glucopyranosyl residues at O-4 of other dextrans is reviewed.

INTRODUCTION

We have previously reported the ^{13}C-n.m.r. spectra of numerous unusual members of the dextran class of α-D-glucans[2–5]. Among these dextrans are a series branching through 2,6-di-*O*-substituted α-D-glucopyranosyl residues[4] and a series branching through 3,6-di-*O*-substituted residues and also containing 3-mono-*O*-substituted α-D-glucopyranosyl residues[5]. For each of these types of α-D-glucopyranosyl residue, characteristic ^{13}C-n.m.r. resonances have been found that are diagnostic of the presence of these residues in dextrans. For the dextran series containing 2,6-di-*O*-substituted branch-point residues, correlation of ^{13}C-n.m.r. spectra to the results of methylation-structural analysis indicated that the intensity of the diagnostic branching-resonances, relative to the resonances normally associated with linear dextran, parallels the degree of linearity established by methylation-structural analysis. Dextran fraction L from *Leuconostoc mesenteroides* B-742 (see ref. 3) and dextran fraction S[Ł] from *Streptobacterium dextranicum* B-1254 (the designation "S[Ł]" is explained in ref. 3) have been shown by methylation-fragmentation analysis to branch through 4,6-di-*O*-substituted D-glucopyranosyl residues. ^{13}C-N.m.r. spectra, previously recorded at 34°, showed certain resonances, both for dextrans B-742 fraction L (see ref. 3) and B-1254 fraction S[Ł] (see ref. 2), that do not appear in the spectra of dextrans that, by methylation-structural analysis, have been shown to be free from 4,6-di-*O*-substituted glucopyranosyl residues. The spectra of these two dextrans, when recorded under our standard conditions, located these characteristic resonances at 101.6, 100.1, and 80.4 p.p.m.

Five additional dextrans have now been selected for further study, on the criterion that a resonance occurs at 80.4 p.p.m. in their ^{13}C-n.m.r. spectra. These are native dextrans, previously prepared and characterized[7,8], from NRRL strains *Acetobacter capsulatum* B-1225, *L. mesenteroides* B-1307, and *L. dextranicum* B-1420, and dextran fractions S and I from *Streptococcus* sp. B-1526. We have observed that spectral resolution is enhanced at elevated recording-temperatures[2], and, for direct comparison with ^{13}C-n.m.r. data for other dextrans, all values reported here, unless otherwise noted, refer to recording conditions in deuterium oxide at 90°.

RESULTS AND DISCUSSION

The ^{13}C-n.m.r. spectra of the five newly-selected dextrans are shown in Fig. 1 and compared with that of dextran B-1254 fraction S[Ł]. The chemical shifts of

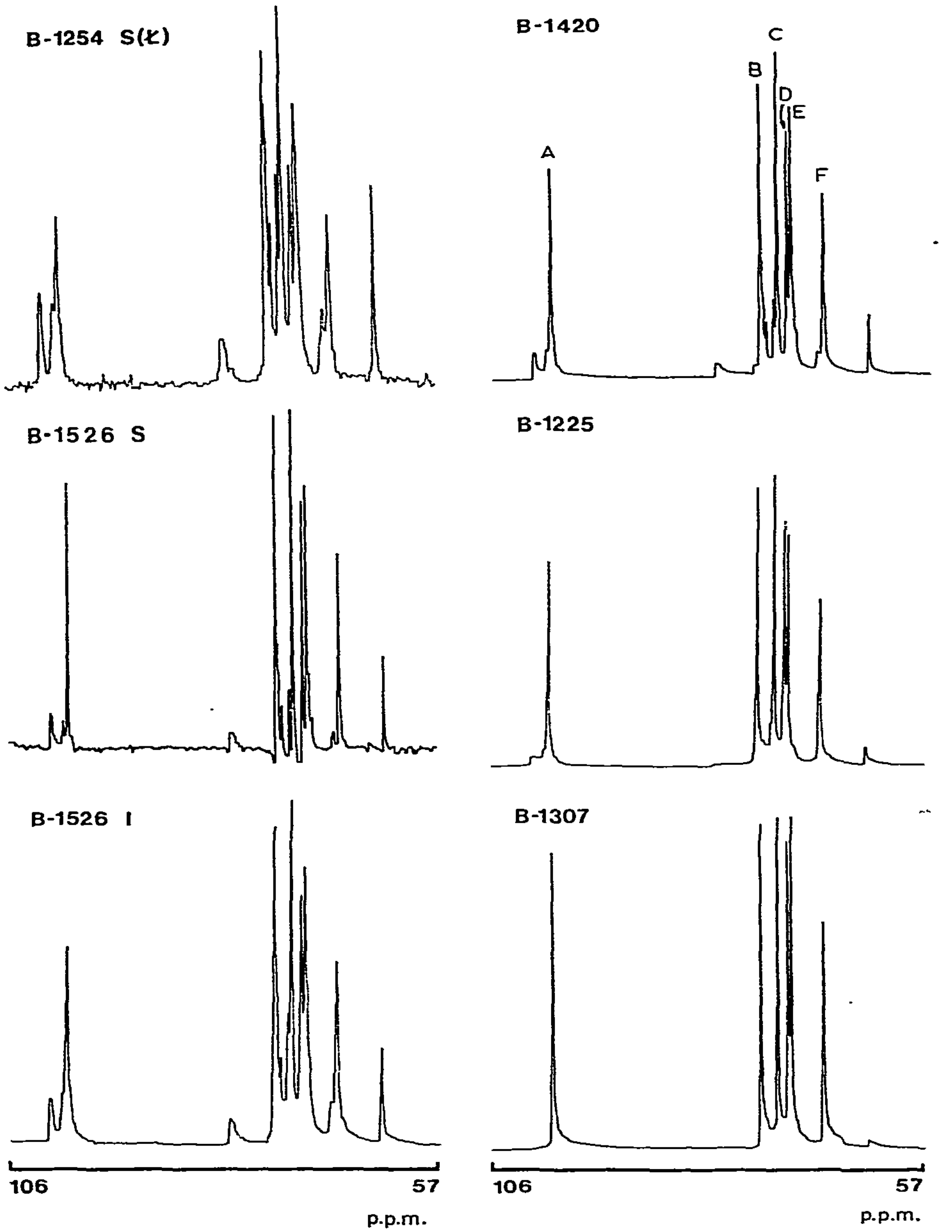


Fig. 1. ^{13}C-N.m.r. spectra at 90° for dextrans B-1254 fraction S[Ł], B-1526 fraction S (spectrum processed by c.d.r.e.), B-1526 fraction I, B-1420, B-1225, and B-1307.

these six dextrans are tabulated in Table I and compared with those of dextran B-742 fraction L.

Each of the seven dextrans in this series displays a ^{13}C-n.m.r. resonance at $\sim$80.4 p.p.m. The results of methylation-structural analysis performed on dextrans

TABLE I

CHEMICAL SHIFTS FOR ^{13}C-N.M.R. SPECTRA[a] OF DEXTRANS CONTAINING 4,6-DI-*O*-SUBSTITUTED α-D-GLUCOPYRANOSYL RESIDUES

Major resonance	*NRRL number of the organism producing the dextran*						
	B-1254	*B-742*	*B-1526*	*B-1526*	*B-1420*	*B-1225*	*B-1307*
	Dextran fraction						
	S[Ł]	*L*	*S*	*I*			
	101.58	101.59	101.59	101.58	101.55	101.61	101.55
	100.07	100.13	100.09	100.07	100.04	100.12	100.86
A[b]	99.42	99.54	99.48	99.48	99.44	99.51	99.48
							82.85[c]
	80.40	80.42	80.49	80.37	80.31	80.42	80.51
	79.32				77.52[c]		
B	75.07	75.17	75.14	75.14	75.10	75.16	75.14
	74.87(s)[d]	74.97(s)	74.96(s)[e]	74.96(s)	74.90(s)	74.97(s)	
	74.37(s)	74.43(s)	74.43(s)	74.41(s)	74.38(s)	74.43(s)	74.42(s)
	73.50(s)	73.53(s)	73.56(s)	73.56(s)	73.52(s)	73.55(s)	73.55(s)
C	73.12	73.16	73.17	73.18	73.12	73.18	73.15
D	71.95	71.99	71.98	71.99	71.92	71.98	71.96
E	71.43	71.58	71.55	71.57	71.52	71.59	71.57
	71.22(s)	71.28(s)	71.26(s)[f]		71.24(s)		
	68.47(s)	68.50(s)	68.47(s)	68.59(s)	68.45(s)	68.53(s)	
F	67.68	67.80	67.75	67.80	67.71	67.80	67.77
	62.43	62.51	62.49	62.51	62.45	62.51	62.50

[a] Spectra recorded at 90°; shifts expressed in p.p.m. relative to tetramethylsilane. [b] The letters A through F refer to the major resonances of linear dextran (see ref. 2). [c] These resonances indicate traces of non-(1→4)-branching. [d] The symbol s designates a shoulder on the major peak. [e] The resonance at 74.96 p.p.m. is followed by a second, less-intense shoulder at 74.55 p.p.m. [f] The resonance at 71.26 p.p.m. is followed by a second, less-intense shoulder at 70.96 p.p.m.

TABLE II

MOLE PERCENTAGE OF METHYLATED D-GLUCOSE 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*	*2,3,6*	*2,3*	*2,4*	*3,4*
Streptobacterium dextranicum	B-1254	S[Ł]	22.1[a]	55.0		3.4	19.5		
Streptococcus sp.	B-1526	I	16.3	62.1			19.3	2.3	
Leuconostoc mesenteroides	B-742	L	14.4[b]	72.5	0.7		12.4		
Leuconostoc dextranicum	B-1420		14.4	71.5			12.0	1.1	1.0

[a] Data taken from ref. 6. [b] Data taken from ref. 9.

B-1420 and B-1526 fraction I are shown in Table II and compared with those for dextrans B-742 fraction L and B-1254 fraction S[L̸]. Dextrans B-1225, B-1307, and B-1526 fraction S were not examined by methylation-structural analysis, because ^{13}C-n.m.r. spectroscopy indicated that (*a*) dextrans B-1225 and B-1307 have low mole-percentages of branching residues (and identified the locus of the branch points), and (*b*) dextran B-1526 fraction S closely resembles fraction I of this strain. The structural features common to these dextrans are discussed in the following section, in order to facilitate later correlation with the ^{13}C-n.m.r.-spectroscopic data.

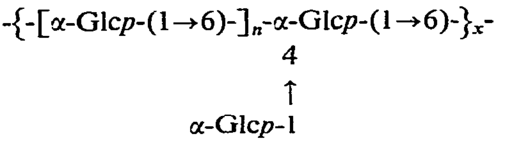
Methylation structural analysis

Evaluation of results obtained. — The results of methylation-fragmentation analysis (see Table II) show, by the high proportion of 2,3-dimethyl ether and the general equivalence of the percentages of 2,3,4,6-tetramethyl ether, that the dextrans named in Table II branch almost exclusively through 4,6-di-*O*-substituted D-glucopyranosyl residues. These dextrans constitute a series, and, in the order enumerated in Table II, show a decreasing degree of branching, except that, for dextrans B-742 fraction L and B-1420, the percentages of the various methyl ethers are essentially identical. However, the disparity in the solubility, viscosity, and gum characteristics[7] of dextrans B-742 fraction L and B-1420 indicates that significant differences exist in their structural detail.

The relative degree of branching in the dextrans reported in Table II has also been calculated by digital integration of the anomeric resonances of the ^{1}H-n.m.r. spectra[1]. On this basis, the order of decreasing degree of branching in these dextrans is B-1254 fraction S[L̸], B-742 fraction L, B-1526 fraction I, and B-1420. This order accords with that indicated by the percentage of maltose isolated[10] from acetolyzates of these dextrans, and is compatible with the physical properties of dextrans B-742 fraction L and B-1420. The lower intensity of the diagnostic resonances observed in the ^{13}C-n.m.r. spectrum of dextran B-1420 is also in agreement with the dextran B-1420 being less branched than dextran B-742 fraction L. Because of the consistency of the indications from these four other types of observation on the relative order of linearity of dextran B-742 fraction L, this order, rather than that obtained from methylation-structural analysis, is used in the tabulation that follows.

Of possible import to the relationship of data from methylation-fragmentation analysis (which employs several, intermediate dialyses) to other structural-analysis data is an observation made on the unusually sensitive papergram that analyzes the action of isomaltodextranase from *Arthrobacter globiformis* T6 on these dextrans[11]. Bound D-glucose has been shown to be present in the dextrans, but the free sugar found is not a product of this enzymolysis[11], the proportion being relatively small for dextran B-742 fraction S, and larger for dextran B-1420. Apparently, this free D-glucose had not been removed from the dextrans by the purification procedures[7,8], which did not include dialysis as a final step. The presence of this contaminant would also increase the percentage of formic acid obtained by periodate-oxidation analysis and, thereby, increase the apparent content of (1→6)-like linkages. However, we have not thus far observed in the ^{13}C-n.m.r. spectra of any dextran any resonances that can be attributed to free D-glucose.

The main structural features of this series of dextrans that branch through 4,6-di-*O*-substituted α-D-glucopyranosyl residues, as revealed by methylation-structural analysis (see Table II) are depicted in structure *A*, where *n* is the average number



A

$$\text{-}\{\text{-}[\alpha\text{-Glc}p\text{-}(1\rightarrow6)\text{-}]_n\text{-}\underset{\substack{\uparrow \\ \alpha\text{-Glc}p\text{-}1}}{\overset{}{\alpha\text{-Glc}p}}\text{-}(1\rightarrow6)\text{-}\}_x\text{-} \quad (\text{branch at } 4)$$

of linearly (1→6)-linked residues in the main backbone chain between branch points; for dextran B-1254 fraction S[L′], $n = 2.5$; for dextran B-742 fraction L, $n = {\sim}5$; for dextran B-1526 fraction I, $n = 3.8$; and for dextran B-1420, $n = 5.0$. This designation of *n* is based on the assumption that all branches are one residue long, that is, that no (1→6)-linked residues occur in the side chains. Numerically, *n* is the ratio of the percentages of 2,3,4-trimethyl to 2,3,4,6-tetramethyl ether derivatives that appear in the chromatograms of the hydrolyzates of the methylated polymers. In this sense, *n* is a measure of the linearity of the dextran.

General confirmation for the data reported here for dextran B-742 fraction L is provided by the results of a study based on preliminary methylation analysis and examination of the sugar fragments liberated by partial hydrolysis of the methylated dextran with acid[12,13]. The conclusions were (*a*) the repeating-unit value is 5 (thus, *n*, as defined in our work, is 3), (*b*) the branch-point attachments are at O-3 and O-4, in the ratio of ~1:2, and (*c*) branches one unit long predominate. These data indicate that the proportion of residues having an O-3 link is significantly higher than the ~1:18 molar ratio of 2,4,6-tri- to 2,3-di-methyl ether from Table II. The ratio 1:18 also implies a much lower content of O-3-linked residues than is indicated by partial acetolysis[10] and by enzymolysis[11], as is detailed in the following section.

Minor methylation products: possible significance, and comparison with results from other structural methods. — The small percentages of dimethyl ethers other than the preponderant 2,3-dimethyl ether, which are shown for each of the dextrans (see Table II), cannot be fitted into structure *A*. The possible origins and significance of these minor, structural components are elucidated by observations resulting from other methods of structural analysis. Any evidence from ^{13}C-n.m.r. spectroscopy is mentioned briefly here, but discussed further in the section on spectroscopy.

The presence of the 2,3,6-trimethyl ether in the hydrolyzate of methylated dextran B-1254 fraction S[L′] (see ref. 6) indicates that the dextran contains 4-mono-*O*-substituted D-glucopyranosyl residues; this indication was confirmed by ^{13}C-n.m.r. spectroscopy, as is discussed later. The corrected results of periodate-oxidation analysis[14] also support the possibility that the 2,3,6-trimethyl ether originated from 4-mono-*O*-substituted D-glucopyranosyl residues. These periodate-oxidation data (77% of the residues linked through O-1 and both O-1 and O-6, and 23% linked through O-4 and both O-4 and O-6) agree precisely with the methylation-structural analysis if the percentages of the 2,3,6-tri- and the 2,3-dimethyl ethers are combined. The presence of (1→3)-linked, α-D-glucopyranosyl residues in dextran B-1254 fraction S[L] was established by Suzuki and Hehre[10], who isolated, from a partial acetolyzate, 0.9% of the dextran as nigerose (3-*O*-α-D-glucopyranosyl-D-glucose). Partial acetolysis, as applied semiquantitatively by these investigators[10], is more sensitive than structural analysis by permethylation and ^{13}C-n.m.r. spectroscopy for detecting non-(1→6)-linkages in dextrans (see ref. 4 for a discussion). Partial acetolysis has recognized limitations that result from (*a*) partial rupture of non-(1→6)-bonds at rates that differ with the type of linkage[15], and (*b*) inability to differentiate between a non-(1→6)-linkage situated in the linear chain or at a branch point.

The presence of 2,4-dimethyl ether derivatives in the hydrolyzate of methylated dextran B-1526 fraction I (see Table II) indicates that the dextran contains 3,6-di-*O*-substituted residues; in confirmation, 0.4% of this dextran was recovered from a partial acetolyzate as nigerose[10].

The 2,4,6-trimethyl ether present in the hydrolyzate of methylated dextran B-742 fraction L might have originated from (*a*) failure to methylate the 3-hydroxyl group because of its known resistance to methylation[9], or (*b*) 3-mono-*O*-substituted D-glucosyl residues, the presence of which would be compatible with the recovery, from a partial acetolyzate, of 1.1% of this dextran as nigerose[10]. Further proof of the presence of 3-mono-*O*- or 3,6-di-*O*-substituted α-D-glucopyranosyl residues in dextran B-742 fraction L is provided by papergrams of the enzymolyzate resulting from the action of isomaltodextranase from *A. globiformis* T6 on this dextran[11]. (This isomaltodextranase apparently does not differentiate between a non-(1→6)-linkage situated in a linear chain or at a branch point.) Readily detectable amounts of 6-*O*-α-nigerosyl-D-glucose [*O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose] appear, as well as somewhat larger amounts of 6-*O*-α-maltosyl-D-glucose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose].

For dextran B-1420, a weak resonance at 77.52 p.p.m. in the ^{13}C-n.m.r. spectrum is associated with the presence of 2,6-di-*O*-substituted D-glucopyranosyl residues; this observation accords with the presence of 3,4-di-*O*-methyl-D-glucose (1.0 mole percent) in the hydrolyzate of the methylated dextran (see Table II). Partial acetolysis, however, revealed no evidence of O-2 linked residues[10]; usually, dextrans from which maltose was obtained did not also yield kojibiose[10] (2-*O*-α-D-glucopyranosyl-D-glucose); however, 0.7% of dextran B-1420 was recovered as nigerose from the partial acetolyzate[10]. The presence of 2,4-di-*O*-methyl-D-glucose in the hydrolyzate of the methylated dextran (see Table II) is likewise in accord with indications of O-3-linked residues in dextran B-1420. Further confirmation is provided by the appearance, on papergrams of the enzymolyzate resulting from the action of isomaltodextranase from *A. globiformis* T6 on this dextran, of 6-*O*-α-nigerosyl-D-glucose, in addition to a larger proportion of 6-*O*-α-maltosyl-D-glucose[11]. The papergram did not show any evidence for the presence of 6-*O*-α-kojibiosyl-D-glucose [*O*-α-D-glucopyranosyl-

(1→2)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose], and thus confirmed the result of acetolysis.

As is discussed fully in the section on ^{13}C-n.m.r. spectroscopy, spectra of dextran B-1307 have resonances diagnostic both of 4,6- and 3,6-di-*O*-substituted α-D-glucopyranosyl residues. The presence of (1→3)-linkages, presumably at branch points, has been demonstrated several times by study of partial acetolyzates of dextran B-1307 as well as of dextran from strain "B", as the strain was initially designated by Sugg and Hehre[16] (see ref. 17 for a review of these acetolysis results, and ref. 7 for the relationship of the strains). Suzuki and Hehre[10] reported, as the percent of dextran from strain "B" recovered from the partial acetolyzate, 0.3% of kojibiose, 2.9% of nigerose, and 1.0% of maltose. Some variation of these proportions might occur in different preparations, such as that[7] from strain B-1307.

Proof has not been acquired as to whether the small proportions of linkages indicated in Table II occur in contaminants of the major product depicted in structure *A*, or as integral irregularities in the major product. However, it was stated[10] that "The recovery of nigerose among the acetolysis products of all the dextrans examined . . . suggests the possibility that α-(1→3)-linkages in larger or smaller proportions may be a regular feature of all sucrose-derived dextrans, possibly of all dextrans".

Other considerations regarding dextrans B-742 fraction L and B-1254 fraction S[Ł]. — The indications of the presence of 3-mono-*O*-substituted residues in dextran B-742 fraction L and of 4-mono-*O*-substituted residues in dextran B-1254 fraction S[Ł] are of unusual significance. This is the first evidence of occurrence of 4-mono-*O*-substitution in a dextran; 3-mono-*O*-substituted residues are, however, a major constituent in dextran B-1355 fraction S and several related dextrans[5]. The observations reported here imply that mono-*O*-substituted residues may occur in still other dextrans. Proof has not yet been acquired, however, that these mono-*O*-substituted residues are (*a*) integral parts of the major, molecular species of the dextrans, and (*b*) present consistently in a dextran produced by a given strain.

Additional research on these dextrans (and others) will be needed in order to provide structural information commensurate with the exacting requirements of the biochemical researches in which they are used. An outstanding need is to apply techniques of established reliability to the determination of branch lengths, in order to support indirect evidence reported here and to provide an absolute basis for new techniques[1].

The structure of dextran B-742 fraction L has frequently been questioned in connection with immunochemical studies reviewed by Torii *et al.*[19] and Wu *et al.*[20]. When tested in precipitin-type and oligosaccharide-inhibition studies, the results of which were correlated with the (1→6)-like linkages of the dextrans as determined by periodate-oxidation analysis[7] (see also, ref. 14), this particular dextran acted as though the content of linkages given by the latter was too high. Correlation with the proportion of residues having (1→6)-like linkages [that is, the sum of the (1→)- and the (1→6)-linkages] would be especially susceptible to error in dextrans in which the ratio of these two types of residue is low, as in dextran B-742 fraction L. The achievement of correlation of results from other dextrans[20], however, implied that access to the (1→6)-linked residues in dextran B-742 fraction L is blocked or hindered. A similar conclusion was reached by Torii *et al.*[19] in a study of human antiserum obtained by immunization with dextran B-742 fraction L; the antiserum showed[19] specificity for relatively short (2–3 D-glucosyl residues) "terminal chains of α-(1→6)-linked glucose residues". Inhibition tests with oligosaccharides complemented previous findings[20], and were interpreted as indicating interruption of sequences of (1→6)-linked residues by branching or by a non-(1→6)-linkage[19].

In their reaction with certain myeloma proteins having α-(1→6)-specificity, dextrans B-742 fraction L and B-1254 fraction S[L′] showed a weak activity that was less than that expected from their proportion of α-D-(1→6)-linkages[19]. Dextran B-1254 fraction S[L′] showed notable behavior, however, with another myeloma protein[21] and with several other immunoglobulins[22].

^{13}C-*Nuclear magnetic resonance spectroscopy*

We have previously made the following observations regarding the ^{13}C-n.m.r. spectra of dextrans[2–5]. (*a*) The spectrum of linear dextran has six resonances (designated A through F, see Table I) which are also present in the spectra of branched dextrans. (*b*) The spectra of branched dextrans display additional resonances that are diagnostic of the type and degree of dextran branching. (*c*) For dextrans containing 2,6-di-*O*-substituted[4] and 3-mono-*O*-substituted[5] α-D-glucopyranosyl residues, the chemical shifts of these diagnostic, branching resonances are independent of the degree of branching of the polysaccharide, and the intensities of these branching resonances are generally proportional (compared to the A through F resonances) to the degree of branching. In conjunction with these observations, we have employed the following hypotheses. (*i*) Each type of *O*-substituted α-D-glucopyranosyl residue has six unique resonances. (*ii*) The resonances of the different, *O*-substituted residues are (to a first approximation) independent of neighboring residues. (*iii*) The total spectrum for a given polysaccharide is a composite of the spectra of the individual residues present in the polymer. (*iv*) A 25-MHz spectrum recorded at 90° provides sufficient resolution to permit observation of many of these resonances. A polysaccharide having structure *A* will then have three different types of residue, with equimolar proportions of branching residues and terminal groups. Based on the foregoing assumptions and observations, a well-resolved spectrum of a compound having a structure of type *A* would contain 18 resonances. Twelve of the resonances are associated with the positions of the carbon atoms of the branch-point residue or the terminal group; however, a simple, ^{13}C-n.m.r. spectrum provides no means of separating these resonances into their respective sets (assigned to branch point or terminal positions).

Table I presents the chemical shifts for the compounds that, by methylation-structural analysis (see Table II), have been established to branch primarily through 4,6-di-*O*-substituted D-glucopyranosyl residues. In addition, Table I includes dextrans B-1225, B-1307, and dextran B-1526 fraction S, the ^{13}C-n.m.r. spectrum of each of which contains a resonance at ~80.4 p.p.m. The spectrum of dextran B-1526 fraction S (see Fig. 1) was recorded with a high signal-to-noise ratio (~35,000 acquisitions), and the resulting data were processed by Convolution Difference Resolution Enhancement (c.d.r.e.). The c.d.r.e. treatment does not create new peaks, but narrows the observed resonance-width and is a convenient technique to allow rapid visual discrimination between poorly defined resonances (under normal plotting conditions) and random noise. The resonances of dextran B-1526 fraction S, listed in column 3 of Table I, plus the additional resonances identified by c.d.r.e. (70.96 and 74.55 p.p.m.) make a total of 17 resonances observed, out of the expected 18 resonances.

The chemical shifts of the resonances of the polysaccharides listed in Table I are quite consistent. This situation is similar to that for the resonance relationships we have observed for data from classes of dextrans containing 2,6-di-*O*-substituted (see Table II in ref. 4), and 3-mono-*O*- with 3,6-di-*O*-substituted (see Table II in ref. 5), α-D-glucopyranosyl residues, further confirming the concept that, for dextrans, the diagnostic chemical-shifts are independent of the degree of branching. However, the chemical shifts for the various classes (different types of branching) differ from one class to another. This persistence of specific chemical-shifts throughout the spectra of different polysaccharides of the same class (*e.g.*, the class of dextrans branching through the 2,6-di-*O*-substituted residues) provides more-definitive evidence that a given resonance is diagnostic of the presence of a specific residue than does the isolated occurrence of such a resonance in the spectrum of a single polymer.

The variations in chemical shifts observed for resonances of the different classes of dextran can be small (especially for the 70–75-p.p.m. region) and are near the limit of accuracy of the XL-100-15 spectrometer. We therefore precisely describe the process of constructing Table I. In recording data obtained with the XL-100-15 spectrometer, we have observed that the chemical-shift values can change by ~0.2 p.p.m. on a month-to-month basis. However, spectra sequentially recorded during a single week have more-consistent chemical-shifts, and therefore, the spectra were recorded in blocks of ~10 at a time. For each set of spectra, a preliminary spectrum of a dextran having a low degree of branching was recorded as a reference. Any changes on a month-to-month basis (which were generally less than 0.2 p.p.m., and were linear across the observed spectrum) were then adjusted by the uniform addition of a small correction to each chemical shift, for all spectra of a given set. These spectra were then separated, according to polysaccharide class, and employed in Table II in ref. 4, Table II in ref. 5, and Table I in the present paper. Similar data were also obtained for levans[23], comb-like amylose derivatives[24], and a yet-to-be-discussed class of dextrans branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues. For any given resonance, the deviation of any chemical shift from the average can be seen by comparing the values in any given line in Table I. We consider these deviations to be essentially a measure of spectrometer stability for a given week, and also of our reproducibility of sample preparations. It would appear that chemical-shift differences somewhat under 0.1 p.p.m. (possibly, near 0.05 p.p.m.) have meaning for these cross-referenced spectra. Conversely, care should be taken with the comparison of chemical-shift differences smaller than ~0.3 p.p.m. when these data have been recorded at different times (with instruments similar to the XL-100-15 spectrometer) and have not been corrected.

The chemical-shift data from ^{13}C-n.m.r. spectra, recorded at 90°, of dextrans branching through 4,6-di-*O*-substituted residues may be summarized as follows. The presence of easily recognized resonances at 80.4 p.p.m., 100.1 p.p.m., and 101.6 p.p.m., which had previously been proposed as diagnostic of this branching type, was further confirmed. The absence of resonances downfield from 102 p.p.m. indicates that all anomeric resonances observed correspond to α-D-linked glucopyranosyl residues[25]. The identity of the 80.4-p.p.m. resonance, previously assigned to C-4 of the branch-point residue, was further confirmed. Also, the weak, 68.5-p.p.m. resonance persists in these spectra, and this resonance had been assigned, on the basis of chemical shift and of intensity, to the linked, C-6 atom of the branch-point residue. With the exception of a chemical-shift displacement of ~5.3 p.p.m. between the resonances of free C-6 (62.5 p.p.m.) and linked C-6 (68.5 p.p.m.) atoms, the chemical shifts of the C-6 resonances are normally not influenced by changes in polysaccharide structure. It is possible that the chemical-shift displacement observed for the aforementioned, 4,6-di-*O*-substituted residue results from the relative nearness of the C-4 and C-6 (as contrasted to C-2, or C-3, and C-6). It is also of interest to compare the relative intensity of the resonance (68.5 p.p.m.) of C-6 of the branch-point residue to that (62.5 p.p.m.) of the free C-6 atom of the terminal group. On the basis of the general structure shown in structure *A*, the number of branch-point residues equals the number of terminal groups. This inequality of contribution of resonance intensity from the two different C-6 atoms is not unexpected, as resonance-relaxation parameters can influence line width and total resonance-intensity. In the case of dextrans, and, apparently, for most polysaccharides, most of the carbon atoms contribute to the total spectrum resonances of approximately equal intensity. However, the free C-6 atom of the terminal group has much more freedom of motion than the linked C-6 atom of the branching residue, and consequently contributes a very intense resonance to the spectrum. Finally, the resonances at 70.96 p.p.m. and 71.26 p.p.m. are notable, as these resonances are upfield from resonance E (71.6 p.p.m.), and, for dextrans branching through 2,6- and 3,6-di-*O*-substituted residues, only the C-6 resonances are upfield from peak E.

We had previously concluded[4] that the intensities of the branching resonances (relative to the resonances associated with linear dextran) of dextrans branching through 2,6-di-*O*-substituted residues are proportional to the degree of branching of the dextran. Anomeric resonances were employed for comparison, because of good resolution in their spectral region. For greatest accuracy, it would be best to compare the areas of the resonances, but, for a first approximation, the relative peak-heights of the anomeric resonances were employed. Relative peak-heights were compared, because (*a*) in some cases, the signal-to-noise ratio of the spectra did not justify comparisons of area, and (*b*) ^{13}C-n.m.r. data were being compared to *n* values obtained from structural-methylation analysis, and these values were not precisely

TABLE III

DEXTRANS BRANCHED THROUGH 4,6-DI-*O*-SUBSTITUTED α-D-GLUCOPYRANOSYL RESIDUES: DEGREE OF LINEARITY CALCULATED FROM ^{13}C-N.M.R. SPECTRA, AT 90°, AND FROM G.L.C.–M.S. PERMETHYLATION-ANALYSIS DATA

NRRL strain	*Dextran fraction*	*^{13}C-N.m.r. anomeric resonance ratio*[a]	n *(calculated from g.l.c.–m.s. data*[b]*)*
B-1254	S[L]	1.8[c]	2.5[d]
B-1526	I	4.6	3.8
B-742	L	4.4[e]	5.0[f]
B-1420		8.0	5.0

[a]The peak height of the resonance at 99.5 p.p.m. divided by that of the resonance at 101.6 p.p.m. [b]The amount of 2,3,4-tri-*O*-methyl derivative divided by the amount of 2,3,4,6-tetra-*O*-methyl derivative. [c]Data taken from ref. 2. [d]Data taken from ref. 6. [e]Data taken from ref. 3. [f]Data taken from ref. 9.

known. That these ^{13}C-n.m.r. spectra had been recorded under relatively uniform conditions was one reason for the good correlation previously observed[4] between *n* values from ^{13}C-n.m.r. peak-heights and from methylation-structural analysis. The concentration of the polymer or the signal-to-noise ratios made little difference in the relative peak-heights for a given compound, but differences in recording temperatures could change the peak-height ratio. C.d.r.e. processing is another factor that can influence the peak-height ratio, introducing a difference possibly due to unequal effectiveness in narrowing different resonances in the spectrum. An example of the c.d.r.e. effect is shown in Fig. 1 for the two fractions of dextran B-1526. The normally processed spectra, either of fraction S or I of dextran B-1526, are essentially identical; however, the c.d.r.e.-processed spectrum of fraction S shows more intense A through F resonances than the corresponding, normal spectrum of fraction I.

Table III compares the *n* values obtained from methylation-structural analysis (see column 4) with the relative peak-height ratios [obtained by comparing the peak height of the anomeric resonance of the linear-chain-extending residues (99.5 p.p.m.) to the peak height of an anomeric resonance (101.6 p.p.m.) associated with dextran branching (see column 3)]. The 99.5-p.p.m. resonance is not completely resolved from a second branching-resonance (at 100.1 p.p.m.), a situation that could affect any expected linear relationship between the values in columns 3 and 4 in Table III. However, beyond this possible resonance-overlap effect, there appears to be less correspondence between the degree of linearity obtained from ^{13}C-n.m.r., peak-height data and methylation-structural analysis data than for the classes of dextrans previously studied[4,5]. For example, the intensities of resonances (^{13}C-n.m.r.) indicate that dextran B-1526 fraction I (or S) and dextran B-742 fraction L have approximately the same degree of branching, and that both are more highly branched than dextran B-1420. Conversely, methylation-structural analysis data indicate that dextran B-742 fraction S and dextran B-1420 have about the same degree of branching. The values in Table III are the result of duplicate, or triplicate, determinations both for ^{13}C-n.m.r. spectra and methylation-structural analyses. The series of ^{13}C-n.m.r. data and the series of methylation-structural analysis data are both consistent and reproducible under currently employed conditions, but the results of the two methods do not precisely parallel one another. We are as yet uncertain as to the source of this lack of agreement between the two methods for establishing the degree of branching. As is discussed in other sections of this article, difficulties could arise from inaccurate methylation-structural analysis. We have observed that dextrans (like cellulose) are much more difficult to permethylate and, subsequently, to hydrolyze[3,6] than many other polysaccharides (*e.g.*, mannans[26] and glycogen[24]). The data from methylation-structural analysis allow the determination of an *absolute* value of *n* in terms of the resulting chromatogram. However, the chromatogram is dependent on completeness of methylation and hydrolysis, and it can also depend on material loss; these are problems we have previously discussed in terms of methylation-structural determination of dextrans. A possible source of error for ^{13}C-n.m.r. estimates of the linearity of dextrans could result from incomplete solubility of the polymer. Should the polysaccharide not be homogeneous in regard to the distribution of branching residues, and should these regions of inhomogeneity differ in solubility or in ease of solvation, all regions of the polymer would not contribute equally to the total spectrum. However, such solubility effects are probably minimal for these dextrans, as (*a*) linear dextran is relatively soluble under the ^{13}C-n.m.r. conditions employed, and (*b*) the soluble dextrans that have been studied are all highly branched. The relative, anomeric peak-height ratios of branched dextrans do not give an absolute value for *n*, as different carbon atoms are compared, and it is known that different carbon atoms can give resonances of different intensity. Because of the relatively few examples of dextrans known to branch through 4,6-di-*O*-substituted α-D-glucopyranosyl residues, it is difficult to establish the precise conversion-factor relating anomeric peak-height ratios to an absolute *n* value. In the course of our dextran survey, we have so far observed fewer dextrans branching through 4,6-di-*O*-substituted α-D-glucopyranosyl residues than dextrans branching through the corresponding 2,6- or 3,6-di-*O*-substituted residues; indeed, as discussed later, we have found relatively few dextrans branching through 4,6-di-*O*-substituted residues. This lack of examples of differing degrees of linearity (especially, of highly branched dextrans having $n \leqslant 2$) for dextrans branching through 4,6-di-*O*-substituted α-D-glucopyranosyl residues has presented difficulties as regards total analysis of a ^{13}C-n.m.r. spectrum for a member of this class of compound. In addition, the ratios of the areas of the ^{1}H-n.m.r. anomeric resonances have failed to clarify the relationship of the degrees of branching of these dextrans[1].

Dextrans B-1225 and B-1307 are dextrans having a low degree of branching. These essentially linear dextrans are included in Table I and Fig. 1, because of the presence of a weak, ~80.4-p.p.m. resonance (resonance H in Fig. 2) in the spectrum of each polysaccharide. On the basis of the relatively weak resonances diagnostic of branching, dextran B-1225 is somewhat more branched than dextran B-1307. The

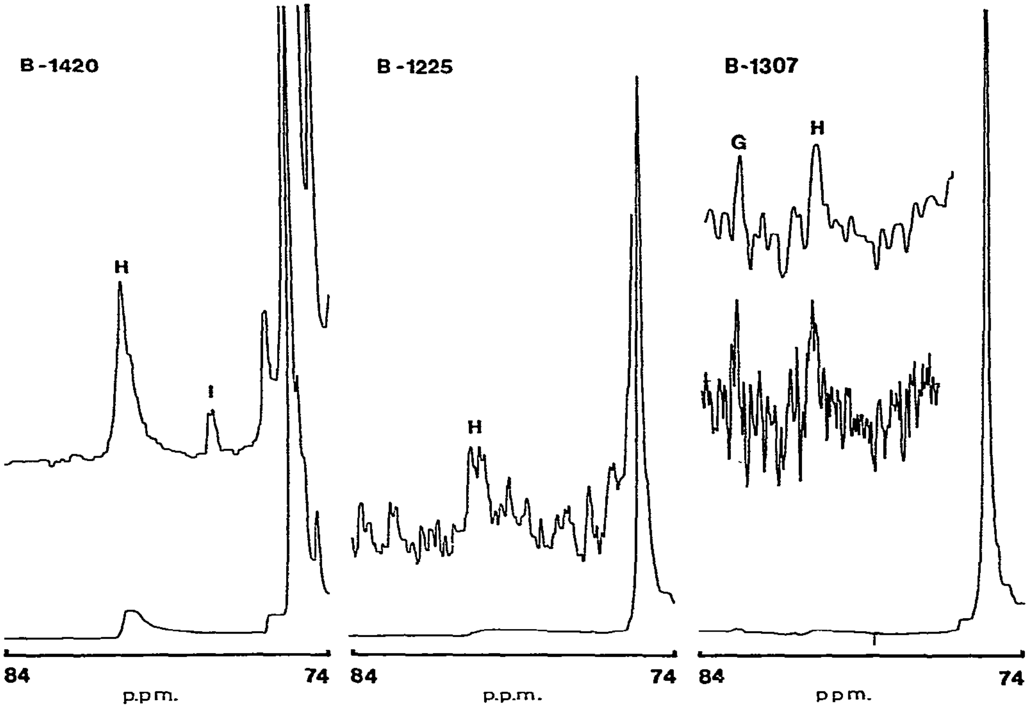


Fig. 2. The expanded, 74–84-p.p.m. region of the ^{13}C-n.m.r.-spectral data presented in Fig. 1 for dextrans B-1420, B-1225, and B-1307. The higher traces represent ten-fold magnification of the amplitude.

74–84-p.p.m., spectral region of dextran B-1307 also shows a weak G resonance (80.51 p.p.m.), indicating the presence of a trace of 3,6-di-*O*-substituted α-D-glucopyranosyl residues. As indicated by the resonance intensity, the total mole-percentage of branching residues in either dextran B-1225 or dextran B-1307 is estimated to be 2–5%. Stacey *et al.*[27] reported a methylation-structural analysis of a dextran that they prepared from *A. capsulatum* NRRL B-1225. Their data showed branching only through 4,6-di-*O*-substituted residues, and indicated that such a branch point occurs at about one in every ten residues of the backbone chain; this is about two to three times the branching frequency suggested by our ^{13}C-n.m.r. data. It should be emphasized that resonances G and H arise from carbon atoms at different positions, and that, therefore, the relative peak-intensities do not necessarily correspond directly to the relative mole-percentages of the residues involved in these branching linkages. However, as highly branched dextrans containing different branching residues [*e.g.*, α-D-(1→3)-linkages in dextran B-742 fraction S, and α-D-(1→4)-linkages in dextran B-742 fraction L] have similar resonance-intensities in the 75–85-p.p.m. region (when corrected for differences in *n* as established by methylation-structural analysis), a comparison of these resonance intensities indicated that the mole ratios of resonances in the 75–85-p.p.m. region correspond to ~20% of the actual mole-percentages present. The upper traces in Fig. 2 represent a ten-fold amplification of the lower

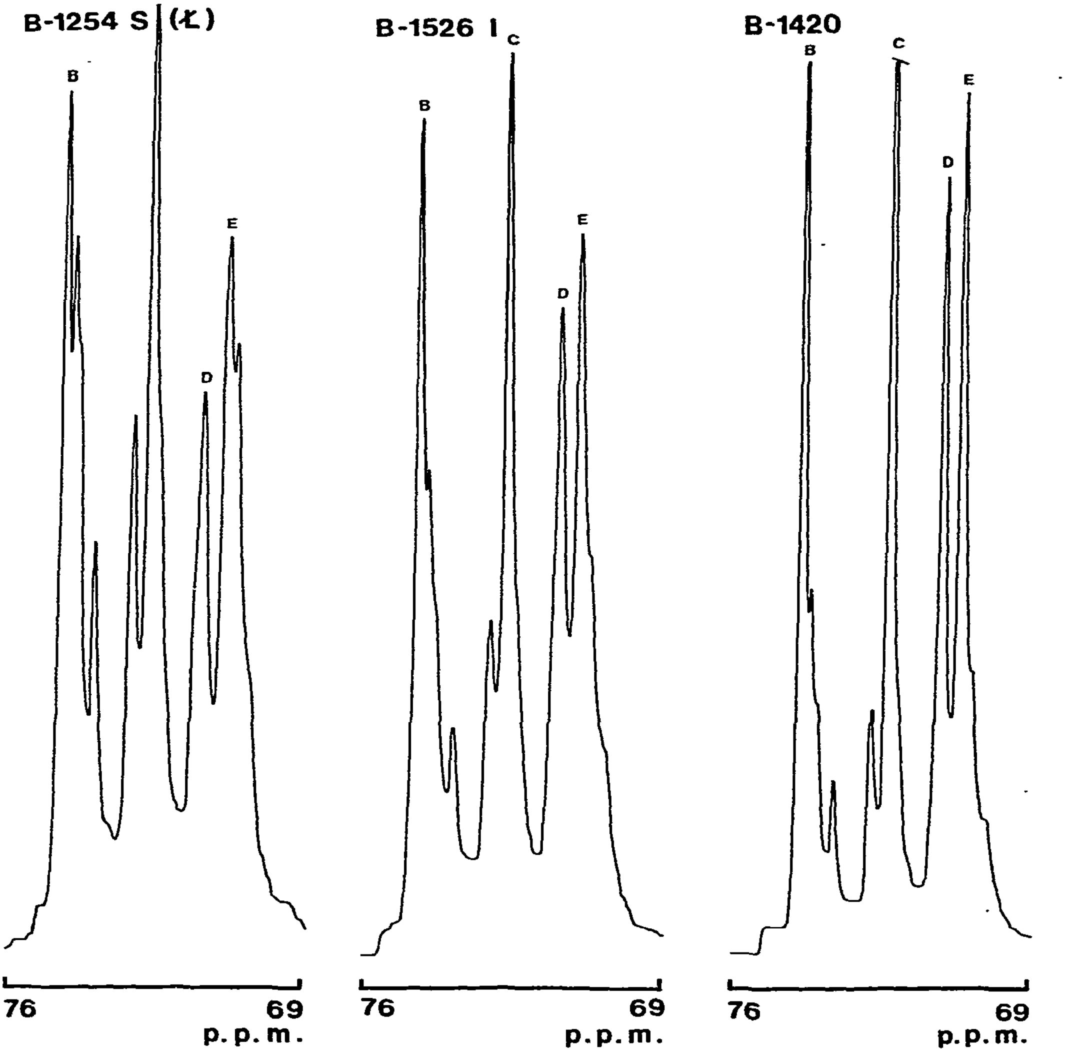


Fig. 3. The expanded, 69–76-p.p.m. region of the ^{13}C-n.m.r.-spectral data presented in Fig. 1 for dextrans B-1254 fraction S[Ł], B-1526 fraction I, and B-1420.

ones, and, for dextran B-1307, the same data set is plotted twice, with two different degrees of digital filtering. The corresponding 74–84-p.p.m., spectral region (see Fig. 2) for the more highly branched dextran B-1420 shows both an H resonance (80.31 p.p.m., indicating 4,6-di-*O*-substituted residues) and a weaker I resonance (77.52 p.p.m., indicating 2,6-di-*O*-substituted α-D-glucopyranosyl residues). This ^{13}C-n.m.r.-spectral evidence is of interest, as it confirms the presence of a trace of 2,6-di-*O*-substituted α-D-glucopyranosyl residues, previously indicated by methylation-structural analysis (see Table II). Both the data from methylation-structural analysis and from the H resonance area to I resonance area indicate a ratio of ~10:1 for 2,6- to 4,6-di-*O*-substituted α-D-glucopyranosyl content in dextran *B-1420*.

Enlarged plots of the 69–76-p.p.m. spectral-region for dextran B-1254 fraction S[L], dextran B-1526 fraction I, and dextran B-1420 are shown in Fig. 3. The resonances associated with linear dextran (resonances B through E) are marked so that (*a*) comparison can be made with the chemical shifts listed in Table I, (*b*) the relative resolution of the less-intense branching resonances can be observed, and (*c*) it may be seen that, as *n* for a given polysaccharide increases, there is a decrease of the relative intensities of the branching resonances.

The resonance regions defined as G, H, and I [indicating, respectively, α-D-(1→3)-, -(1→4)-, and -(1→2)-linkages] require further comment. We have previously shown that the G-region resonances (~83 p.p.m.) separate into two groups, corresponding to the C-3 resonance of the 3-mono-*O*-substituted α-D-glucopyranosyl residue (~83.3 p.p.m.) and the C-3 resonance of the 3,6-di-*O*-substituted α-D-glucopyranosyl residue (~82.9 p.p.m.). In a similar way, a distinction can be made for the H-region resonances, (~80 p.p.m.), as the spectra of polysaccharides containing mainly 4-mono-*O*-substituted α-D-glucopyranosyl residues[24] (*e.g.*, glycogen) have a resonance at ~79.4 p.p.m., whereas the spectra of those containing 4,6-di-*O*-substituted α-D-glucopyranosyl residues (*e.g.*, the dextrans reported here) have a resonance at ~80.4 p.p.m.; this further confirms the importance of employing the concept of specifically *O*-substituted residues, rather than linkage-types, for polysaccharide structural correlations. These minor differences in chemical shift with regard to types of *O*-substituted residue are of interest for the analysis of the spectrum of dextran B-1254 fraction S[L]. The 75–85-p.p.m., spectral region of dextran B-1254 fraction S[L] contains a prominent resonance at 80.40 p.p.m., associated with 4,6-di-*O*-substituted α-D-glucopyranosyl residues, and a weaker, incompletely resolved resonance centered at 79.3 p.p.m. that has been associated with the 4-mono-*O*-substituted α-D-glucopyranosyl residue. The latter weak resonance, in conjunction with evidence of other poorly defined resonances in the anomeric spectral region of this polysaccharide, supports the concept that the 2,3,6-tri-*O*-methyl derivative found in the hydrolyzate of permethylated dextran B-1254 fraction S[L] represents a 4-mono-*O*-substituted residue in the original polysaccharide.

The occurrence of (1→4)-linked α-D-glucopyranosyl residues in dextrans

The first dextran to be subjected to methylation-structural analysis[28,29] gave 2,3,4,6-tetra-:2,3,4-tri-:2,3-di-*O*-methyl-D-glucose in the ratios of 1:3:1. (The methoxyl content found for the product was that calculated for complete substitution.) *L. mesenteroides*, culture 4, was used; this strain, when obtained later from two different culture-collections, was the parent strain of NRRL B-742 and NRRL B-1142, respectively[7]. Strain B-742 gave a polydisperse product[7,8]; the L-type fraction seems to differ from the original dextran from culture 4 in being less branched; the S-type fraction has an ~50% content of 3,6-di-*O*-substituted residues[4,9]. Dextran B-1142, obtained in poor yield[7], seemed essentially homogeneous, and equivalent to B-742 fraction S. As has been discussed previously[7], culture 4 changed during long maintenance under routine conditions. Other dextran-producing strains have also been found to change, usually to give dextrans having an increased content of (1→3)-linked residues[6]. Thus, duplication of a dextran product from a strain, after long maintenance in a culture collection, cannot be assured without careful attention to the activity of the strain and to characterization of the product.

The dextran from *Betacoccus arabinosaceous*, Birmingham strain, was the second dextran to be subjected to reliable, methylation-structural analysis[30]. When initially studied, it gave 2,3,4,6-tetra-:2,3,4-tri-:2,3-di-*O*-methyl-D-glucose in the ratios of 1:3:1 (see ref. 30). Later study resulted in the ratios of 2,3,4,6-tetra-:2,3,4-tri-:2,4-di- of 1:3:1.5 (see ref. 31). This Birmingham strain was the parent of NRRL B-1375; the ~20% of non-(1→6)-linkages in dextran B-1375 (see refs. 7 and 14) have been shown[11] to be (1→3)-linkages (see ref. 11).

From a large number of dextrans for which the proportion of linkages was determined by analytical, periodate oxidation[7], all except one were indicated to have (1→4)-like linkages [that is, (1→4)- or (1→2)-] in the reported[7] range of 2–50%. The exception was the water-insoluble dextran from NRRL strain B-1120, which showed no (1→4)-like linkages. Later, the presence of (1→2)-linkages in 22 of the dextrans was established by serological analysis[32], and this was supported by the optical rotatory shift in cuprammonium solution[33] (see also, ref. 4 for further discussion). For the rest of the dextrans (~78 in number), the (1→4)-like linkages indicated by periodate-oxidation analysis were then considered to be truly (1→4).

Evaluation of the periodate-oxidation analyses on the basis of methylation-structural analyses[14] has now shown that the percentage of (1→4)-linkages indicated by periodate oxidation was usually too high, that of the (1→3)-linkages was too low, and in certain ranges of percentage, that for the (1→)- plus the (1→6)-linkages was sometimes too low. The deviations from the true values (that is, from the permethylation-structural analyses) result from the formation of stable, cyclic hemi-acetal structures that inhibit complete periodate oxidation[14]. The periodate-oxidation values, after being corrected by systematic calculation[14], accord well with the values from methylation analyses. The low percentage of non-(1→6)-linkages in several dextrans is thus identified as being (1→3), instead of (1→4) [examples are dextrans B-640 and B-1308, which have 95% of (1→)- and (1→6)-linked residues (as determined by periodate-oxidation analysis)[7]]. The 5% of non-(1→6)-linkages in dextran B-512(F) was shown earlier, by methylation analysis[34], to be (1→3), instead of (1→4). Similar correction is probably applicable to numerous other dextrans having a relatively low content of non-(1→6)-linked residues[7]. Dextran B-1307, for which periodate-oxidation analysis indicated 9% of (1→4)-linkages, and which is here shown to contain both (1→3)- and (1→4)-linkages, is considered to be representative of numerous other dextrans[7]. Still other dextrans, represented by B-1191 (77% of (1→6)-like linkages] and B-742 fraction S [57% of (1→6)-like linkages], for which periodate-oxidation analyses indicated both (1→4)- and (1→3)-linkages, are now known to contain only (1→3)-linkages[14]. Thus, the effect of correcting the identity and proportion of linkages indicated for dextrans by periodate-oxidation analysis is to (*a*) decrease significantly the number of dextrans known to contain (1→4)-linkages, (*b*) increase the number of dextrans known to contain (1→3)-linkages [in addition to (1→4)], and (*c*) necessitate re-evaluation of any data derived from use of these dextrans, should interpretation and correlations involve the percentage of linkages as initially reported from periodate-oxidation analysis, as, for example, in immunochemical studies. For dextrans other than those for which corrected, periodate-oxidation data have been reported[14], individual analysis will be necessary in order to establish the presence and proportions of (1→3)- and (1→4)-linked residues.

Dextrans containing significant proportions of 4,6-di-*O*-substituted residues usually do not also contain 2,6-di-*O*-substituted residues[10]; they do, however, often contain 3,6-di-*O*- (and, possibly, 3-mono-*O*-) substituted residues, although in relatively small proportions[10]. Among dextrans now known, those having a relatively high content of 4,6-di-*O*-substituted residues, such as those reported here, appear to be relatively rare.

Interaction with various immunoproteins

Antigenicity of dextran B-1307 was demonstrated when sucrose-grown cells of strain "B" (the parent strain of NRRL B-1307) produced in rabbits[16] antiserum that was strongly precipitated by high dilutions of the dextran.

The dextran from strain "B" also reacted strongly at high dilution with rabbit antisera[16] to Pneumococcus types 2 and 20, but not with that to type 12. The dextran was, thus, the prototype of "serotype B" dextrans, which differ from serotype A dextrans in being unreactive (or only weakly reactive) with type 12 pneumococcal antisera, and in the absence of (1→2)-linkages[10,32] (see also, the discussion in ref. 4). All dextrans (including the serotype A) cross-react, with various degrees of activity, with types 2 and 20 anti-pneumococcal sera[32]; this activity stems from (1→6)-linked α-D-glucopyranosyl residues[32,35].

Dextran B-1307 and other dextrans having 4,6-di-*O*-substituted residues interact with numerous types of substances, such as antidextrans of various structural specificity, nonspecific naturally occurring components of blood sera, and myeloma proteins. The studies made are listed among those cited for dextrans of all structural types[36].

EXPERIMENTAL

Materials. — Previously prepared dextrans[7] and dextran fractions[8] were used. The dextran from *A. capsulatum* NRRL B-1225, in accordance with the specific, carbohydrate-substrate requirement of the strain[37], was produced from a starch dextrin. In contrast, all of the other bacterial strains represented in our studies[7] required sucrose for dextran production. The only other strain known to produce dextran from starch dextrin is *A. viscosus* NRRL B-1226 (see refs. 7 and 37). The dextran products from *Streptococcus* sp. NRRL B-1526 are not comparable to the S- and L-type fractions from other strains that have been used in our previous studies and that are referred to here. The B-1526 products were not fractionated by means of aqueous alcohol; instead, they occurred, respectively, in a soluble phase in the fermentation culture, and in a sedimented gum-phase (fraction I). The product from the soluble phase is designated here as fraction S in order to simplify the terminology. Originally[7], this product was not designated a fraction, because it was, by definition[8], the main dextran product. The same distinction also applies to the pairs of products from strains B-1380, B-1420, and B-1394 (see ref. 7). The attempts thus made to establish the significance of the phase separation were indeterminant.

Methods. — Methods previously described were used for the methylation[9] of the dextrans and for structural analysis[6,38] by combined g.l.c.–electron-impact mass spectrometry of the peracetylated aldononitriles. For each permethylation, three successive Hakomori[39] methylations were employed on an initial, 40-mg sample, with ~80% (final weight) recovery of each permethylated dextran. Successive formolysis and acetic acid hydrolysis were employed, and, after each step, the resulting solutions were clear, colorless, and free from suspended material. 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 II were obtained with a Barber–Coleman Series 5000 g.l.c. instrument equipped with hydrogen-flame detectors. On-column injection with glass columns (2 mm i.d. × 1.23 m) was employed for all chromatography.

The ^{13}C-n.m.r. conditions and the methods for the 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. The dextran samples, ~0.3 g/4 mL of deuterium oxide, were maintained at 90°. Chemical shifts are expressed in p.p.m. relative to external tetramethylsilane, but were actually calculated by reference to the solvent lock-signal. The convolution-difference resolution-enhancement (c.d.r.e.) technique has been described[40].

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REFERENCES

1 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 74 (1979) 77–92.
2 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 51 (1976) 179–194.
3 F. R. Seymour, R. D. Knapp, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 68 (1979) 123–140.
4 F. R. Seymour, R. D. Knapp, E. C. M. Chen, A. Jeanes, and S. H. Bishop, *Carbohydr. Res.*, 71 (1979) 231–250.
5 F. R. Seymour, R. D. Knapp, E. C. M. Chen, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 74 (1979) 41–62.
6 F. R. Seymour, M. E. Slodki, R. D. Plattner, and A. Jeanes, *Carbohydr. Res.*, 53 (1977) 153–166.
7 A. Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, M. J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya, and C. E. Rist, *J. Am. Chem. Soc.*, 76 (1954) 5041–5046.
8 C. A. Wilham, B. H. Alexander, and A. Jeanes, *Arch. Biochem. Biophys.*, 59 (1955) 61–75.

9 F. R. Seymour, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, 68 (1979) 113–121.
10 H. Suzuki and E. J. Hehre, *Arch. Biochem. Biophys.*, 104 (1964) 305–313.
11 T. Sawai, T. Tohyama, and T. Natsume, *Carbohydr. Res.*, 66 (1978) 195–205.
12 R. J. Dimler, R. W. Jones, W. C. Schaefer, and J. W. Van Cleve, *Abstr. Pap. Am. Chem. Soc. Meet.*, 129 (1956) 2D.
13 A. Jeanes, in G. F. Springer (Ed.), *Polysaccharides Biol. Trans. Conf., 3rd*, (1958) 130–147, 151–153.
14 A. Jeanes and F. R. Seymour, *Carbohydr. Res.*, 74 (1979) 31–40.
15 K. Fujimoto, K. Matsuda, and K. Aso, *Tohoku J. Agric. Res.*, 13 (1962) 61–67.
16 J. Y. Sugg and E. J. Hehre, *J. Immunol.*, 43 (1942) 119–128.
17 R. L. Sidebotham, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
18 F. R. Seymour and R. L. Julian, *Carbohydr. Res.*, 74 (1979) 63–75.
19 M. Torii, E. A. Kabat, and H. Weigel, *J. Immunol.*, 96 (1966) 797–805.
20 A. M. Wu, E. A. Kabat, and M. G. Weigert, *Carbohydr. Res.*, 66 (1978) 113–124.
21 M. A. Leon, N. M. Young, and K. R. McIntire, *Biochemistry*, 9 (1970) 1023–1030.
22 H. J. Sage, A. Ransby, and M. A. Leon, *J. Immunol.*, 90 (1963) 347–357.
23 F. R. Seymour, R. D. Knapp, and A. Jeanes, *Carbohydr. Res.*, 72 (1979) 222–228.
24 F. R. Seymour, R. D. Knapp, T. E. Nelson, and B. Pfannemüller, *Carbohydr. Res.*, 70 (1979) 125–133.
25 D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, 93 (1971) 4463–4472.
26 F. R. Seymour, M. E. Slodki, R. D. Plattner, and R. M. Stodola, *Carbohydr. Res.*, 48 (1976) 225–237.
27 S. A. Barker, E. J. Bourne, G. T. Bruce, and M. Stacey, *J. Chem. Soc.*, (1958) 4414–4416.
28 F. L. Fowler, I. K. Buckland, F. E. Brauns, and H. Hibbert, *Can. J. Res., Sect. B*, 15 (1937) 486–497.
29 I. Levi, W. L. Hawkins, and H. Hibbert, *J. Am. Chem. Soc.*, 64 (1942) 1959–1962.
30 M. Stacey and G. Swift, *J. Chem. Soc.*, (1948) 1555–1559.
31 S. A. Barker, E. J. Bourne, G. T. Bruce, W. B. Neely, and M. Stacey, *J. Chem. Soc.*, (1954) 2395–2399.
32 E. J. Hehre, *Bull. Soc. Chim. Biol.*, 42 (1960) 1581–1585.
33 T. A. Scott, N. N. Hellman, and F. R. Senti, *J. Am. Chem. Soc.*, 79 (1957) 1178–1182.
34 J. W. Van Cleve, W. C. Schaefer, and C. E. Rist, *J. Am. Chem. Soc.*, 78 (1956) 4435–4438.
35 J. W. Goodman and E. A. Kabat, *J. Immunol.*, 84 (1960) 333–346.
36 A. Jeanes, "Dextran Bibliography: Extensive Coverage of Research Literature (Exclusive of Clinical) and Patents, 1861–1976", *U. S. Dep. Agric., Agric. Res. Serv., Misc. Publ. No. 1355*, (1978) 370 pp.
37 E. J. Hehre (with the assistance of D. M. Hamilton), *J. Biol. Chem.*, 192 (1951) 161–174.
38 F. R. Seymour, R. D. Plattner, and M. E. Slodki, *Carbohydr. Res.*, 44 (1975) 181–198.
39 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
40 I. D. Campbell, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J. Magn. Reson.*, 11 (1973) 172–181.