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CORRELATION OF THE STRUCTURE OF DEXTRANS TO THEIR ^{1}H-N.M.R. SPECTRA*

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ABSTRACT

Dextran fractions from NRRL strains *Leuconostoc mesenteroides* B-742, B-1299, B-1355, and B-1501, *Streptobacterium dextranicum* B-1254, *Streptococcus* sp. B-1526, and also the native dextrans from *L. mesenteroides* B-1142, B-1191, B-1402, and *L. dextranicum* B-1420 were examined in aqueous solution at 90° by Fourier-transform, ^{1}H-n.m.r. spectroscopy. Branching of dextrans through the 2,6-, 3,6-, and 4,6-di-*O*-substituted α-D-glucopyranosyl residues was correlated to changes in spectral patterns. The major, spectral differences for these dextrans were in the anomeric (4–6-p.p.m.) region. In general, the degree of branching of a dextran can be determined by comparing anomeric-peak integrals; however, the integrals of the displaced, nonlinear, anomeric resonances are also dependent on the type of branching residues present in the dextran. Although the ^{1}H-n.m.r. resonances for the anomeric region identify the presence of non-6-mono-*O*-substituted α-D-glucopyranosyl residues, these additional resonances provide little discrimination between other residue types, the exception being the 2,6-di-*O*-substituted residue, which has a clearly displaced resonance. Techniques for the suppression of the interfering resonance of deuterium hydroxide are also discussed.

INTRODUCTION

This series of articles on dextrans has examined the structures of dextrans in terms of g.l.c.–m.s.[2,3], ^{13}C-n.m.r.-spectroscopic[4–6], periodate oxidation[7], and

*Unusual Dextrans, Part X. For Part IX, see ref. 1.

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difference Fourier-transform i.r. data[1]. We now consider the relationships between the ^{1}H-n.m.r. spectra of a selected group of dextrans having various types of branching. Previous ^{1}H-n.m.r. studies examined various dextrans[8], and established that, in common with most hexoglycans, such spectra of dextrans have resonances grouped in the 3–4-p.p.m. region, and have the hemiacetal, C-1 resonances displaced into the 4–6-p.p.m. region. Pasika and Cragg[9,10] noted that, for the ^{1}H-n.m.r.-spectral anomeric region, linear dextran gives a single peak (a doublet) at ~4.9 p.p.m., and that branched dextrans have additional resonances in the 4.9–5.3-p.p.m. spectral region. The interpretation of the spectra of dextrans was difficult, due to poor resonance resolution and a lack of well-characterized, branched dextrans. The currently available 100-MHz, Fourier-transform, n.m.r. spectrometers provide slightly improved resolution and the definite advantages of greater flexibility in data-acquisition techniques and ease of manipulation of data.

In our previous examination of a large number of dextrans from the NRRL (Northern Regional Research Center, Peoria, Illinois) collection, we have: (*a*) confirmed the presence of a preponderance of linear chains of 6-mono-*O*-substituted α-D-glucopyranosyl residues, (*b*) shown that branching occurs through three types of α-D-glucopyranosyl residue (2,6-, 3,6-, and 4,6-di-*O*-substituted), (*c*) identified a series of dextrans of various degrees of linearity which mainly (or, possibly, exclusively) branch through each of these di-*O*-substituted residues, (*d*) identified a fourth class of biopolymers[11] which are associated with, and similar to, these three types of dextran, but which contain large percentages of 3-mono-*O*-substituted α-D-glucopyranosyl residues, and (*e*) established that all dextran residues are α-D-linked.

Permethylation–fragmentation analysis *via* the g.l.c.–m.s. of peracetylated aldononitrile derivatives has provided fundamental data for establishing the type and degree of branching for each dextran. In addition, ^{13}C-n.m.r. spectra have complemented and extended the fundamental g.l.c.–m.s. data. The general approach for ^{13}C-n.m.r.-spectral analysis included: (*a*) employing elevated recording-temperatures, to improve spectral resolution, (*b*) considering a polymer spectrum as a composite spectrum composed of an assembly of the spectra of differently *O*-substituted α-D-glucopyranosyl residues, (*c*) assuming, to a first approximation, that the relative positions of the residues (*e.g.*, neighboring-group effects) need not be considered, (*d*) assuming that the relative intensity of any set of resonances associated with a specific residue is proportional to the percentage of that residue in the polymer, (*e*) employing the concept that most of the diagnostic resonances in the spectrum of a dextran are those associated with the carbon atoms directly involved in the intra-residue linkages [*e.g.*, C-1 and C-4 for a (1→4)-linkage], and (*f*) assuming that residues involved in similar linkages (*e.g.*, 3-mono-*O*-substituted and 3,6-di-*O*-substituted α-D-glucopyranosyl residues) will have more-similar diagnostic resonances than other residue types (*e.g.*, the 3-mono-*O*-substituted and 4,6-di-*O*-substituted α-D-glucopyranosyl residues). The validity of these assumptions and of this general approach to the analysis of the ^{13}C-n.m.r. spectra of dextrans has been confirmed by comparison of the information from the ^{13}C-n.m.r. spectra with the

structural information derived from other sources for approximately thirty different dextrans.

Contrasted to ^{13}C-n.m.r. spectroscopy, ^{1}H-n.m.r. spectroscopy, being ~5000 times as sensitive, has an important advantage as regards sample size, as many dextrans are of low solubility. However, ^{1}H-n.m.r. spectroscopy has two disadvantages: poor spectral resolution (in fact, 100-MHz spectra may provide less information than difference Fourier-transform, i.r. spectrometry), and of interference by deuterium hydroxide in the anomeric spectral-region. Dental-plaque dextrans are an example of insoluble materials that have been studied[12] by ^{1}H-n.m.r. spectroscopy. To promote solubilization, solutions of carbohydrates in anhydrous[13] or aqueous[12] dimethyl sulfoxide have been studied. To allow direct comparison to our previous, ^{13}C-n.m.r.-spectral studies, and as our dextrans were quite soluble in water, we limited the current study to use of pure deuterium oxide as the solvent.

Several reports[14–18] have extended the initial observations of Pasika and Cragg[9]. Sidebotham *et al.*[15,16] employed fractions of the 4.96- and 5.32-p.p.m. peak areas in the ^{1}H-n.m.r. spectra of various dextrans as a measure of polysaccharide linearity. It was concluded that the low solubility of the S and L fractions of the dextran from *Leuconostoc mesenteroides* B-1299 gave problems with continuous-wave, ^{1}H-n.m.r. spectroscopy. Usui *et al.*[17] also examined a B-1299 dextran, produced by the NRRL B-1299 microbial strain, but from a different production than the B-1299 dextran described here. The B-1299 polymer was examined by Usui *et al.* by methylation, and ^{13}C-n.m.r. and ^{1}H-n.m.r. spectroscopy. All of these methods of characterization indicated that this polymer contains ~7% of α-D-(1→3)-linked residues, a content much larger than we have found in the NRRL B-1299 dextran fractions. In addition to noting that high recording-temperatures improve ^{1}H-n.m.r.-spectral resolution, Usui *et al.* recognized[18] that, compared to the linear dextran (6-mono-*O*-substituted α-D-glucopyranosyl residues), the branch-point (2,6-di-*O*-substituted α-D-glucopyranosyl) residues are chemically unique, and could contribute distinctly different ^{1}H-n.m.r., anomeric chemical-shifts.

Our previous analysis of polysaccharides by ^{13}C-n.m.r. spectroscopy was based on the following approach. Firstly, the total spectrum of the polymer is the sum of the individual contributions of the different types of residue present. Secondly, the total number of branch-point residues essentially equals the total number of terminal-group residues. Therefore, the well resolved, anomeric region of a dextran of moderate degree of branching would contain three resonances: a major resonance representing the chain-extending residues, and two minor resonances, of similar intensity, representing the branching and terminal residues. It is possible that neighboring-group effects could result for residues before and after the branch-point residue, and such an effect would be expected to split, or broaden, the resonances of the chain-extending residues. Such a neighboring-group effect has not been noted for the ^{13}C-n.m.r. spectra of dextrans, although it has been observed for the polysaccharide pullulan[19], which contains different linkage types [*e.g.*, α-D-(1→4)- and -(1→6)-linkages] incorporated directly into the back-bone chain. As the ^{1}H-n.m.r. resonances are less

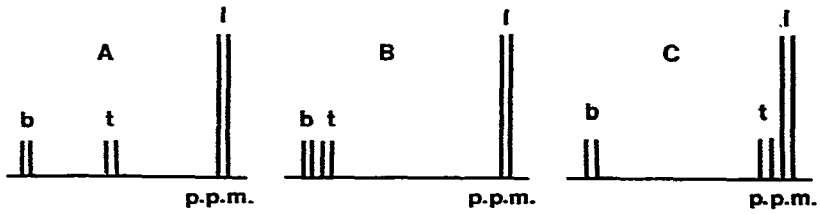


Fig. 1. A schematic representation of the ^{1}H-n.m.r. anomeric-resonance (~5–6-p.p.m.) region, showing possible sets of anomeric-resonance doublets that can arise on introduction of a branch-point into linear dextran. (The double lines represent resonances from branch-point (b), terminal (t), and linear (l) residues. In all cases, resonances b and t can be interchanged without affecting the discussion.)

resolved, and apparently less dependent on chemical environment, than the ^{13}C-n.m.r. resonances of the corresponding dextran, it was assumed that such neighboring-group effects are negligible.

The ^{13}C nuclei are essentially decoupled from the attached protons under the conditions used for recording ^{13}C-n.m.r. spectra, and each ^{13}C nucleus gives a single resonance. For ^{1}H-n.m.r. spectroscopy, such decoupling is impractical; therefore, each proton is coupled to protons held by adjacent carbon atoms, with complex resonance-splitting as the result. Fortunately, the anomeric-hydrogen resonances are not only displaced downfield from the other proton resonances of the saccharide, but, due to the presence of a single, adjacent hydrogen atom (H-2), the anomeric-hydrogen resonances are split into rather closely spaced (~0.1 p.p.m.) doublets. In general, the relative areas of the ^{1}H-n.m.r. resonances correspond to the percentages of protons present in the compound which gives rise to the various resonances. Attempts have been made to correlate the degree of branching of a dextran to the ratio of the areas of anomeric resonances in the spectra of polysaccharides. However, several effects must be considered before such data can be directly interpreted.

The generalized structure of a branched dextran is depicted in **1**, where Glc

$$-[(1\rightarrow6)\text{-}\alpha\text{-Glc}^{b}\text{-}\{(1\rightarrow6)\text{-}\alpha\text{-Glc}^{l}\text{-}\}_{n}\text{-}]_{x}\text{-}$$
$$\overset{m}{\uparrow}$$
$$\alpha\text{-Glc}^{t}\text{-}1$$

1

represents the D-glucopyranosyl residue; *n*, the number of D-glucopyranosyl residues between branch-points; *m*, the number of the carbon atom at the position of branching; *b*, a branch-point residue; *t*, a terminal group, and *l*, a linear-chain-extending residue. Such a branched dextran could exhibit three of the possible ^{1}H-n.m.r.-spectral, anomeric-hydrogen resonance patterns shown in Fig. 1, where each type of anomeric hydrogen atom is represented as a doublet, and the symbols *l*, *b*, and *t* respectively identify the resonances corresponding to the linear-chain-extending residues, branch-

point residues, and terminal residues. At low resolution, pattern A is readily distinguishable from patterns B and C, but the last two patterns are not readily distinguishable from one another. For polysaccharides of identical degree of branching, where one polysaccharide has anomeric-resonance pattern A, and the other, pattern B, the

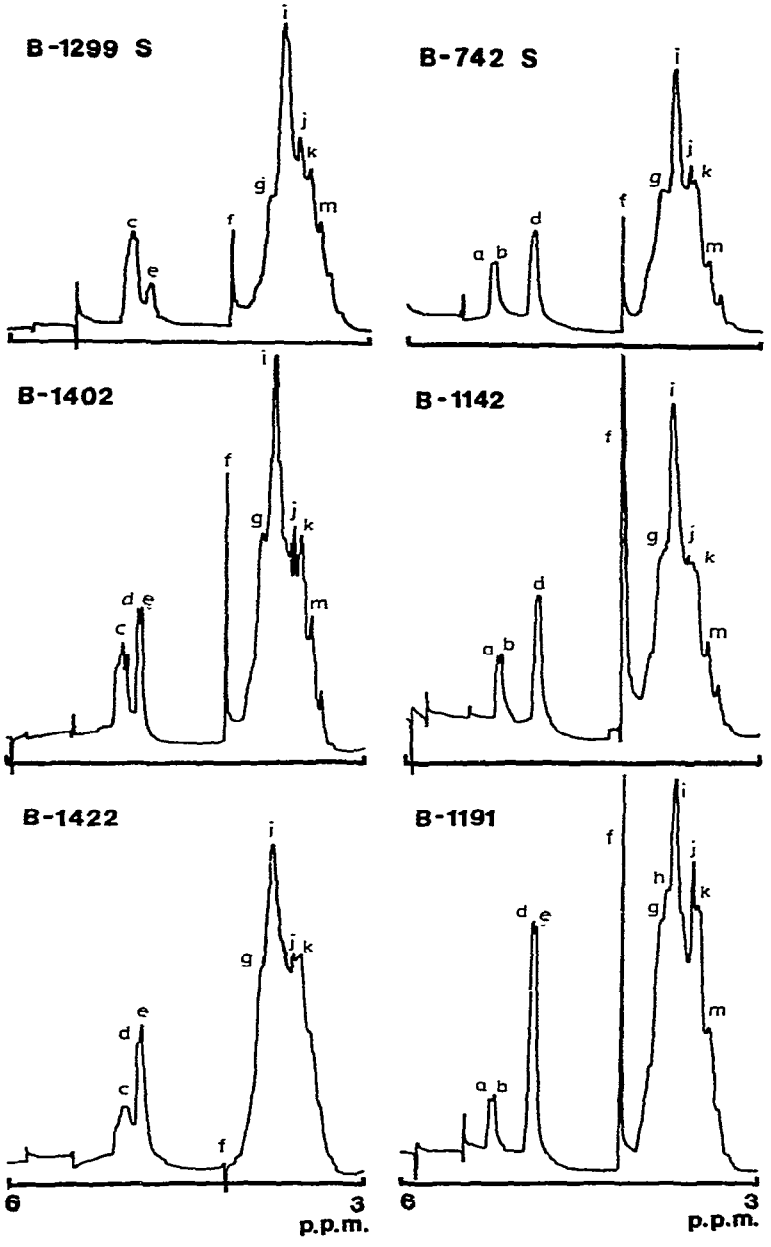


Fig. 2. The 3–6-p.p.m. region of the ^{1}H-n.m.r. spectra of dextran B-1299 fraction S, dextran B-1422, dextran B-1402, dextran B-742 fraction S, dextran B-1142, and dextran B-1191 at 90°.

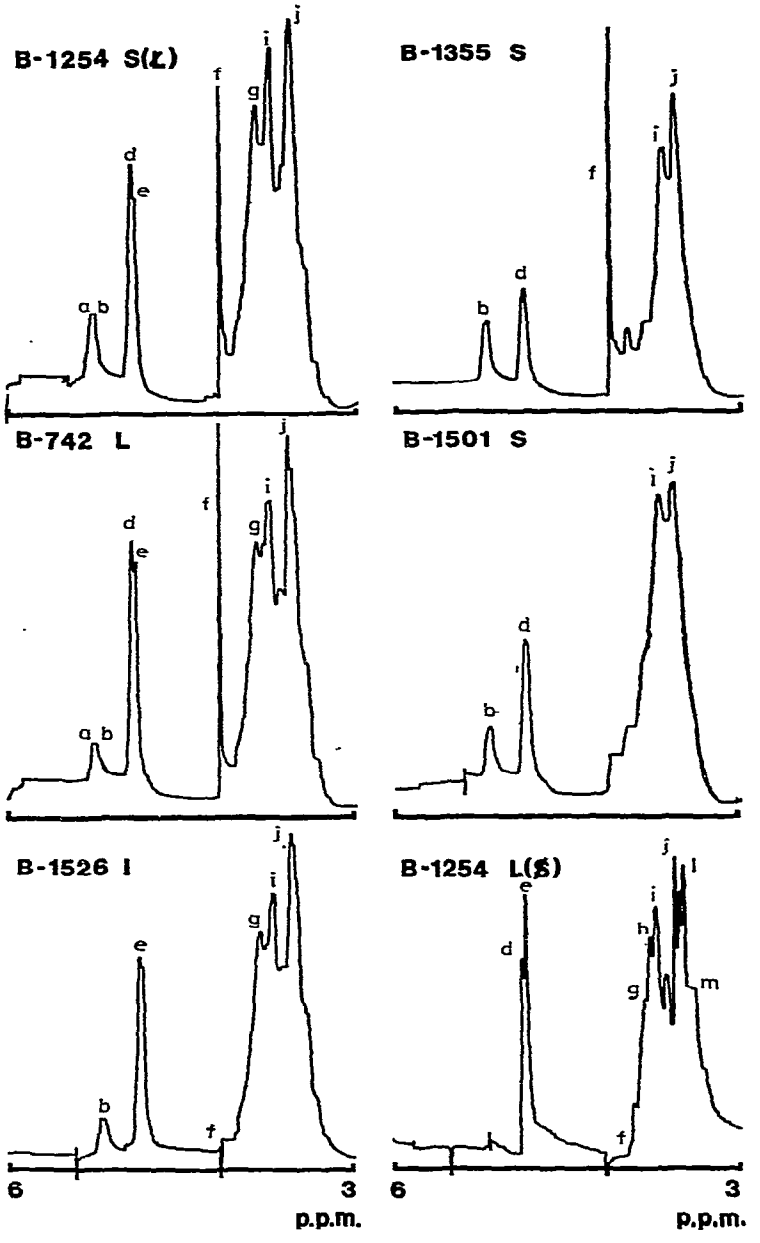


Fig. 3. The 3–6-p.p.m. region of the ^{1}H-n.m.r. spectra of dextran B-1254 fraction S[Ł], dextran B-742 fraction L, dextran B-1526 fraction I, dextran B-1355 fraction S, and dextran B-1254 L[Ś] at 90°.

ratio of resonance integrals (and assumed degree of branching) could differ by a factor of two. For ^{13}C-n.m.r. spectra, it has been observed that change in m (2, 3, or 4) can greatly alter the pattern of anomeric resonances. Therefore, to establish the degree of branching of dextrans by such resonance fractions or ratios, it is necessary to have some understanding of the origin and nature of these resonances.

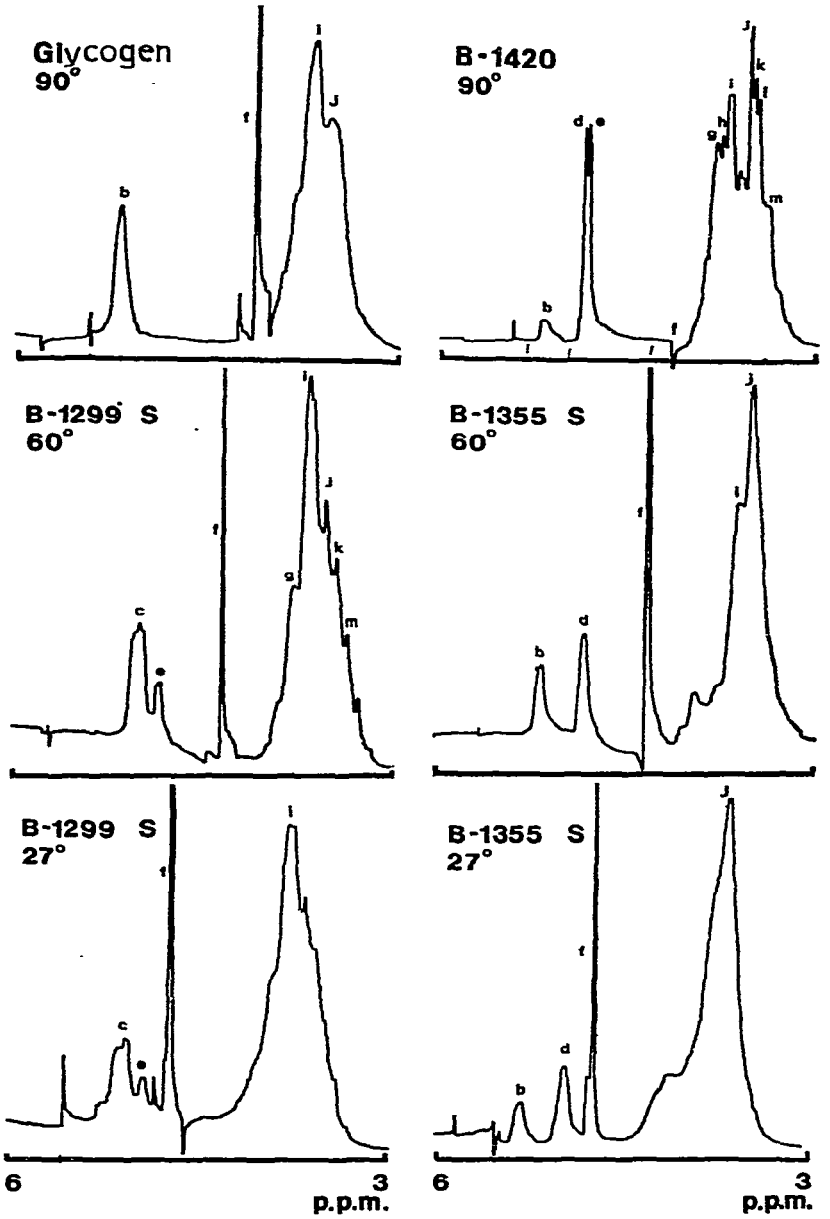


Fig. 4. The 3–6-p.p.m. region of the ^{1}H-n.m.r. spectra of rabbit-liver glycogen and dextran B-1420 at 90°. In addition, the 3–6-p.p.m. region of the ^{1}H-n.m.r. spectra of dextran B-1299 fraction S and dextran B-1355 fraction S at 27° and 60° are given.

RESULTS AND DISCUSSION

Dextran fractions from NRRL strains *Leuconostoc mesenteroides* B-742, B-1299, B-1355, and B-1501, *Streptobacterium dextranicum* B-1254, *Streptococcus* sp. B-1526,

TABLE I

CHEMICAL SHIFTS FOR ^{1}H-N.M.R. SPECTRA OF DEXTRANS[a] AT 90°

NRRL strain	*Dextran fraction*	*Resonance identification-letter*[b]											
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*
B-1299	S			5.03		4.87	4.17	3.85		3.73	3.61	3.52		3.43
B-1402				5.06[c]	4.93	4.89	4.17	3.87		3.77	3.60	3.55		3.46
B-1422				5.08	4.92	4.90	4.17	3.86		3.77	3.60	3.53		
B-742	S	5.26	5.23		4.92		4.17	3.85		3.75	3.63	3.58		3.46
B-1142		5.26	5.23		4.91		4.17	3.84		3.75	3.63	3.60		3.46
B-1191		5.26	5.22		4.92	4.89	4.17	3.86	3.81	3.75	3.60	3.56		3.46
B-1254	S[Ł]	5.27	5.24		4.92	4.89	4.17	3.87		3.76	3.60			
B-742	L	5.26	5.23		4.92	4.89	4.17	3.86		3.75	3.59			
B-1526	I		5.22			4.89	4.17	3.84		3.73	3.57			
B-1420			5.23		4.90	4.87	4.17	3.84	3.80	3.75	3.57	3.54	3.50	3.46
B-1355	S		5.23		4.92		4.17			3.73	3.63			
B-1501	S		5.23		4.92					3.74	3.62			
B-1254	L[Ś]				4.90	4.89	4.17	3.84	3.79	3.75	3.58	3.54	3.51	3.45

[a]The chemical shift in p.p.m. relative to DSS. [b]These letters are referenced to the corresponding dextran spectra in Figs. 2, 3, and 4. [c]The anomeric region of dextran B-1402 is well resolved, and additional resonances are identified in Fig. 6.

TABLE II

CHEMICAL SHIFTS FOR ^{1}H-N.M.R. SPECTRA OF DEXTRANS[a] AT 27°, 60°, AND 90°

Temperature (degrees)	*Resonance identification-letter*[b]									
	b	*c*	*d*	*e*	*f*	*g*	*i*	*j*	*k*	*m*
Dextran B-1299 fraction S										
90		5.03		4.87	4.17	3.85	3.73	3.61	3.52	3.43
60		5.06		4.90	4.42	3.88	3.76	3.64	3.55	3.46
27		5.12		4.92	4.70		3.77			
Dextran B-1355 fraction S										
90	5.23		4.92		4.17		3.73	3.63		
60	5.26		4.93		4.42		3.74	3.63		
27	5.30		4.95		4.70			3.64		

[a]The chemical shift in p.p.m. relative to DSS. [b]These letters are referenced to the corresponding spectra in Figs. 2, 3, and 4.

and the native dextrans from *L. mesenteroides* B-1142, B-1191, B-1402, B-1422, and *L. dextranicum* B-1420 in D_2O were examined by Fourier-transform (F.t.), ^{1}H-n.m.r. spectroscopy at 90° (see Figs. 2, 3, and 4, and Table I). The recording temperature and solvent were so chosen as to allow compatibility with our published, ^{13}C-n.m.r.

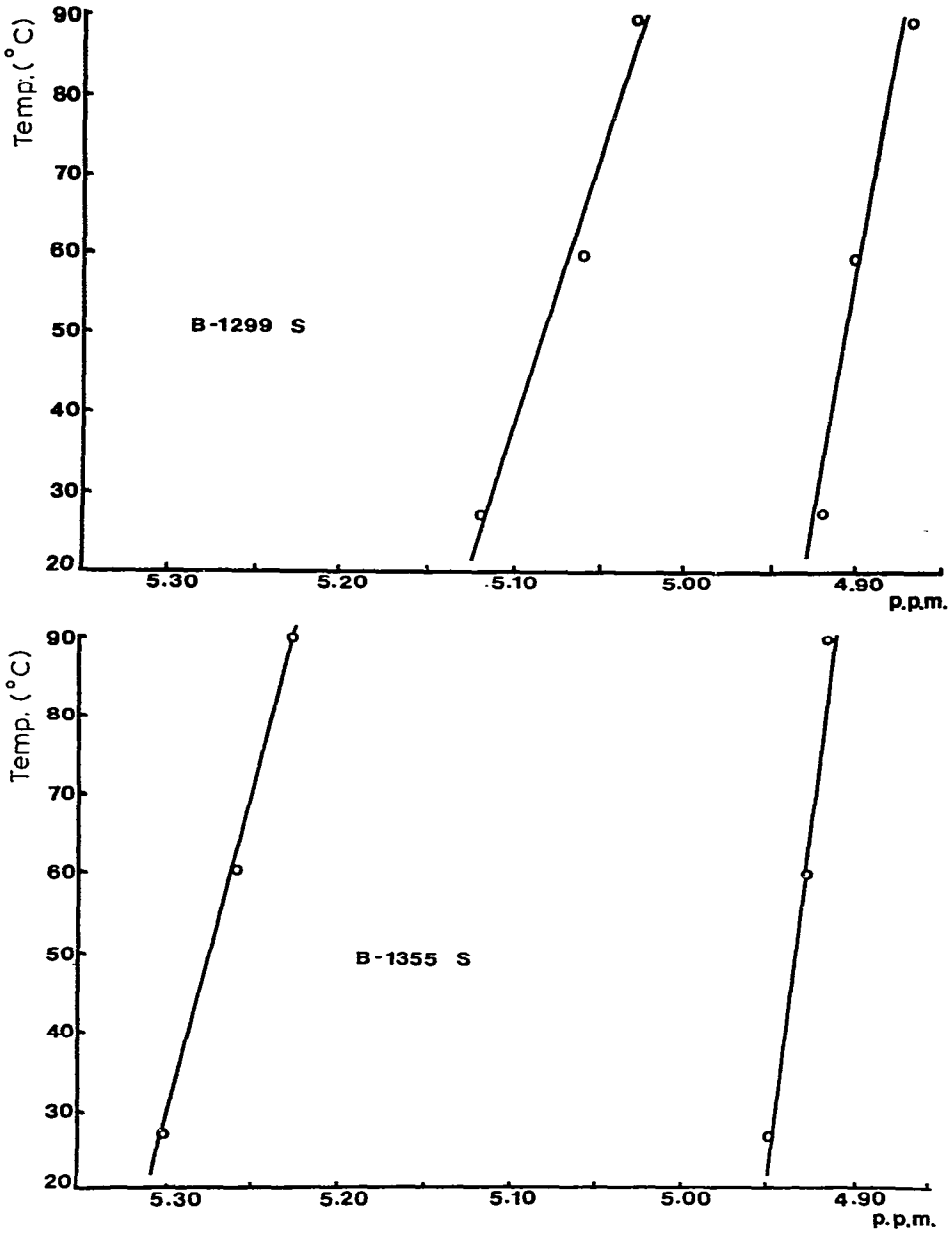


Fig. 5. Temperature dependence (°C) of the chemical shifts for the 4.85–5.35-p.p.m. spectral-region (^{1}H-n.m.r.) of dextran B-1299 fraction S and dextran B-1355 fraction S.

spectra of these dextrans. In addition, Usui *et al.* reported[17] that elevated recording-temperatures sharpen the ^{1}H-n.m.r. lines of D-glucan spectra. To examine the temperature-dependence of ^{1}H-n.m.r. resonances, two D-glucans, namely, dextran B-1299 fraction S and dextran B-1355 fraction S, were also studied at 27° and 60° (see Table II and Fig. 4). The effect of dependence of chemical shift on the recording

temperature ($\Delta\delta/\Delta T$) was plotted for the anomeric resonances of dextran B-1299 fraction S and dextran B-1355 fraction S (see Fig. 5). On comparing the total spectral-width of the ^{1}H- and ^{13}C-n.m.r. spectra[4] of the dextrans, it was found that the $\Delta\delta/\Delta T$ values for these two types of spectroscopy show that changes in recording temperatures have similar effects on the precision of the interpretation of these data. Increase in the recording temperature results in a general improvement in the ^{1}H-n.m.r.-spectral resolution for the polysaccharide (see Figs. 2–4) and a small upfield displacement for each dextran resonance.

The most notable feature of changing the ^{1}H-n.m.r.-spectral recording-temperature is the displacement of the resonance of the deuterium hydroxide, which has a $\Delta\delta/\Delta T$ value $\sim$10 times that of the typical, polysaccharide resonance. This large $\Delta\delta/\Delta T$ value is useful, as the deuterium hydroxide resonance is an undesired and interfering resonance that can obscure critical portions of the ^{1}H-n.m.r.-spectral regions of a dextran. The removal, or suppression, of this deuterium hydroxide resonance is necessary for the utilization of the F.t.-n.m.r. capability to record the spectra of small, or dilute, samples, as, otherwise, this resonance will become relatively large and will obscure the dextran spectrum. We have employed three techniques for suppressing the effects of the deuterium hydroxide resonance.

Firstly, the samples ($\sim$5 mg) were repeatedly dissolved in deuterium oxide ($\sim$1 mL) and then freeze-dried. However, after the third cycle of deuterium oxide treatment, little additional diminution in the intensity of the ^{1}H-n.m.r. resonance for deuterium hydroxide was observed. Secondly, a special, data-acquisition sequence [a modified form of the Water Elimination in Fourier-Transform (WEFT) procedure[20]] was employed. The WEFT procedure is a technique, specific for Fourier-transform acquisition, that permits the suppression of a resonance of a solvent by choosing an appropriate time-delay between pulsing and data acquisition. For very short ($\sim$50 msec) delay times, an "inverted" spectrum results, and, as the delay time is lengthened, the various resonances decrease in a negative manner, become null, and then increase in a positive manner. Due to differences in T_1 values, each resonance will null at a different delay-time, and, as the T_1 value of the ^{1}H-n.m.r. resonance of deuterium hydroxide is greatly different from the T_1 values of dextran, it is possible to choose a data-acquisition delay-time that approximately nulls the deuterium hydroxide resonance, and that has no detectable effect on the relative areas of the resonances of dextran. The spectra in Figs. 2–4 still show the deuterium hydroxide resonance (4.17 p.p.m., at 90°), but this has been lessened to levels acceptable for spectral analysis, and could be further lowered by choosing a pulse delay-time closer to the actual null value; an average, pulse-delay value (3.00 sec) was employed for these spectra, but the actual delay necessary to null the deuterium hydroxide resonance will differ somewhat for different samples, as this delay is dependent on several physical properties (*e.g.* viscosity) of the solution. Thirdly, the deuterium hydroxide resonance-effect can be avoided by employing the afore-described, $\Delta\delta/\Delta T$ effect. By use of the deuterium oxide exchange procedure and the modified WEFT technique, the region of spectral perturbation could readily be

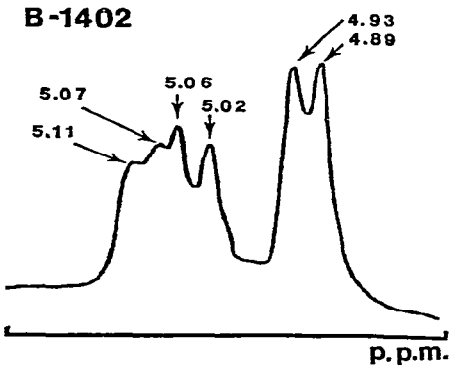


Fig. 6. The expanded, ~4.8–5.2-p.p.m. region of the ^{1}H-n.m.r. (90°) spectrum of dextran B-1402, indicating the chemical shifts (p.p.m.) for the six maxima. (See Fig. 2 for the total spectrum of dextran B-1402.)

lessened to a width of less than 0.2 p.p.m. However, this deuterium hydroxide resonance can be shifted by ~0.5 p.p.m. by changing the recording temperature from 27° to 90°. Therefore, in the presence of a lowered deuterium hydroxide resonance, recording of the ^{1}H-n.m.r. spectrum at 27° and at 90° allows the identification of any saccharide resonance in the 4–5-p.p.m. region.

The dextrans examined by ^{1}H-n.m.r. spectroscopy were so chosen as to provide a broad diversity in both type and degree of branching. The results in Table I show that the resonances in the 3–4-p.p.m. region are similar for all (branched and unbranched) dextrans. The first three dextrans listed in Table I (dextran B-1299 fraction S, dextran B-1402, and dextran B-1422) are examples of dextrans primarily branching through 2,6-di-*O*-substituted α-D-glucopyranosyl residues, where *n* (see **1**) equals, respectively, 0.7, 2, and 3. These spectra, all of dextrans branching through 2,6-di-*O*-substituted residues, contain the anomeric resonance at ~4.90 p.p.m. (resonances d and e) observed in the spectrum of the linear dextran (dextran B-1254 fraction L[$]). However, in addition, these dextrans branching through 2,6-di-*O*-substituted residues also show a resonance at ~5.3 p.p.m. (resonance c). Resonance (d + e) represents the anomeric resonance of the chain-extending residue, and resonance c is much larger than resonance (d + e) for dextran B-1299 fraction S (with *n* ~0.7), indicating that dextrans branching through 2,6-di-*O*-substituted residues have an anomeric spectral-pattern as indicated in Fig. 1, B. An anomeric spectral-pattern of type B for the dextrans branching through 2,6-di-*O*-substituted residues is further suggested by the spectrum of dextran B-1402, which is unusually well resolved (as is the corresponding, ^{13}C-n.m.r. spectrum of this dextran). The anomeric spectral-region of dextran B-1402 (see Fig. 6) shows four partially resolved resonances in the region designated resonance c, with the two upfield and the two downfield resonances separated by ~0.03 p.p.m., a magnitude similar to that observed for the d and e peaks for the linear residue, and further suggesting the B type of spectral pattern. It is of interest to compare this ^{1}H-n.m.r. anomeric-region of dextran B-1402 to the published spectrum[12] of the D-glucan OMZ 176; the anomeric regions of these

spectra are extremely similar, although direct comparison is not possible, due to different recording-conditions (conditions of Meyer *et al.*[12]: 80°, 9 : 1 Me_2SO-d_6–D_2O).

The ^{1}H-n.m.r. spectra of dextrans branching through 3,6-di-*O*-substituted residues (dextran B-742 fraction S, dextran B-1142, and dextran B-1191) differ little from those of dextrans branching through 4,6-di-*O*-substituted residues (dextrans B-1254 fraction S[L], dextran B-742 fraction L, dextran B-1526 fraction I, and dextran B-1420, as all of the spectra contain resonances a and b, in addition to the linear-dextran resonances d and e. In a few cases, the digital slope-detector failed to detect a split resonance, but this was probably due to lack of resolution rather than to any consistent, spectral feature.

Dextran B-742 fraction S, which is highly branched through 3,6-di-*O*-substituted residues, has a spectrum having only two anomeric peaks [(a + b) and c] of approximately equal intensity (see Fig. 2). As dextran B-742 fraction S has been shown by permethylation analysis to have few linear-dextran (6-mono-*O*-substituted) residues, it follows that dextrans branching through 3,6-di-*O*-substituted residues display the anomeric spectral-pattern C of Fig. 1. The two examples of dextran-like

TABLE III

THE RATIOS OF PEAK INTEGRALS FOR ^{1}H-N.M.R. SPECTRA OF DEXTRANS AT 90°

NRRL strain	*Dextran fraction*	m[a]	n[b] *from g.l.c.*	(A1+A2)/N[c]	A2/A1	*Normalized* n *from* A2/A1	*Normalized* n *from* ^{13}*C-n.m.r. spectra*
B-1299	S	2	0.66	0.170	0.365	0.99[d]	0.87[e]
B-1402		2	1.98	0.184	0.730	1.98[d]	1.98[e]
B-1422		2	2.99	0.191	1.36	3.69[d]	4.6[e]
B-742	S	3	0.10	0.184	1.28	0.73[f]	1.53[g]
B-1142		3	1.05	0.172	1.84	1.05[f]	2.23[g]
B-1191		3	2.72	0.191	2.95	1.68[f]	3.42[g]
B-1254	S[L]	4	2.49	0.192	1.95	2.49[h]	1.96[i]
B-742	L	4	5.03	0.196	3.42	4.37[h]	5.03[i]
B-1526	I	4	3.81	0.171	4.46	5.70[h]	4.81[i]
B-1420		4	4.97	0.164	7.04	8.99[h]	8.75[i]
B-1355	S			0.183	1.26		
B-1501	S			0.185	1.78		

[a]The symbol *m* indicates the number of the carbon atom of the D-glucopyranosyl residue through which branching occurs. [b]The symbol *n* indicates the average number of chain-extending, 6-*O*-substituted α-D-glucopyranosyl residues per branch-point residue. [c]The terms **A1**, **A2**, and **N** refer, respectively, to the integrated resonances in the spectral regions 5.06–5.30-p.p.m., 4.75–5.06-p.p.m., and 3.21–4.12-p.p.m. [d]Normalized by multiplying the values in column 6 by 2.71. [e]Data taken from ref. 5; the peak height of the resonance at 98.7 p.p.m. divided by the peak height of the resonance at 97.2 p.p.m. (normalized by × 1.50). [f]Normalized by multiplying 0.57 times the values of column 6. [g]The integral of the resonance at 99.7 p.p.m. divided by the integral of the resonance at 101.0 p.p.m. (F. R. Seymour and R. D. Knapp, unpublished data). [h]Normalized by multiplying the values in column 6 by 1.28. [i]The peak height of the resonance at 99.5 p.p.m. divided by the peak height of the resonance at 101.6 p.p.m. (normalized by × 1.09) (F. R. Seymour and R. D. Knapp, unpublished data).

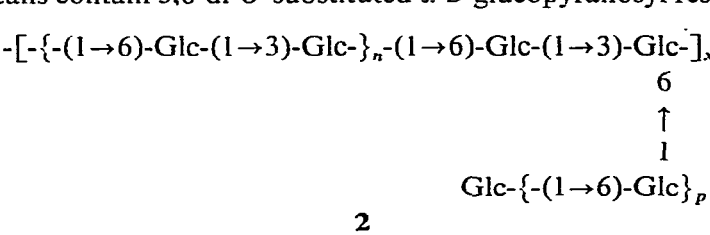
polysaccharides (fractions S of dextrans B-1355 and B-1501) that contain large percentages of 3-mono-*O*-substituted α-D-glucopyranosyl residues display anomeric resonances with chemical shifts similar to those for dextrans branching through 3,6- and 4,6-di-*O*-substituted residues. However, dextran B-1355 fraction S and dextran B-1501 fraction S have ^{1}H-n.m.r. spectra much less resolved than those of other dextrans, no doubt another indication of the increased structural complexity of this class of D-glucans. Unfortunately, we have not identified any dextran that is branched through 4,6-di-*O*-substituted residues which is as highly branched as dextran B-1299 fraction S or dextran B-742 fraction S. However, the general, anomeric-integral ratio *vs.* methylation data (see later) indicate that this type of branching also yields the anomeric spectral-pattern C of Fig. 1.

Figs. 2, 3, and 4 show that the resonances of all branched dextrans may be divided into three groups: anomeric resonances associated only with branched dextrans (resonances a, b, and c, with a collective integral defined as A1), the anomeric resonances associated with unbranched dextran (resonances d and e, with a collective integral defined as A2), and the non-anomeric resonances in the 3–4-p.p.m. region (the g through m resonances, with a collective integral defined as N). Each D-glucopyranosyl residue contains six non-anomeric hydrogen atoms attached to carbon atoms, and one hydrogen atom attached to the anomeric carbon atom. Therefore, if each hydrogen atom of a D-glucopyranosyl residue contributes a resonance of equal intensity to the ^{1}H-n.m.r. spectrum, the fraction (A1 + A2)/N should equal 0.166. Column 5 in Table III shows that this measured resonance-fraction, (A1 + A2)/N, (*a*) is within 15% of the predicted value of 0.166 for all dextrans, and (*b*) provides a general indication for the accuracy of the following A2/A1 fractions. With the exception of linear dextran B-1254 fraction L[$], the spectrum of each dextran in Figs. 2, 3, and 4 shows well defined and clearly separated A1 and A2 peaks. The fraction A2/A1 is tabulated for each dextran spectrum in Table III, column 6, and is compared to the *n* values established by permethylation g.l.c.–m.s. (in column 4) and to the ^{13}C-n.m.r. anomeric-resonance ratios (in column 8). Table III contains three sets of differently branched dextrans, a set of three dextrans having $m = 2$ (see **1**), a set of three dextrans having $m = 3$, and a set of four dextrans having $m = 4$. As already discussed, the anomeric spectral-pattern of a dextran can differ with different types of branching residues, and, therefore, each set of specific, branch-type dextrans was normalized to an *n* value (permethylation g.l.c.–m.s.), for a dextran, of ~2. Such an intermediate value was chosen, as the *n* value by permethylation becomes less precise for dextrans of low degree of branching, due to the imprecision involved in the measurement of small, g.l.c.-peak integrals, and also as it is possible that very highly branched dextrans present special permethylation problems. The normalization procedure was employed as follows: for $m = 2$, dextran B-1402 ($n = 1.98$); for $m = 3$, dextran B-1142 ($n = 1.05$); and for $m = 4$, dextran B-1254 fraction S[K] ($n = 2.49$). The normalization for dextrans having $m = 2$ required a large A2/A1 normalization-factor (2.71), which is in general accord with the expected normalization factor of 2.0 for a type B (Fig. 1) anomeric spectral-pattern.

Dextrans having $m = 3$ required a much smaller A2/A1 normalization-factor (0.57), which is in general accord with the expected normalization factor of 1.0 for a type C (Fig. 1) spectral-pattern. Dextrans having $m = 4$ require an A2/A1 normalization-factor of 1.28, a value near 1.0, which suggests that this series has an anomeric spectral-pattern of type C (Fig. 1).

Comparison of permethylation data with ^{13}C-n.m.r. data for those dextrans branching through 4,6-di-*O*-substituted residues gave ambiguous results with regard to the relative degrees of branching of dextran B-742 fraction L, dextran B-1526 fraction I, and dextran B-1420, these ^{1}H-n.m.r. data being in general accord with the ^{13}C-n.m.r. data. Some question persists about the degree of branching of both dextran B-742 fraction S and dextran B-1299 fraction S. Dextran B-1299 fraction S has a ^{13}C-n.m.r. spectrum essentially identical to the ^{13}C-n.m.r. spectrum of the corresponding L fraction, and permethylation data indicated that $n = 1.0$ for dextran B-1299 fraction L. Similarly, permethylation data indicated $n = \sim 0.1$ for dextran B-742 fraction S, and $n = \sim 1.0$ for dextran B-1142; however, the A2/A1 fraction indicated that these polysaccharides have a more-similar degree of branching. ^{13}C-N.m.r. resonance-intensity relationships are not particularly useful in establishing the precise value of n for these highly branched polysaccharides, as those dextrans branching through 3,6-di-*O*-substituted residues have all of the ^{13}C-n.m.r. resonances that are attributed to the 6-mono-*O*-substituted, chain-extending residue partially obscured by other resonances. However, a high-resolution, convolution-difference, resolution enhancement (c.d.r.e.) processed[21] dextran B-742 fraction S spectrum suggested that $n = \sim 0.3$ for this dextran.

The D-glucans designated dextran B-1355 fraction S and dextran B-1501 fraction S are apparently not true dextrans, as these biopolymers contain large percentages of 3-mono-*O*-substituted α-D-glucopyranosyl residues which are probably incorporated into the backbone chain of the polysaccharide[11] (see **2**). These two D-glucans contain 3,6-di-*O*-substituted α-D-glucopyranosyl residues, in addition to the



(For dextran B-1355 fraction S, $n = 5.1$, $p = 0.7$, and for dextran B-1501 fraction S, $n = 3.4$, $p = 3.9$.)

3-mono-*O*-substituted residues, but no difference could be observed between the anomeric chemical-shifts of the resonances of these residues, nor could anomeric chemical-shift-differences be observed on comparing the spectra of these D-glucans to the spectra of dextrans branching only through 3,6-di-*O*-substituted residues (*e.g.*, dextran B-1142). Although slope detection identifies only one resonance in the ~5.2-p.p.m. region for the dextran B-1355 fraction S type of D-glucan spectrum, this

is probably due to the general lack of spectral resolution for these polysaccharides. The ^{1}H-n.m.r. spectrum of rabbit-liver glycogen, recorded under conditions identical to those used for the dextrans (Fig. 4: b, 5.24 p.p.m.; f, 4.17 p.p.m.; i, 3.72 p.p.m., and j, 3.60 p.p.m.), shows that this dendritically branched D-glucan has ^{1}H-n.m.r. resolution similar to that of the D-glucans depicted in **2**, and that ^{1}H-n.m.r. spectra at this field strength cannot distinguish between the anomeric resonances of 4-mono-*O*-substituted α-D-glucopyranosyl residues (the predominant residues in glycogen) and the 4,6-di-*O*-substituted α-D-glucopyranosyl residues of dextrans of structure **1**, where $m = 4$.

In conclusion, ^{1}H-n.m.r. spectroscopy at 100 MHz provides much less discriminatory ability as regards the structure of polysaccharides than ^{13}C-n.m.r. spectroscopy at similar field-strength. Although dextrans of structure **1**, with $m = 2$, can be readily distinguished from the corresponding dextrans having $m = 3$ and $m = 4$, the last two classes of dextran cannot be readily distinguished by ^{1}H-n.m.r. spectroscopy. However, our alternative, spectroscopic method for structure analysis employing small samples of dextran is F.t.-i.r. spectrometry[1], which is least efficient in distinguishing between dextrans of structure **1** having $m = 2$ and $m = 3$. Therefore, a combination of difference F.t.-i.r. spectrometry and ^{1}H-n.m.r. spectroscopy can provide complementary information on milligram samples. The foregoing discussion and data also show that it is necessary to understand the nature of the anomeric spectral-pattern for a specific type of polysaccharide branching if an estimate of the degree of branching is to be made from anomeric-resonance integrals.

However, the fundamental assumption that specific positions of hydrogen atoms would contribute resonance integrals proportional to their percentages present in the polysaccharide may not be correct; or, conversely, our previous estimation of degree of branching based on permethylation analysis and ^{13}C-n.m.r. may, in some cases, be incorrect. Even when differences in anomeric spectral-patterns are considered, a relationship close to 2, 1, 1 would be expected for the ^{1}H-n.m.r., anomeric normalization-factors for dextran types having, respectively, $m = 2$, 3, and 4, rather than the 2.7, 0.6, 1.3 relationship actually found; this difference in normalization factors required for dextrans of different branch-types suggests that some anomeric hydrogen atoms do not contribute proportionally to the spectral integrals. Finally, these ^{1}H-n.m.r. data for these polymers, when compared to our previous structural determinations by alternative methods, again show that no single method for the determination of structure can provide complete insight into the structure of a specific class of polysaccharides. Furthermore, the cross-referencing of data provided by a variety of structural methods and afforded by a variety of different, but structurally similar, polysaccharides would seem to be the best method for ultimately establishing the structure of any specific polysaccharide.

EXPERIMENTAL

The preparation and characterization of the dextrans[22] and dextran fractions[23]

has been described. The rabbit-liver glycogen was obtained from Sigma Chemical Co., St. Louis, Missouri.

^{1}H-N.m.r. spectra were obtained in the F.t. mode with a Varian XL-100-15 n.m.r. spectrometer equipped with a Nicolet TT-100 data system. A data-collection delay-time of 100 μsec was utilized, to prevent spurious signals from pulse ringdown. Dextran samples ($\sim$5 mg) were dissolved in 99.8% deuterium oxide (0.8 mL). Solvent deuterium was used for the field lock, and the spectra were measured at 100.1 MHz. Chemical shifts are reported relative to internal 3-(trimethylsilyl)propanesulfonic acid sodium salt (DSS). To suppress the deuterium hydroxide signal, the following pulse sequence was used in acquiring each spectrum: [180°-T-90°-0.5 sec] (where T equals 2.75 to 3.30 sec). A spectral width of 600 Hz, with 4,096 data points and 64 acquisitions, was employed, the samples being spun at 50 Hz in 5-mm tubes. Temperatures were controlled by the Varian temperature-controller to within ± 1°.

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