Carbohydrate Research, 81 (1980) 105–129

STRUCTURAL ANALYSIS OF DEXTRANS, FROM STRAINS OF LEUCONOSTOC AND RELATED GENERA, THAT CONTAIN 3-*O*-α-D-GLUCOSYLATED α-D-GLUCOPYRANOSYL RESIDUES AT THE BRANCH POINTS, OR IN CONSECUTIVE, LINEAR POSITIONS*

FRED R. SEYMOUR,

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

AND 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.)

(Received September 25th, 1979; accepted for publication, October 16th, 1979)

ABSTRACT

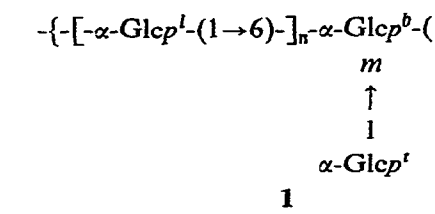
Dextran fractions from NRRL strains *Leuconostoc mesenteroides* B-523, B-742, B-1149, *Betabacterium vermiforme* B-1139, and *Streptococcus viridans* B-1351 were examined by ^{13}C-n.m.r. spectroscopy. The native, structurally homogeneous dextrans from NRRL strains of *L. mesenteroides* B-1191, B-1192, and B-1142 were also examined by ^{13}C-n.m.r. spectroscopy, and the spin–lattice relaxation values of dextran B-1142 were measured. Methylation–fragmentation, structural analyses were performed on dextrans B-1142 and B-1191. Except for the A fractions of dextrans B-523 and B-1149, all of these dextran fractions differ from linear dextran by branching primarily, if not exclusively, through 3,6-di-*O*-substituted α-D-glucopyranosyl residues. Dextran B-1149 fraction A is spectroscopically different from the dextrans branching through the 3,6-di-*O*-substituted residues, and apparently contains significant mole percentages of contiguously linked 3-mono-*O*-substituted α-D-glucopyranosyl residues.

INTRODUCTION

We have previously applied methylation–fragmentation analysis (employing g.l.c.–m.s.)[2,3] and ^{13}C-n.m.r.-spectrometric analysis[4,5] for a general survey of the extracellular, microbial dextrans produced by bacteria in the ARS Culture Collection at the Northern Regional Research Center. The results of an initial, structural survey of this dextran collection, based primarily on periodate-oxidation studies[6], were used to direct the subsequent g.l.c.–m.s. and ^{13}C-n.m.r.-spectrometric survey, and many of the dextrans studied in the second survey are listed in Table I of ref. 7.

*Unusual Dextrans, Part XIII. For Part XII, see ref. 1.

Most of the dextrans that have been studied can be described in terms of the general repeating-unit **1**, where Glc*p* represents a D-glucopyranosyl residue or group; *n*, the



1

number of D-glucopyranosyl residues between branch-point residues; and *m*, the carbon atom number of the position of branching (*O*-substitution); and *t* identifies a terminal group; *b*, a branch-point residue; and *l*, a linear, chain-extending residue.

We originally employed **1**, on a hypothetical basis, for the correlation and comparison of structural data obtained by different methods. However, in Part XII of this series[1], a variety of data from different sources and for different dextrans were surveyed, and it was concluded that **1** may truly be an accurate representation for many dextrans. It should be noted that *n* represents an *average* distance between branch-point residues (*b*-residues), and our current methods provide little information on the frequency distribution of specific *n* values for any given polysaccharide. A side chain of a single residue is shown in **1**, and although such a structure is most compatible with our current, ^{13}C-n.m.r., chemical-shift and spin–lattice relaxation data for dextrans, it is possible that longer side-chains exist in some, or all, of these polysaccharides. We have found no dextrans that contain large percentages of branching through different *m* values in the same dextran, and few dextrans that contain spectroscopically significant percentages of residues branching through a second *m* type of residue. Most of the ^{13}C-n.m.r. spectra recorded for these dextrans are capable of identifying branch-point residues at ~5 mole percent, and **1** therefore represents three major classes of dextran, having $m = 2$, 3, or 4. We have previously discussed dextrans and dextran fractions that belong to the class[8] of **1**, $m = 2$, and also to the class[9] of **1**, $m = 4$. We now deal with compounds represented by **1** when $m = 3$, namely the last of these three groups.

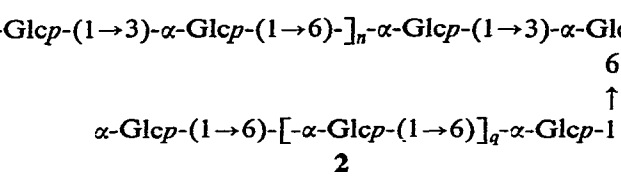
For brevity, and simplicity of expression, we now propose to group the dextrans into a limited number of classes based on operational definitions. Dextrans that deviate from linear dextran by the presence of di-*O*-substituted α-D-glucopyranosyl residues will be described as class I dextrans. The group of class I dextrans can be further divided on the basis of the carbon-atom number of the branch-point position of the di-*O*-substituted residue [*e.g.*, a class I ($m = 2$) dextran refers to a dextran deviating from linear dextran by branching occurring through 2,6-di-*O*-substituted α-D-glucopyranosyl residues]. Class I dextrans can operationally be identified by the presence of di-*O*-methyl derivatives of D-glucose in the hydrolyzate of a permethylated dextran, with 3,4-di-*O*-methyl derivatives indicating a class I ($m = 2$) dextran, 2,4-di-*O*-methyl derivatives indicating a class I ($m = 3$) dextran, and 2,3-di-*O*-methyl derivatives indicating a class I ($m = 4$) dextran. Traces (less than 3 mole

percent) of di-*O*-methyl derivatives in the chromatogram of the hydrolyzate are not considered to have significance in this classification. ^{13}C-N.m.r. spectrometry has been referenced to the methylation–fragmentation, structural-analysis data for dextrans, and the diagnostic resonances of the 75–85-p.p.m. region of the ^{13}C-n.m.r. spectra (unless otherwise stated, all ^{13}C-n.m.r. values refer to recording conditions at 90° for aqueous solutions) indicated a correspondence to the dextran classes as follows: ~77.8 p.p.m., class I (m = 2) dextran; ~80.4 p.p.m., class I (m = 4) dextran; and ~82.9 p.p.m., class I (m = 3) dextran.

For those examples of extracellular dextran that have been obtained from various strains of *L. mesenteroides* and related genera, and have been structurally studied, the following generalizations can be made. Most dextrans known to contain (1→2)- or (1→4)-linkages can be described in terms of **1**, where m = 2 or m = 4; dextran B-1254 fractions S[L] is the exception, and may contain significant (>2%) proportions of 4-mono-*O*-substituted residues. However, dextrans containing (1→3)-linkages display greater diversity in structure, although all current evidence indicates that all such linkages are present as (1→3)-α-D-linkages.

The class I (m = 3) dextrans differ from the class I (m = 2) dextrans and class I (m = 4) dextrans in several ways. Firstly, structural analysis by acetolysis, which is more sensitive than ^{13}C-n.m.r. or methylation–structural analysis to traces of non-6-mono-*O*-substituted residues, indicates that many (or possibly all) extracellular dextrans contain (1→3)-α-D-linkages[10]. Unfortunately, methylation–fragmentation, structural analysis is not a good method for the confirmation of traces of (1→3)-α-D-linkages, as OH-3 of an α-D-glucopyranosyl residue tends to resist complete *O*-methylation[3]. Secondly, class I (m = 3) dextrans having a moderate (~5%) content of 3,6-di-*O*-substituted residues have been extensively studied[11]; for example, *L. mesenteroides* dextran NRRL B-512(F). Thirdly, with the exception of dextran B-742 fraction S (which has been described in previous parts of this series), these highly branched, class I (m = 3) dextrans have been the subject of few investigations, in contrast to the relatively extensive studies on class I (m = 2) dextrans and class I (m = 4) dextrans (see refs. 8 and 9, and references cited therein). And, finally, the greater diversity of dextran structures into which the (1→3)-α-D-linkage [as contrasted to the (1→2)-α-D- and the (1→4)-α-D-linkages] has been found incorporated has contributed difficulties to the identification of class I (m = 3) dextrans.

Several dextrans (*e.g.*, dextran B-1355 fraction S) yielded periodate-oxidation data that indicated relatively large percentages of (1→3)-α-D-linkages, but were later shown to contain relatively large percentages of 3-mono-*O*-substituted α-D-glucopyranosyl residues, in addition to 3,6-di-*O*-substituted α-D-glucopyranosyl residues (see ref. 15, and references therein). It was also concluded that the 3-mono-*O*-substituted residues were not consecutively linked, and that excess 6-mono-*O*-substituted residues were consecutively linked, and situated in side chains[1]. The structure of this group of polysaccharides, as exemplified by dextran B-1355 fraction S, can be represented by **2**; for convenience and brevity, this group



of dextrans (which also includes the S fractions of dextran B-1498 and dextran B-1501) may be referred to as class II dextrans. In terms of an operational definition, class II dextrans yield (*a*) significant proportions of 2,4,6-tri-*O*-methyl-α-D-glucose in the hydrolyzate from such a permethylated polysaccharide, and (*b*) ^{13}C-n.m.r. spectra containing diagnostic resonances at ~83.3 and ~101.5 p.p.m.

It is also possible to differentiate class I ($m = 3$) dextrans from class II dextrans on the basis of immunochemical reactions[12] and by Fourier-transform, infrared difference-spectrometry[13], but not by ^{1}H-n.m.r. spectrometry[14] at 100 MHz. Furthermore, the difficulties involved in the structural analysis of dextrans containing (1→3)-linkages are increased by the possible existence of yet another group of dextrans, exemplified by dextran B-1149 fraction A, which will be further discussed in a subsequent section herein.

Examination of ^{13}C-n.m.r. spectra obtained for class I ($m = 2$) dextrans, class I ($m = 4$) dextrans, and class II dextrans indicated that, in addition to the six resonances of linear dextran, the spectra of each class of dextrans contain diagnostic resonances that differ for each class of dextrans, and that are essentially invariant (a $\Delta\delta$ range of ~0.04 p.p.m.) in terms of chemical shift, but that have intensities (relative to the resonances of linear dextran) that are proportional to the degree of branching for each dextran[8,9]. As branching from the linear dextran structure results in two new residues, the *b*- and *t*-residues, it is anticipated that each type of branching for class I dextrans could result in 12 (2 × 6) diagnostic resonances. Two class I ($m = 3$) dextrans have been spectroscopically examined[15], namely, the very highly branched dextran B-742 fraction S and the relatively linear dextran B-1351 fraction L. These preliminary data indicate that the spectra of class I ($m = 3$) dextrans contain diagnostic resonances that differ from those in the spectra of the other class I dextrans and the class II dextrans.

Previous, structural investigations on the dextrans produced by the NRRL strains discussed herein include acetolysis studies of dextran B-523 and dextran B-1149 fraction A by Suzuki and Hehre[10], the initial examination of the ^{13}C-n.m.r. spectrum of dextran B-742 fraction S by Colson *et al.*[6], and the use of isomaltodextranase by Sawai *et al.*[17] to examine dextran B-1191 and dextran B-742 fraction S. The NRRL dextrans have also been extensively studied by immunochemical techniques, and we have previously summarized many of these observations in terms of the major, structural features of these polysaccharides now known[2,8,15]. Dextran B-742 fraction S (which has also been described as fraction C3R) and dextran B-1142 were the representative examples of class I ($m = 3$) dextrans employed in these immunochemical studies[18–22]. The general literature pertaining to immunology and dextrans has also

been collated in Part XIX of ref. 11. In addition to dextrans from NRRL strains, there have been other reports of highly branched polymers that apparently fulfil the definition of class I ($m = 3$) dextrans. Preobrazhenskaya *et al.* (see ref. 23, and references cited therein) described the production, from a strain of *L. mesenteroides*, of dextran 44b-2, for which both periodate-oxidation and methylation–fragmentation data indicated the structure to be **1** with $m = 3$ and $n = \sim 3.0$. Ito and Schuerch[24] reported the chemical synthesis of polysaccharides branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues, and recorded the ^{13}C-n.m.r. spectra of these compounds. Dextrans containing 3,6-di-*O*-substituted α-D-glucopyranosyl residues, and of relatively high degree of branching, have been identified as the soluble fractions in the *in vitro* production of dextran by enzyme systems from *S. mutans* by Hare *et al.*[25], who established, by methylation–fragmentation, structural analysis, that the D-glucan OMZ 176 GTF-S corresponds to **1** with $m = 3$, and $n = \sim 1.1$.

^{13}C-N.m.r. assignments of resonances to the carbon-atom position (or to the residue type) have also been made by use of spin–lattice relaxation measurements[1,26,27] (T_1 values). In general, it has been assumed that the dipole–dipole, spin–lattice relaxation value (T_1^{DD}) of a resonance is proportional to the segmental motion of the carbon-atom position associated with that resonance, and that, for polysaccharides, the T_1^{DD} value is proportional to the observed spin–lattice value (T_1^{obs}). Therefore, the T_1^{obs} value obtained for each resonance is assumed to correspond to the relative degree of motion of the carbon-atom position associated with that resonance, and (in general) resonances having large T_1^{obs} values are assigned to carbon-atom positions of residues in side chains.

RESULTS AND DISCUSSION

Data from nine homogeneous dextrans or dextran fractions have been examined. These dextran fractions had been produced by NRRL strains *Leuconostoc mesenteroides* B-523, B-742, B-1149, *Betabacterium vermiforme* B-1139, and *Streptococcus viridans* B-1351. The structurally homogeneous dextrans were produced by NRRL strains of *L. mesenteroides* B-1191, B-1192, and B-1142.

Class I (m = *3*) *dextrans*

Methylation–fragmentation analysis. — G.l.c.–m.s. data are currently available for four dextrans (see Table I) that are known to differ from linear dextran by yielding chromatograms, from the hydrolyzates of the permethylated polysaccharides, that contain significant proportions of 2,4-di-*O*-methyl derivatives, and therefore fulfil the operational definition of a class I ($m = 3$) dextran. Dextrans B-1142 and B-1191 were permethylated, and the products hydrolyzed, in parallel with other polysaccharides. These dextrans resisted permethylation and were only slowly hydrolyzed, in contrast both to highly branched mannans and (the dendritically branched) glycogen. Although minor components of *O*-methylated dextrans afford products present in

TABLE I

MOLE PERCENTAGE OF METHYLATED D-GLUCOSES IN HYDROLYZATES OF METHYLATED DEXTRANS[a]

NRRL strain	*Dextran fraction*	*Methyl ethers of D-glucose*					
		2,3,4,6	*2,3,4*	*2,4,6*	*2,3*	*2,4*	*3,4*
B-742[b]	S	45.3	4.4			50.2	
B-1142		33.3	35.0	1.3		30.4	
B-1191		21.8	59.6		0.8	17.2	0.6
B-1351[c]	S	5.8	83.3			10.5	0.3

[a]The mole percentages are expressed as the uncorrected areas of the peaks in the chromatogram of the PAAN derivatives, employing a hydrogen-flame detector. [b]Data taken from ref. 3. [c]Data taken from ref. 2.

the chromatograms of the hydrolyzates thereof, it is possible that these minor components represent a small degree of incomplete methylation, rather than traces of repeating units described by **1**, $m = 3$. Both dextran B-742 fraction S and dextran B-1351 fraction S were previously analyzed[2,3] by processes of permethylation, hydrolysis, and g.l.c.–m.s. essentially identical to those employed for dextrans B-1142 fraction A and dextran B-1191.

^{13}C-Nuclear magnetic resonance spectrometry. — Spectra have been recorded at both 34° and, for increased resolution and shorter acquisition times, at 90°, for solutions in deuterium oxide. Unless specifically noted, the discussion of the ^{13}C-n.m.r. spectra will refer to 90° recording-conditions. ^{13}C-N.m.r. spectra were recorded for dextrans B-1142, B-1191, and B-1192, and also for dextran B-1139 fraction S, dextran B-1149 fraction A, and dextran B-1351 fraction L (see Table II, and Figs. 1 and 2). In addition, Table II and Figs. 2 and 3 contain data for B-1351 fraction S. The initial, ^{13}C-n.m.r. investigation[16] of dextran B-742 fraction S (the fraction was not identified, but the original material was from a source identical to the S fraction studied herein) indicated, by comparison (at pH 14) to a relatively insoluble, (1→3)-α-D-linked D-glucan from *Penicillium patulum*, that this dextran contained (1→3)-α-D-linkages. The resonances in Table II and Figs. 1 and 2 are referenced to the identifying numbers employed in Part XII of this series[1]. We have previously described our technique of cross-referencing chemical-shift data that have been recorded at different times[9]; however, the majority of the data in Table II were acquired in a single time-period of less than a week, and are uncorrected. With the exception of dextran B-1149 fraction A, only a limited number of diagnostic chemical-shifts are present in the spectrum of each polysaccharide listed in Table II, and an inspection of any line in Table II indicates that few sets of resonances deviate by more than ±0.03 p.p.m. from an average value. As dextran B-1142, dextran B-1191, dextran B-742 fraction S and dextran B-1351 fraction S have been shown by methylation–fragmentation analysis to contain the residues corresponding to **1**, $m = 3$, it is there-

TABLE II

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

		Dextran							
		B-742 Fraction	*B-1142*	*B-1191*	*B-1192*	*B-1139*	*B-1351*	*B-1351*	*B-1149*[b]
		S				*S*	*L*	*S*	*A*
		Acquisitions[c]							
		16	*4*	*6*	*4*	*8*	*12*	*8*	*208*[d]
	5[e]	100.81(250)[f,g]	100.88(253)[f]	100.88	100.87	100.87	100.87	100.88	100.96
A[h]	7	99.56(150)	99.50(152)	99.50	99.50	99.50	99.48	99.48	99.50
	8								98.16[i]
	9								97.29[i]
	11	82.89(110)	82.81(125)	82.87	82.84	82.84	82.82	82.85	
	11a								82.49
	15								77.71[i]
B	16	75.09(140)	75.13(186)	75.14	75.13	75.14	75.15	75.17	75.13
	17	74.79(180)	74.78(226)	74.79	74.78	74.79	74.79	74.79	74.78
	21[j]	73.57(180)	73.58	73.61				73.61	
	22[j]	73.50	73.51(211)	73.55	73.55	73.50	73.52	73.50	73.45
C	24	73.14(140)	73.15(180)	73.17	73.15	73.15	73.16	73.18	73.15
D	27		71.79s[k]	71.97	71.93	71.97	71.95	71.98	71.97
	28	71.74(110)	71.83(129)	71.83s				71.80s	
E	29		71.57(130)	71.57	71.57	71.59	71.58	71.59	71.56
	29a	71.41(170)	71.42s					71.40s	
F	32	67.67(63)	67.72(83)	67.76	67.72	67.62	67.74	67.77	67.76
	33	62.49(206)	62.45(231)	62.46	62.45	62.40	62.42	62.45	62.41

[a]The spectra are expressed in p.p.m. relative to tetramethylsilane. [b]The (1→3)-α-D-linkages of this compound are probably not with 3,6-di-*O*-substituted residues. [c]Acquisitions in thousands. [d]Fig. 2 shows an additional spectrum of 16,000 acquisitions. [e]These numbers were assigned in ref. 1, and are referenced to resonances in Figs. 1, 2, 3, and 5. [f]The numbers in parentheses refer to T_1^{obs} values (in ms) for the corresponding resonances. [g]Data taken from ref. 1. [h]The letters A through F designate the major resonances of linear dextran (see ref. 4). [i]These resonances indicate small percentages of (1→2)-α-D-linkages (see ref. 8). [j]Resonances 21 and 22 could not be resolved for individual T_1 measurements. [k]The letter s designates a shoulder, or weak resonance.

fore concluded that all dextrans listed in Table II (except for dextran B-1149 fraction A) are class I ($m = 3$) dextrans. The spectra listed in Table II contain no resonances identifiable with di-*O*-substituted residues having $m = 2$, or $m = 4$. Dextran B-1149 fraction A and dextran B-523 fraction A apparently do not belong to the class I ($m = 3$) dextran group, and will be discussed separately.

High-resolution spectra, processed by convolution-difference, resonance-enhancement[28] (c.d.r.e.) techniques, show the persistence of well defined, diagnostic resonances in the spectra of class I ($m = 3$) dextrans when n becomes large. Such consistency in the chemical shifts of these diagnostic resonances, even when such resonances dominate the spectrum of a highly branched polysaccharide, can be observed in the expanded-scale, 69–76-p.p.m. region of the spectra in Fig. 3 and

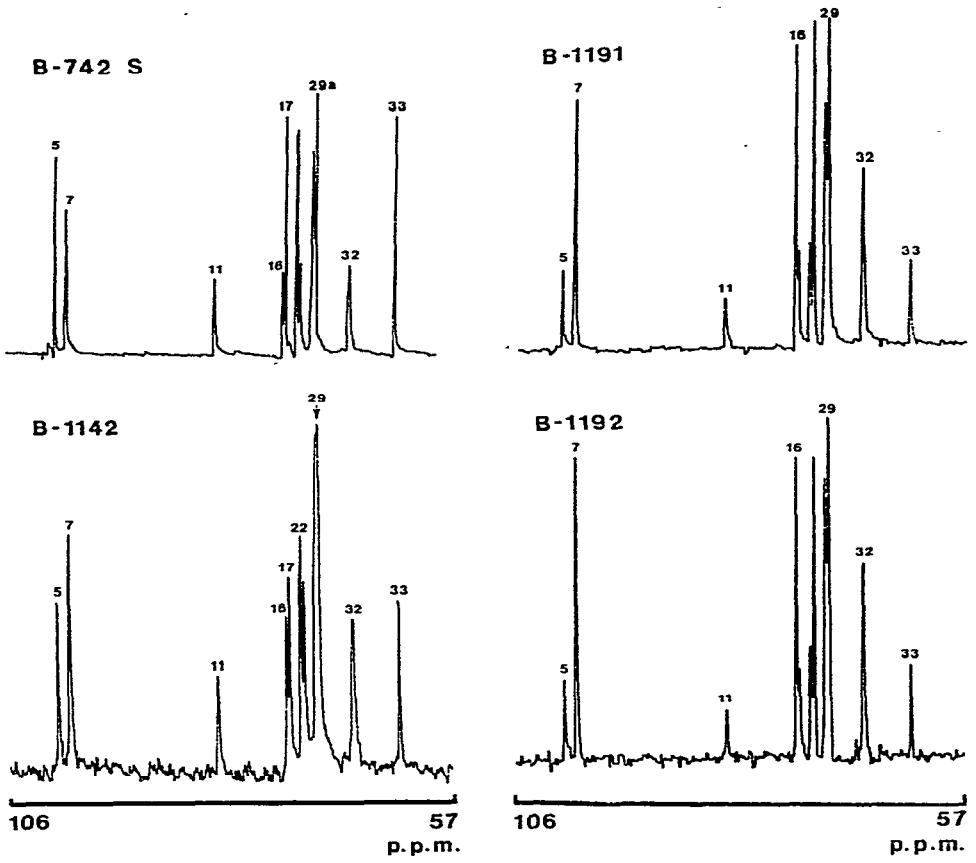


Fig. 1. ^{13}C-N.m.r. spectra, at 90°, of dextran B-742 fraction S, dextran B-1142, dextran B-1191, and dextran B-1192. (The number of acquisitions for each spectrum is listed in Table II. Except for those of dextran B-1142, all data were processed by c.d.r.e. before plotting. The identifying resonance-numbers are referenced to the resonances in Table II.)

Table II. For example, all of the spectra given in Fig. 3 show the closely separated resonances 21 and 22, although relatively long acquisition-times and c.d.r.e. processing are necessary in order to separate these resonances clearly. The presence of these well defined, minor resonances in the spectra of class I ($m = 3$) dextrans having a low degree of branching, and the absence of additional, minor resonances from the spectra of highly branched, class I ($m = 3$) dextrans, indicate that neighboring-residue effects are not evident for the resonances associated with residues lying immediately before or after a *b*-residue. We have previously discussed this general lack of neighboring-residue effect, which has been observed for all class I ($m = 2$, 3, or 4) dextrans, and concluded that such an effect is compatible for dextrans having backbone chains composed exclusively of (1→6)-α-D-linked D-glucopyranosyl residues[1].

The dextrans of intermediate *n* value provide a convenient reference for comparing the resonances of highly branched dextrans with those of the dextrans of low

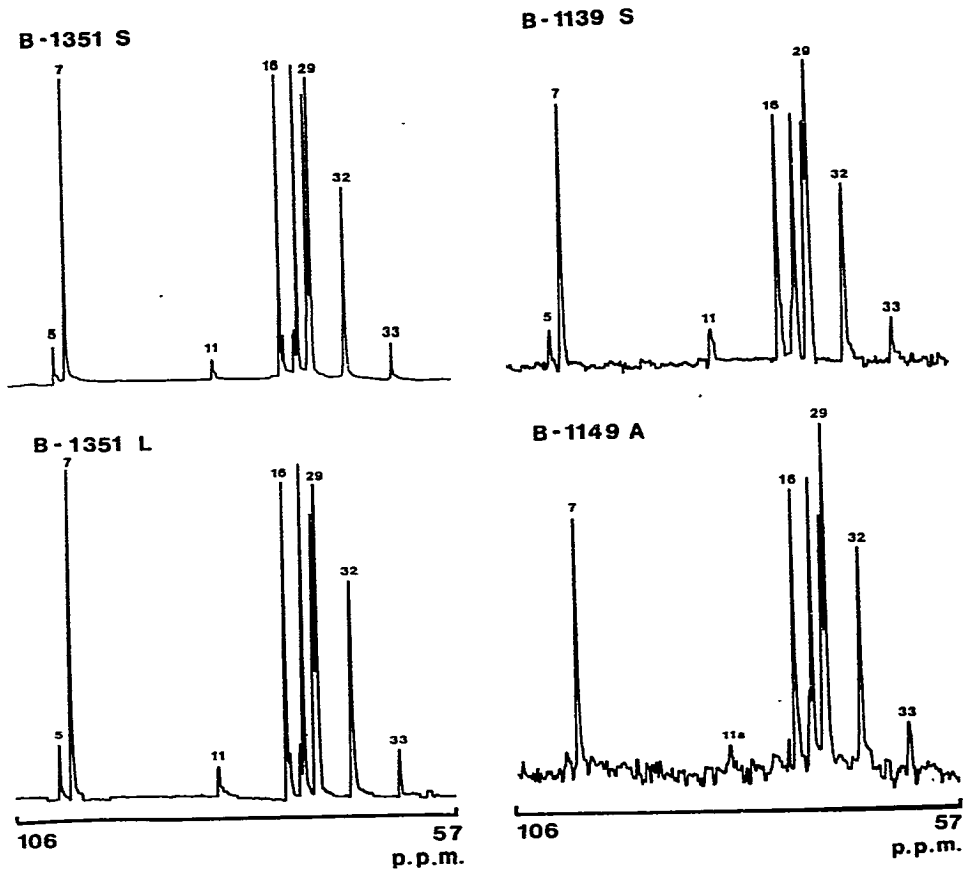


Fig. 2. ^{13}C-N.m.r. spectra, at 90°, of dextran B-1351 fractions S and L, dextran B-1139 fraction S, and dextran B-1149 fraction A. [Except for the spectrum (16,000 acquisitions) of dextran B-1149 fraction A, the number of acquisitions for each spectrum is listed in Table II. All data were processed by c.d.r.e. The identifying resonance-numbers are referenced to the resonances in Table II.]

degree of branching. For example, resonances 28 and 29a of dextran B-742 fraction S are similar, and equally spaced, to resonances 27 and 29 of dextran B-1351 fraction S, and such differences (of ~0.15 p.p.m.) could be attributed to spectrometer inaccuracy. However, the spectrum of dextran B-1142 (see Table II and Fig. 3) shows resonances 27, 28, 29, and 29a present in the same spectrum, further confirming the hypothesis that each, differently *O*-substituted, α-D-glucopyranosyl residue will give ^{13}C-n.m.r. resonances having unique chemical-shifts, and that these differences (from residue type to residue type) will lie within, or near to, the limits of resolution at 25 MHz. The diagnostic resonances associated with class I (m = 3) dextrans are: 62.45, 71.42, 71.83, 71.52, 73.60, 74.78, 82.84, and 100.88 p.p.m. These eight resonances provide a spectral pattern that, at 25 MHz, is clearly distinguishable from the spectral patterns of class I (m = 2, or 4) dextrans[8,9] or class II dextrans[15].

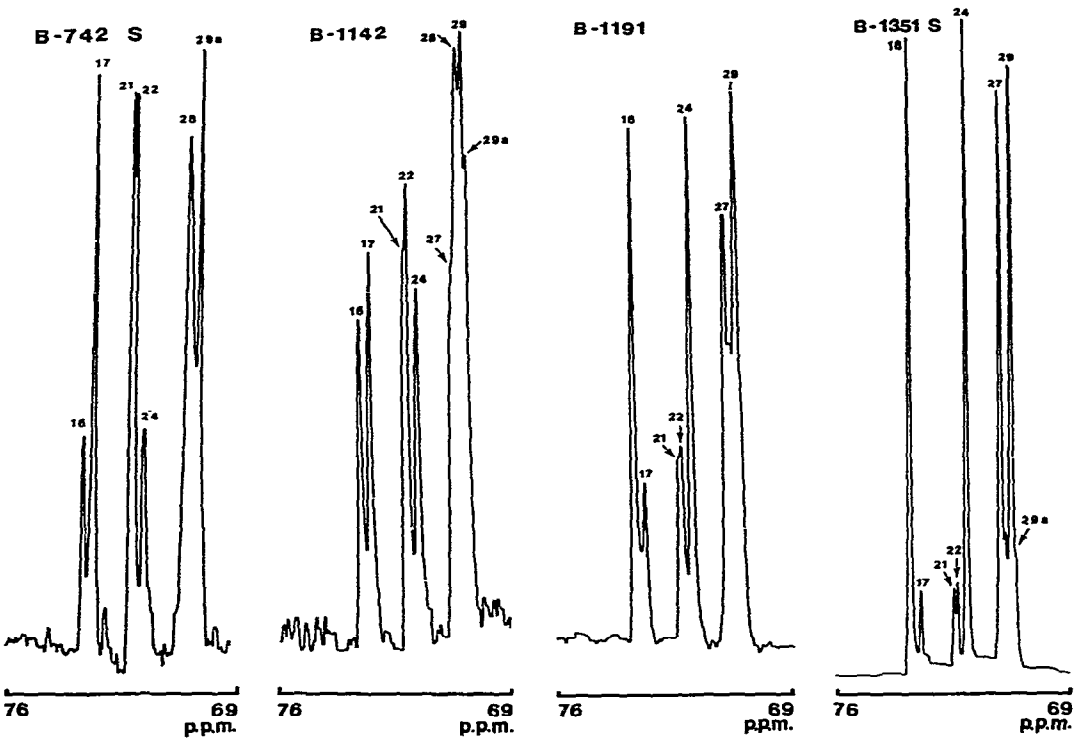


Fig. 3. The expanded, 69–76-p.p.m. region of the ^{13}C-n.m.r. spectra, at 90°, of dextran B-742 fraction S, dextran B-1142, dextran B-1191, and dextran B-1351 fraction S. (All data were processed by c.d.r.e., and the number of acquisitions for each spectrum is listed in Table II. The identifying resonance-numbers are referenced to the resonances in Table II.)

If our hypothesis that branching produces 12 additional resonances (6 resonances from the *t*-residue and 6 resonances from the *b*-residue) is correct, certain of the branching resonances in the spectra in Table II are not identified. We have previously[1] established that, for class I ($m = 3$) dextrans, the anomeric resonances of the *b*-residue and the *l*-residue are not resolved. The relative insensitivity of the chemical shift of the resonances of C-6 results in no resolution between the resonances of the linked C-6 atom of the *b*-residue and the *l*-residue (*i.e.*, resonance 32). Therefore, only 2 of the expected 12 diagnostic resonances for the class I ($m = 3$) dextrans have not been accounted for, and it is assumed that these resonances are unresolved in the 70–75-p.p.m. region. In our initial, ^{13}C-n.m.r. study of dextrans[4], we observed, in the spectrum of dextran B-1351 fraction S, a resonance at 67.5 p.p.m. that was identified as the C-6 resonance of the *b*-residue. However, this 67.5-p.p.m. resonance was an artifact of that specific preparation, apparently due to an impurity, because subsequent, duplicate, spectrometric determinations of dextran B-1351 fraction S have given only two resonances (61.3 and 66.6 p.p.m., under ambient recording-conditions) in the 60–65-p.p.m. region.

TABLE III

THE DEGREE OF BRANCHING OF DEXTRANS CONTAINING 3,6-DI-*O*-SUBSTITUTED RESIDUES AS ESTABLISHED BY METHYLATION–FRAGMENTATION (G.L.C.–M.S.) ANALYSIS AND BY ^{13}C-N.M.R. SPECTROSCOPY

Dextran		n *from*	^{13}*C-N.m.r. anomeric peak-height*[b]		^{13}*C-N.m.r. anomeric peak-area*[b]	
NRRL strain	*Fraction*	*g.l.c.–m.s.*[a]	*Ratio*	*Normalized* n	*Ratio*	*Normalized* n
B-742	S	0.10[d]	0.89	0.10	1.53	0.10
B-1142		1.05	1.39	0.60	2.23	0.80
B-1191		2.72	3.46	2.67	3.42	1.99
B-1192			3.42	2.63	3.40	1.97
B-1139	S		5.90	5.11	5.77	4.34
B-1351	L		5.69	4.90	7.91	6.48
B-1351	S	14.4[e]	8.73	7.94	9.90	8.47

[a]The amount of the 2,3,4-tri-*O*-methyl derivative divided by the amount of the 2,3,4,6-tetra-*O*-methyl derivative. [b]The resonance height (or area) at 99.5 p.p.m. divided by the resonance height (or area) at 100.1 p.p.m. The spectra that were measured had not been processed by c.d.r.e. [c]The anomeric ratios were linearly normalized, and referenced to the value of $n = 0.10$ for B-742 fraction S. [d]G.l.c.–m.s. data taken from Table I. [e]When the amount of 2,3,4-tri-*O*-methyl derivative is divided by the amount of 2,3-di-*O*-substituted derivative, n equals 7.93.

We have previously discussed the peak-intensities of the diagnostic resonances, relative to the intensities of the A through F resonances of linear dextran, as a measure of the degree of polysaccharide branching. We have correlated branching data for several dextrans, including dextran B-742 fraction S, dextran B-1191, and dextran B-1142, by the use of difference F.t.-i.r.[13] and ^{1}H-n.m.r.[14] techniques. Methylation–fragmentation analysis is the method of choice for use in the comparison of data on degree of branching, as the g.l.c.–m.s. technique can provide an n value essentially independent of polymeric reference-standards (see Table III). Data have been correlated from g.l.c.–m.s. and from ^{13}C-n.m.r. spectroscopy for degree of branching in dextrans of class I ($m = 2$) and class II, and we have found a general correlation, in terms of relative degrees of branching, for sets of polysaccharides in these two classes of dextran. For ^{13}C-n.m.r. resonances, it is necessary to introduce a resonance-intensity correction-factor, as different carbon-atom positions do not all have resonances of equal intensity. For class I ($m = 4$) dextrans, the correlation between data for degree of branching given by g.l.c.–m.s. and by ^{13}C-n.m.r. spectroscopy has been less close than data from the foregoing classes (although maintaining a general correlation) and, at present, we are not certain as to the cause of this lack of agreement[9]. However, the data presented for the degree of branching for class I ($m = 3$) dextrans (see Table III) show a similar correlation between degree of branching from g.l.c.–m.s. data and from ^{13}C-n.m.r.-spectral data.

As all of the data from ^{13}C-n.m.r. spectroscopy had been obtained with a high signal-to-noise ratio, both the peak heights and the peak areas in the anomeric region were compared. For more-direct comparison of the sets of data, these comparisons were made for spectra for which the data which had not been treated by c.d.r.e.

processing. As each carbon-atom position can give a ^{13}C-n.m.r. resonance of different intensity, and, as the anomeric resonances of the *l*- and *b*-residues are not resolved for class II ($m = 3$) dextrans, the following, normalization approach was employed for comparing resonance 5 to resonance 7. Methylation–fragmentation-analysis data indicated that $n = \sim 0.1$ for dextran B-742 fraction S, and so the anomeric resonances of the spectrum of this polysaccharide were normalized on the basis that resonance 7 contained a 10% contribution (by peak height or by area, respectively) from the small proportion of *l*-residues present. Therefore, in Table II, the resonance-intensity ratio (0.89 for peak height, and 1.53 for peak area) was subtracted from the resonance intensity ratios for each of the other polysaccharides (either the ratios of the peak height or of the peak area), and the value of 0.1 was then added to this value in order to give a normalized, n value. In general, there is a good correlation between the degree of branching obtained by the two methods, especially if the value $n = \sim 8$ (the tri- to di-methyl ether ratio) is employed for the value of n, as established by g.l.c.–m.s. for dextran B-1351 fraction S.

Ito and Schuerch[24] have recently reported the ^{13}C-n.m.r. spectra of synthetic polysaccharides that can be described by **1**, $m = 3$. Their method of synthesis of the polysaccharides assures that (*a*) the polymer has a linear, backbone chain, (*b*) all linkages in the backbone chain are (1→6)-D-linkages, (*c*) all chain branching occurs through 3-*O*-substitution, and (*d*) each side chain is only one residue long. Within the limitations of somewhat different recording-conditions (the synthetic polysaccharides were studied at ambient temperature), it would appear that the diagnostic chemical-shifts of the synthetic dextrans and of the dextrans in Table II (except for dextran B-1149 fraction A) are identical.

Such spectral identity of natural and synthetic polysaccharides is advantageous for two reasons. Firstly, the spectral similarity of these two groups of compounds supports our suggestion that the natural class I ($m = 3$) dextrans have backbones composed exclusively of (1→6)-α-D-linked residues, and side chains that are only one residue long. Secondly, such a spectral similarity, especially for highly branched polysaccharides ($n = \sim 1$), indicates that the natural polysaccharides can have the *b*-residues well distributed throughout the backbone chain. Although the previously studied dextrans of this series have been described as "structurally homogeneous", as they could not be analytically fractionated, it is, nevertheless, difficult to prove that such polysaccharides are not mixtures (in different proportions) of a highly branched and a linear polysaccharide, or that these natural dextrans are not "block" polymers composed of long segments of unbranched residues followed by long segments of branched residues. Technically, it would be possible to reproduce all spectra in Table II and Figs. 1, 2, and 3 (except for dextran B-1149 fraction A) by employing solutions containing different percentages of dextran B-742 fraction S and dextran B-1254 fraction L[$], although such a mixture could be detected by analytical fractionation. Therefore, when dealing with the lack of an observable "neighboring-residue effect" for these Class I dextrans, it was necessary to consider the possibility that these natural dextrans were "block"-type polymers, and, therefore,

no neighboring-residue effect need be expected. However, the spectra of the synthetic dextrans confirm the hypothesis that the *b*-residues could be well distributed throughout the structures of the various class I ($m = 3$), natural dextrans.

The data in Table II further confirm previous observations and resonance assignments[1] that were made for class I ($m = 3$) dextrans, and that were primarily based on data for dextran B-742 fraction S. Spin–lattice, resonance-relaxation measurements (T_1^{obs}) were performed on dextran B-1142 (see Table II), and these results, both in terms of absolute values and relative order of magnitude, are in close agreement with those of the corresponding resonances observed in the spectrum of dextran B-742 fraction S. This close agreement further confirms the accuracy of the T_1^{obs} values of the former compound (see ref. 1), and also indicates that the absolute magnitude of the T_1^{obs} values of polysaccharides are essentially independent of small changes in solvent properties that are encountered in preparing aqueous solutions (*e.g.*, differences in amounts of trace metals and dissolved oxygen). The bacterial strains B-742 and B-1142 are closely related, and the origin of these strains has previously been discussed[9].

TABLE IV

CHEMICAL SHIFTS FOR ^{13}C-N.M.R. SPECTRA, AT 34°, OF DEXTRANS CONTAINING (1→3)-α-D-LINKAGES[a]

		Dextran				
		B-1351	*B-1191*	*B-1139*	*B-523*	*B-523*[b]
		Fraction				
		S		*S*	*A (culture)*	*A (culture)*
		Acquisitions[c]				
		57	*52*	*26*	*50*	*42*
	5[d]	100.29[e]	100.24	100.23		101.30
A[f]	7	98.71	98.67	98.67	98.68	99.17
	11	81.55	81.53	81.55		83.33
B	16	74.38	74.35	74.36	74.36	75.09
	17		73.88s[g]	73.86		
	22		72.70s	73.13		73.48s
C	24	72.40	72.38	72.37	72.37	72.83
D	27	71.16	71.13	71.14	71.17	71.57
E	29	70.52	70.51	70.52	70.53	71.02
		67.54[h]				
F	32	66.55	66.54	66.58	66.58	66.86
	33	61.33	61.39	61.35	61.45s	62.13
						58.43[i]

[a]The spectra are expressed in p.p.m. relative to tetramethylsilane. [b]This spectrum was recorded at pH ~14. [c]Acquisitions in thousands. [d]These numbers were assigned in ref. 1, and are referenced to resonances in Fig. 4. [e]Data taken from ref. 4. [f]The letters A through F designate the major resonances of linear dextran (see ref. 4). [g]The letter s designates a shoulder, or weak resonance. [h]A resonance not observed in later determinations. [i]An impurity arising from the addition of base.

In addition, the similarity of magnitude of the T_1^{obs} values for the anomeric resonances (resonance 5) that have been identified as being associated with the *t*-residues of both dextran B-742 fraction S and dextran B-1142 further suggests that most, if not all, of the spectroscopically observable side-chains of dextran B-1142 are only one residue long. The extremely highly branched structure of dextran B-742 fraction S requires a structure principally composed of side chains that are one residue long. The magnitude of the T_1 value of a given resonance is essentially dependent on the degree of freedom of the carbon-atom position associated with that resonance. Were the side chains of dextran B-1142 more than one residue long, their associated *t*-residues would have an average degree of freedom greater than that of the *t*-residues of dextran B-742 fraction S. These differences in degrees of freedom between the terminal residues of the two polysaccharides would then be reflected in the T_1^{obs} values of the resonances associated with the anomeric positions of the *t*-residues of these dextrans, an effect not observed (see Table II).

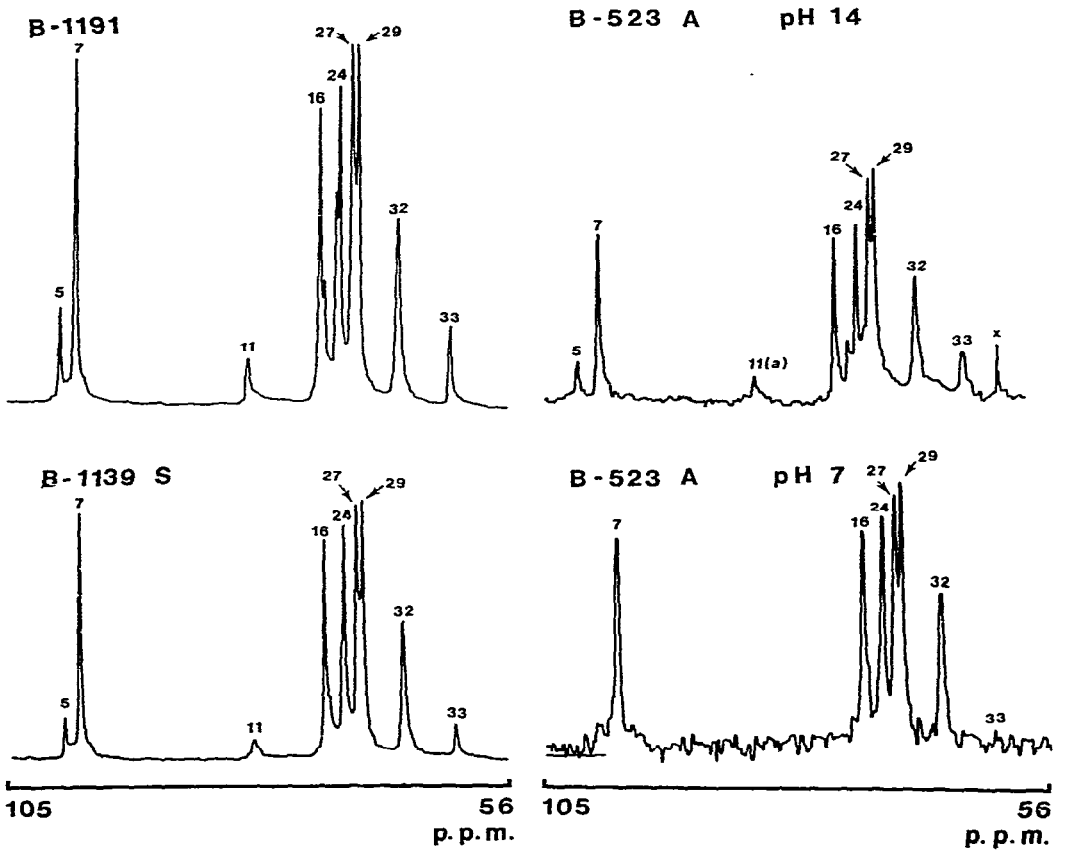


Fig. 4. ^{13}C-N.m.r. spectra, at 34°, of dextran B-1191, dextran B-1139 fraction S, and dextran B-523 fraction A (at pD 7 and at pD ~14). (The number of acquisitions for each spectrum is listed in Table IV, and the identifying resonance-numbers are referenced to the resonances in Table IV.)

Additional spectra were recorded at 34° (see Table IV and Fig. 4) for dextran B-1191 and Dextran B-1139 fraction S, and these spectra are quite similar to previous dextran spectra[2,5,8] recorded at 34° (see ref. 1 for a discussion of ambient temperatures of 27° and 34°). The latter spectra allow comparison of the class I dextrans to other dextrans described in the following section. Spectra recorded at 34°, compared to 90°, have less resolution, and require ~5 times the number of acquisitions to afford similar, signal-to-noise ratios.

Class III dextrans

Data for dextran B-1149 fraction A and dextran B-523 fraction A are included in Tables II and IV, and Figs. 2, 3, and 4. However, although these two dextrans are fundamentally composed of (1→3)-α-D- and (1→6)-α-D-linkages, evidence has slowly accumulated that there exists a remaining set of dextrans, as yet not discussed in this series on unusual dextrans, and that these two dextrans are members of this group. Commencing with the acquisition of structural and physicochemical data on a general collection[6] of dextrans, it became apparent that there exists a sub-group of dextrans that are relatively insoluble, and that contain moderate to large proportions of "1,3-like" linkages (see refs. 6 and 7 for a discussion of the term "like"). Dextrans could be categorized by use of the i.r. absorption (employing dispersive instrumentation) at 793 cm^{-1} (designated, Type II Infrared) into groups containing 1,3-like linkages[6]. However, the insoluble dextrans, known by periodate-oxidation studies to contain 1,3-linkages, contain an additional, i.r. band at 822 cm^{-1}; one member of this group, dextran B-1149 fraction A, was found by periodate-oxidation analysis to contain a large proportion of 1,3-like linkages (~40%) and to give an i.r. band at 822 cm^{-1}, but not at 793 cm^{-1}. Therefore, the i.r. band at 822 cm^{-1} appeared to have some relationship to the low solubility of dextrans, but not necessarily a direct relationship to the total, 1,3-like-linkage content of these dextrans. The sample of dextran B-523 fraction A studied herein is the same as the material previously studied by periodate-oxidation analysis, and reported without the fraction designation (see Tables I and III of ref. 6). Compared to the other dextrans examined herein, dextran B-523 fraction A is very difficult to dissolve, or disperse, in an aqueous solution at concentrations required for the recording of ^{13}C-n.m.r. spectra.

Dextran B-523 fraction A, in deuterium oxide, at pD 7 and ambient temperature, forms a white paste, and it is possible to record for this compound (see these data, at pD 7, in Table IV and Fig. 4) a ^{13}C-n.m.r. spectrum that indicates an essentially linear dextran. However, when the pD of this solution is adjusted (with NaOD) to ~14, the spectrum (see the data, at pD ~14, in Table IV and Fig. 4) now contains resonances diagnostic of (1→3)-α-D-linkages, and the dispersion is clear, although extremely viscous. The chemical shifts associated with the major (A through F) resonances are displaced downfield (by ~1 p.p.m.) from the values observed at pD 7, an effect previously discussed by Colson *et al.*[29] and similar to that caused by elevating the recording temperature, causing difficulty in making precise comparisons of the diagnostic chemical-shifts of this spectrum to those of the other spectra in

Tables II and IV. In contrast to elevating the recording temperature, which narrows the resonances and improves the spectral resolution, an increase of the pD of the solution slightly broadens the resonances and results in spectral resolution similar, or slightly inferior, to that at pD 7. When the pD of the solution was readjusted to 7, a flocculent precipitate resulted, and the sample gave a weaker ^{13}C-n.m.r. spectrum which, again, contained no diagnostic, nonlinear dextran resonances. We conclude that this weak, linear-dextran spectrum of the solution of dextran B-523 fraction A at pD 7 results from either (*a*) a small proportion of a linear dextran associated with the major component of dextran B-523 fraction A, or (*b*) relatively linear segments of dextran B-523 fraction A, which, after neutralization, remain sufficiently solvated to yield a spectrum. The relative intensities of the diagnostic resonances of dextran B-523 fraction A are in general accord with the percentage (24%) of 1,3-like linkages as established by periodate oxidation[6]. For example, the ratio of the peak heights of resonance 5 to resonance 7 of the spectrum of dextran B-1191 (14%, 1,3-like-linkage content by periodate oxidation) at pD 7 (see Fig. 4) is ~27, whereas the corresponding resonance-intensity ratio for the spectrum of dextran B-523 fraction A at pD ~14 is ~0.24.

The examples of class I and class II dextrans that we have studied can be dissolved, or, more accurately, dispersed, in aqueous medium at 90° without the use of a strong base, to yield clear or opalescent solutions, or dispersions, at high (~100 mg/mL) concentrations. Solubility differences, as defined by the percentage of ethanol needed to precipitate a given dextran from an aqueous ethanol solution[5], do exist for these class I and class II dextrans, and the ability to obtain well resolved, ^{13}C-n.m.r. spectra for such solutions is evidence of the extensive solvation of these polymers. In contrast, attempts to disperse those polysaccharides that yield spectra containing the 822-cm^{-1} band in neutral aqueous solution (at 25–95°) result in either a solvent filled with flocculent precipitate, or a white paste.

We therefore propose an additional category of dextrans, the class III dextrans, which can operationally be defined to include any dextran that gives an i.r. spectrum containing an identifiable band at 822 cm^{-1}. It is probable that such a class of dextrans has a close, structural relationship to the insoluble D-glucans produced by various strains of *Streptococcus mutans*, which are known to be (1→6)-α-D- and (1→3)-α-D-linked, and also to give[29,30] i.r. spectra having bands at 822 cm^{-1}. Representative examples of class III dextrans (as designated by *b* in Table III of ref. 6) and of insoluble D-glucans from *S. mutans* (as listed in Table I of ref. 30), displayed essentially the same solubility properties; that is, it was not possible to dissolve, or disperse, these polysaccharides in neutral, aqueous solution at concentrations approximating the minimal requirements (~5 mg/mL) for ^{13}C-n.m.r. spectrometry. However, these class III dextrans and the D-glucans from strains of *S. mutans* can be dissolved in dimethyl sulfoxide, or in aqueous solutions at high pH.

One member of the group of insoluble dextrans, dextran B-1149 fraction A, displays solubility properties at variance with those of the other members of the group. Dextran B-1149 fraction A is relatively insoluble in water at room temperature,

and exhibits an i.r. spectrum containing the 822-cm^{-1} band. However, in contrast to the other insoluble dextrans discussed in ref. 6, dextran B-1149 fraction A displays no 793-cm^{-1} band in its i.r. spectrum; furthermore, when the temperature is increased, this dextran fraction dissolves, yielding an opalescent solution similar to that of dextran B-1142 or of dextran B-1191. The recording of the ^{13}C-n.m.r. spectrum at 90° of dextran B-1149 fraction A proceeds differently from that of the other polysaccharides listed in Table II; little detail can be observed in a spectrum at 4,000 acquisitions, the diagnostic resonances cannot be readily observed at 16,000 acquisitions (see Fig. 2), and only at ~200,000 acquisitions (see Fig. 5) can the diagnostic resonances in this spectrum be readily observed. Three interesting features are observable in this spectrum of dextran B-1149 fraction A. Firstly, the ^{13}C-n.m.r. spectrum indicates a polysaccharide having a greater contribution of linear-dextran character than the corresponding, periodate-oxidation data suggest [these indicate a large percentage of (1→3)-like linkages]. Secondly, although the major, diagnostic

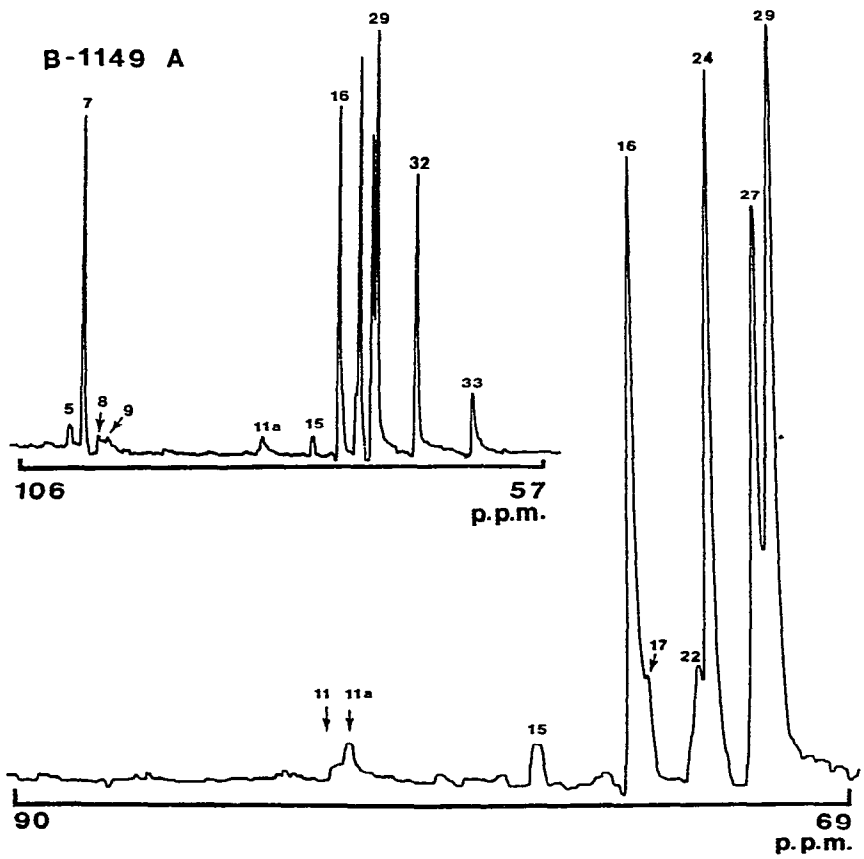


Fig. 5. The ^{13}C-n.m.r. spectrum, at 90°, of dextran B-1149 fraction A at 208,000 acquisitions, and the expanded 69–90-p.p.m. region of the same data. (All data were processed by c.d.r.e. before plotting. The identifying resonance-numbers are referenced to the resonances in Table II.)

resonances, designated resonance 5 and 11a in Table II and Fig. 5, are similar to diagnostic resonances observed for (1→3)-α-D-linkages in class I ($m = 3$) and class II dextrans, they are not the same; resonance 5 (100.96 p.p.m.) is slightly outside the limits of the resonance centered at 100.88 p.p.m. for class I ($m = 3$) dextrans, and resonance 11a (83.49 p.p.m.) is clearly displaced from the diagnostic resonance at 82.84 p.p.m. of the class I ($m = 3$) dextrans. Both resonance 5 and resonance 11a also differ from the diagnostic resonances of the (1→3)-α-D-linkages associated with class II dextrans[10]. Thirdly, the spectrum shows weak, but well defined, resonances associated[8] with 2,6-di-*O*-substituted α-D-glucopyranosyl residues (resonances at 77.71, 97.29, and 98.16 p.p.m.). Therefore, based on data from dextran B-1149 fraction A, it may be anticipated that, if such class III dextrans can be solubilized at 90° (aqueous conditions), the ^{13}C-n.m.r. spectra of these compounds will contain diagnostic resonances at ~83.49 and ~100.96 p.p.m.

At present, we are uncertain as to why the diagnostic resonances of the ^{13}C-n.m.r. spectrum of dextran B-1149 fraction A are so weak, relative to the six resonances (A through F) associated with linear dextran. For example, the ratio of the resonance peak-heights for resonance 16 to resonance 11a (for the data set as plotted in Fig. 5) is ~16, and, even when a correction factor of ~1/3 is applied, owing to the lessened intensity of resonances in the 75–85-p.p.m. region (see ref. 1), such a diagnostic, resonance intensity would indicate an upper limit of <20% of (1→3)-linkages, in contrast to an ~40% content of (1→3)-like linkages indicated by periodate-oxidation analysis. Similarly, the areas of the anomeric resonances for the data set plotted for dextran B-1149 fraction A in Fig. 5 are present in the proportions of: peak 5, 7, 8, 9: 3.6, 24.8, 1.0, 1.2, which would indicate linkage ratios of (1→2)-, (1→3)-, (1→6)-linkages: 1, ~4, 25.

It is of interest to establish the structural feature that these class III dextrans have in common, and that gives rise to the singular, i.r. band and ^{13}C-n.m.r. resonances of this group. The structural feature common to the class III dextrans may be identified by several methods. Firstly, the chemical shifts of the ^{13}C-n.m.r. resonances associated with 3,6-di-*O*-substituted α-D-glucopyranosyl residues and with noncontiguously linked, 3-mono-*O*-substituted α-D-glucopyranosyl residues[15] have been well established. As periodate-oxidation data indicated a large percentage of 1,3-like linkages for dextran B-1149 fraction A, and as the ^{13}C-n.m.r. spectrum of this compound excludes major contributions from structures associated with class I ($m = 3$) dextran or class II dextran, an obvious alternative remaining consists of a structure composed of segments of contiguously linked, (1→3)-α-D-linked residues, and, consequently, also of segments of contiguously linked, (1→6)-α-D-linked residues. Such a structure would be expected to give a ^{13}C-n.m.r. spectrum showing a major contribution from linear-dextran resonances, as does the spectrum of dextran B-1149 fraction A. Secondly, we have found, and will discuss in detail elsewhere*, that the 822-cm^{-1} band of the i.r. spectrum is present only when contiguously linked, (1→3)-linked

*F. R. Seymour and R. L. Julian, unpublished results.

α-D-glucopyranosyl residues are present. Such a conclusion is based on the presence of the 822-cm^{-1} band in the i.r. spectrum of pseudonigeran (a D-glucan that is principally composed of contiguously linked, 3-mono-*O*-substituted, α-D-glucopyranosyl residues), and the absence of this 822-cm^{-1} band from the i.r. spectra of a variety of D-glucans known to contain (1→3)-α-D-linkages but not to contain such linkages in contiguously linked sequences. On this basis, it is, therefore, concluded that the distinguishing, structural feature of class III dextrans is the presence of analytically significant percentages of contiguously linked, (1→3)-α-D-glucopyranosyl residues.

The data obtained for dextran B-1149 fraction A are unusual with regard to the other dextrans; although the results of the various methods of structural analysis are in accord with regard to the identity of the structural features present in this polysaccharide, they do not agree as regards the percentage of nonlinear-dextran contained. Periodate-oxidation analysis originally indicated a high (~40%) content[6] of 1,3-like linkages for dextran B-1149 fraction A. Scott *et al.*[31] employed structural analysis by use of the optical rotation of cuprammonium complexes of dextrans, and also concluded that dextran B-1149 fraction A contains large (~40%) percentages of 1,3-like-linked residues. The cuprammonium-complex technique yielded data for dextran B-1149 fraction A (and also for dextran B-1355 fraction S) that could most readily be interpreted by assuming that a large fraction of the 1,3-linkages in these polysaccharides results from 3-mono-*O*-substituted residues, rather than 3,6-di-*O*-substituted residues (estimated at ~70% of 3-mono-*O*-substituted residues for dextran B-1149 fraction A). However, the cuprammonium-complex method has been but little used for 1,3-linkage determinations, and it is difficult to evaluate the accuracy of such measurements; for example, this method was able to identify, accurately, the presence of 3-mono-*O*-substituted residues in dextran B-1355 fraction S, but the quantitation of these residues differed considerably from the results given by methylation–fragmentation analysis. Suzuki and Hehre[10] applied selective hydrolysis [acetolysis, which preferentially cleaves (1→6)-α-D-linkages] to dextran B-1149 fraction A, and obtained the disaccharides kojibiose (4.4%) and nigerose (6.4%), with an overall recovery of 59% of the total sugar fragments. Such data set lower limits on the percentage of 2-*O*-substituted residues (the nigerose indicating ~3% of such residues); however, as cleavage of non-(1→6)-linkages can occur, such data do not set upper limits for the percentages of the 2- or 3-*O*-substituted residues. However, in terms of the relative intensities of the diagnostic resonances, the ^{13}C-n.m.r. spectrum of dextran B-1149 fraction A indicates a polysaccharide having a relatively low content (~10%) of non-(1→6)-linkages. Such ^{13}C-n.m.r. data accord with the solubility properties of dextran B-1149 fraction A, which are more similar to those of linear dextran than to those of the other, class III dextrans, and with the relatively weak, i.r. band at 822 cm^{-1} (compared to the intensity of the same band for pseudonigeran) in the spectrum of this polysaccharide.

Therefore, the periodate-oxidation data and the ^{13}C-n.m.r. data are not in agreement with regard to the 1,3-linkage content of dextran B-1149 fraction A, and

the acetolysis data support either set of data [as the percent of hydrolysis of non-(1→6)-linkages is not known]. We know of no reason why either set of data is incorrect. One possible explanation that could accommodate many of the observations is as follows. Dextran B-1149 fraction A could contain large percentages of contiguously linked 3-mono-*O*-substituted residues in chains that are periodically interspersed with residues of a different *O*-substitution type (*e.g.*, 6-mono- or 2,6-di-*O*-substituted residues). Upon dispersing, or dissolving, such a polysaccharide, the relatively short chains of contiguously linked 3-mono-*O*-substituted residues are not completely solvated, and the nuclei associated with these insoluble regions therefore do not make a proportional contribution to the total, ^{13}C-n.m.r. spectrum of the polysaccharide (see ref. 9 for a further discussion of such a possibility). Such a situation would contrast with that for the other, known, class III dextrans, which would be presumed to contain longer segments (although not necessarily higher mole percentages) of contiguously linked, 3-mono-*O*-substituted residues, and these longer chains would effectively prevent the dissolution, or dispersion, of these polysaccharides. In conjunction with the foregoing, attempts were made to observe the major resonances in the ^{13}C-n.m.r. spectrum of the (linear) pseudonigeran at low concentrations, but no resonances were observed, even with acquisition times at which 1 mg/mL of solution would have provided observable signals.

The absence of the 793-cm^{-1} band (under dispersive, i.r. recording-conditions), and of any identifiable, ^{13}C-n.m.r. resonances at ~82.8 or 100.88 p.p.m., indicates the essential absence of 3,6-di-*O*-substituted residues [class I (m = 3) dextran, structural feature] in dextran B-1149 fraction A. Were class I (m = 3) dextran, structural features present in appreciable proportions in a given dextran, the unique, ^{13}C-n.m.r. resonances associated with class III dextrans could readily be obscured. Many of the class III dextrans exhibit both the 743- and the 822-cm^{-1} band in their i.r. spectra. Both i.r. bands are present in the spectra of the insoluble (fractions A and B) D-glucans[30] produced by strains of *S. mutans*, but the more-insoluble fractions (B fractions) of the D-glucans contain a more-intense, 822-cm^{-1} band (relative to the 793-cm^{-1} band) than the more soluble fractions (A fractions), suggesting a range of structural contributions from both 3,6-di-*O*-substituted and contiguously linked, 3-mono-*O*-substituted α-D-glucopyranosyl residues. Therefore, the ^{13}C-n.m.r. spectrum of dextran B-1149 fraction A may prove to be unique for this class of compound under these conditions, as the diagnostic resonances of dextrans having less class-III-dextran character will present signal-to-noise problems (or be obscured by the presence of resonances from 3,6-di-*O*-substituted residues), and dextrans having greater class-III-dextran character will be insoluble, and not give a ^{13}C-n.m.r. spectrum.

In summary, it is assumed that the principal, structural feature of the class III dextrans is the presence of contiguously linked, 3-mono-*O*-substituted α-D-glucopyranosyl residues. The position of these chains of contiguously linked 3-mono-*O*-substituted residues, relative to the linear-dextran-like portion of the polysaccharide, is not understood at present.

Relationships between the D-*glucans produced by strains of* L. mesenteroides *and* S. mutans

It has been recognized for some time that a general relationship exists between the structure of the polysaccharides produced by certain cariogenic strains of *Streptococcus mutans* and the dextrans produced by *Leuconostoc mesenteroides*[32]. In recognition of the fact that the polymers, especially the insoluble polymers, produced by strains of *S. mutans* contain significant, or predominant, percentages of (1→3)-α-D-linkages, recent discussions[25,29] of these polysaccharides have employed the term "D-glucan" rather than the term "dextran". However, the relationship between the insoluble dextrans, which we now describe as class III dextrans, and the insoluble D-glucans produced by *S. mutans* has been noted by Meyer *et al.*[30], specifically with regard to the 822-cm^{-1} band of the i.r. spectrum. The literature contains several observations that suggest that the species of *S. mutans* and *L. mesenteroides* are more similar than the nomenclature might indicate. The genera Streptococcus and Leuconostoc are close, both being members of the family Streptococcacea[33]. The strains comprising both *L. mesenteroides* and *S. mutans* show considerable diversity, as demonstrated in this series of reports with regard to the extracellular polysaccharides produced by different strains of *L. mesenteroides*, and as reviewed by Coykendall[34] with regard to the general diversity of the properties associated with various strains of *S. mutans*.

Despite this diversity within the strains comprising *L. mesenteroides* and *S. mutans*, these strains have several properties in common. Firstly, both sets of bacterial strains can employ sucrose as the substrate[32,35]. Secondly, both sets of strains can produce extracellular, or interstitial, dextrans; strains of *L. mesenteroides* produce a characteristic slime when cultured[33], and essentially all of the strains of *S. mutans* that have been studied produce a hard mass of extracellular, insoluble dextran, "a property unique for these strains when compared to other strains of Streptococci" (ref. 35). Thirdly, strains of both *S. mutans* and *L. mesenteroides* often produce levans in conjunction with the production of dextrans. *L. mesenteroides* and *S. mutans* are certainly not identical, and the species differ in several respects, including details of the enzyme systems that produce the extracellular D-glucans; for example, a recent, comparative study of these species shows both differences and similarities[36]. However, in view of the extensive parallels that have been observed for the polysaccharides from *L. mesenteroides* and *S. mutans*, it is possible that elucidation of the structure of the polysaccharides produced by strains of one of these species may indicate the structural features of the corresponding polysaccharides produced by other species. For example, studies have indicated the existence of contiguously linked, 3-mono-*O*-substituted, α-D-glucopyranosyl residues as a major, structural component of the insoluble D-glucans produced by *S. mutans*, and degradative enzyme-studies[16,29,30,37] have also suggested that these contiguously linked, (1→3)-α-D-linked residues comprise the backbone chain of these polysaccharides, the remaining, (1→6)-D-linked residues constituting side chains.

Alternatively, it is possible that the similarities noted between the D-glucans

produced by strains of *S. mutans* and *L. mesenteroides* are merely fortuitous. However, it may be noted that strains from both these species have a predilection to produce D-glucans that have a wide range of physicochemical properties, and that are based on (1→3)- and (1→6)-α-D-linkages. The apparent elegance and simplicity of employing only two types of linkages for these polymer structures can produce confusion if the physicochemical properties of the polymers are discussed in terms of linkage types instead of residue types. For example, as a result of investigations on D-glucans from strains of *S. mutans*, Ceska *et al.*[38] concluded that D-glucans similar to dextran become less soluble as the proportion of (1→3)-α-D-linkages increases; such an observation would appear to be the converse of our observation that dextran solubility increases as the percentage of non-(1→6)-α-D-linkages increases[5]. However, Ceska *et al.* were studying variants of dextran containing contiguously linked, 3-mono-*O*-substituted, α-D-glucopyranosyl residues, whereas, with a single exception, our examples were class I dextrans. In general, strains of both *L. mesenteroides* and *S. mutans* produce insoluble D-glucans that are based on the incorporation of contiguously linked, 3-mono-*O*-substituted residues into a dextran-like structure (class III dextrans), and produce soluble D-glucans based on the 3,6-di-*O*-substituted residue incorporated into a dextran-like structure, although strains of *L. mesenteroides* can also incorporate (1→2)- and (1→4)-α-D-linkages into the soluble dextrans.

The foregoing, solubility-to-structure relationships are a logical development, as (*a*) linear homoglucans tend to be insoluble, and (*b*) linear dextran is an exception. The solubility of dextrans, relative to that of other homoglucans, results from intrasaccharide linkages through the primary alcohol (O-6) position of the D-glucopyranosyl residues (see ref. 39, and references cited therein) which allow a large number of minimum-energy conformations. Therefore, the introduction of branching residues into a dextran decreases the order of the molecule, and provides a greater number of minimum-energy conformations, resulting in greater solubility, whereas, conversely, the introduction of segments of contiguously linked, 3-mono-*O*-substituted residues into a dextran lessens the number of minimum-energy conformations of the polymer, resulting in a product having solubility properties approximating those of pseudonigeran. However, not only must the nature of the *O*-substitution of the individual residues be considered, but also the relative positions of such *O*-substituted residues (*e.g.*, alternating, 3- and 6-mono-*O*-substituted residues of class II dextrans result in quite soluble polysaccharides). Finally, it may be observed that, although considerable diversity exists in the structures of the D-glucans that have been studied, and that are produced by a wide variety of strains of *L. mesenteroides*, there is little, if any, evidence of similar polysaccharide systems in this set of D-glucans that employ either (1→2)- or (1→4)-α-D-linkages to produce both soluble and insoluble variants of the linear-dextran structure.

EXPERIMENTAL

Materials. — Previously prepared dextrans[6] and dextran fractions[40] were used.

Dextran B-523 fraction A and dextran B-1149 fraction A are the same materials as had previously been described without a fraction designation.

Methods. — Previously described methods were used for the methylation[3] of the dextrans and for structural analysis[2,41] by combined g.l.c.–electron-impact, mass spectrometry of the peracetylated aldononitriles. For each permethylation, three successive, Hakomori[42] methylations were employed on an initial sample (40 mg), with ~90% (final) weight recovery of each permethylated dextran. Formolysis and successive hydrolysis with acetic acid 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 III 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. by 1.23 m), was employed for all such chromatography.

The ^{13}C-n.m.r. conditions, and methods for the preparation of dextran samples have been described[4,5]. 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 (~0.1 g/3 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[28]. However, the relative, anomeric peak-areas employed in Table III were taken from data that were not processed by c.d.r.e. The techniques for obtaining T_1^{obs} values have been described[1]. For dextran B-1142, 20 spectra (4,096 acquisitions for each spectrum) were recorded at delay intervals of 1 ms to 30 s, with each successive, delay-time approximately twice as long as the previous delay.

ACKNOWLEDGMENTS

We thank Dr. A. Jeanes, Northern Regional Research Center, Peoria, IL, for providing the dextrans and dextran fractions; Dr. C. Schuerch, State University of New York, Syracuse, NY, for providing a comparison plot of the ^{13}C-n.m.r. spectrum of a synthetic dextran branching through 3,6-di-*O*-substituted residues; Dr. J. H. Nordin, University of Massachusetts, Amherst, MA, for providing a sample of pseudonigeran; and Dr. B. L. Lamberts, Naval Dental Research Institute, Great Lakes, IL, for providing comparison samples of the insoluble D-glucans produced by strains of *S. mutans*. In addition, we thank Dr. L. R. Brown, Jr., University of Texas Dental Science Institute, Houston, TX, Dr. A. Jeanes, and Dr. B. L. Lamberts for helpful discussion and comments in the course of the preparation of the manuscript. This work was supported, in part, by National Institutes of Health Grants (HL-17269, HL-17373).

REFERENCES

1 F. R. SEYMOUR AND R. D. KNAPP, *Carbohydr. Res.*, 81 (1980) 67–103.
2 F. R. SEYMOUR, M. E. SLODKI, R. D. PLATTNER, AND A. JEANES, *Carbohydr. Res.*, 53 (1977) 153–166.
3 F. R. SEYMOUR, E. C. M. CHEN, AND S. H. BISHOP, *Carbohydr. Res.*, 68 (1979) 113–121.
4 F. R. SEYMOUR, R. D. KNAPP, AND S. H. BISHOP, *Carbohydr. Res.*, 51 (1976) 179–194.
5 F. R. SEYMOUR, R. D. KNAPP, S. H. BISHOP, AND A. JEANES, *Carbohydr. Res.*, 68 (1979) 123–140.
6 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.
7 A. JEANES AND F. R. SEYMOUR, *Carbohydr. Res.*, 74 (1979) 31–40.
8 F. R. SEYMOUR, R. D. KNAPP, E. C. M. CHEN, A. JEANES, AND S. H. BISHOP, *Carbohydr. Res.*, 71 (1979) 231–250.
9 F. R. SEYMOUR, R. D. KNAPP, E. C. M. CHEN, A. JEANES, AND S. H. BISHOP, *Carbohydr. Res.*, 75 (1979) 275–294.
10 H. SUZUKI AND E. J. HEHRE, *Arch. Biochem. Biophys.*, 104 (1964) 305–313.
11 A. JEANES, "Dextran Bibliography: Extensive Coverage of Research Literature (Exclusive of Clinical) and Patents, 1861–1976", *U. S. Dept. Agric., Agric. Res. Serv. Misc. Publ. No. 1355*, (1978), 370 pp.
12 L. L. SO AND I. J. GOLDSTEIN, *J. Biol. Chem.*, 242 (1967) 1617–1622.
13 F. R. SEYMOUR AND R. L. JULIAN, *Carbohydr. Res.*, 74 (1979) 63–75.
14 F. R. SEYMOUR, R. D. KNAPP, AND S. H. BISHOP, *Carbohydr. Res.*, 74 (1979) 77–92.
15 F. R. SEYMOUR, R. D. KNAPP, E. C. M. CHEN, S. H. BISHOP, AND A. JEANES, *Carbohydr. Res.*, 74 (1979) 41–62.
16 P. COLSON, H. J. JENNINGS, AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
17 T. SAWAI, T. TOHYAMA, AND T. NATSUME, *Carbohydr. Res.*, 66 (1978) 195–205.
18 P. Z. ALLEN AND E. A. KABAT, *J. Am. Chem. Soc.*, 78 (1956) 1890–1894.
19 P. Z. ALLEN AND E. A. KABAT, *J. Am. Chem. Soc.*, 81 (1959) 4382–4386.
20 J. W. GOODMAN AND E. A. KABAT, *J. Immunol.*, 84 (1960) 347–357.
21 A. LUNDBLAD, R. STELLER, E. A. KABAT, J. W. HIRST, M. G. WEIGERT, AND M. COHN, *Immunochemistry*, 9 (1972) 535–544.
22 J. CISAR, E. A. KABAT, J. LIAO, AND M. POTTER, *J. Exp. Med.*, 139 (1974) 159–179.
23 M. E. PREOBRAZHENSKAYA, E. L. ROSENFELD, AND L. KANDRA, *Carbohydr. Res.*, 66 (1978) 213–223.
24 H. ITO AND C. SCHUERCH, *J. Am. Chem. Soc.*, 101 (1979) 5797–5806.
25 M. D. HARE, S. SVENSSON, AND G. J. WALKER, *Carbohydr. Res.*, 66 (1978) 245–264.
26 F. R. SEYMOUR, R. D. KNAPP, AND S. H. BISHOP, *Carbohydr. Res.*, 72 (1979) 229–234.
27 F. R. SEYMOUR, in W. PASIKA (Ed.), "C-13 N.M.R. and Solving Macromolecular Problems", *ACS Symp. Ser.*, 103 (1979) 27–51.
28 I. D. CAMPBELL, C. M. DOBSON, R. J. P. WILLIAMS, AND A. V. XAVIER, *J. Magn. Reson.*, 11 (1973) 172–181.
29 P. COLSON, H. C. JARRELL, B. L. LAMBERTS, AND I. C. P. SMITH, *Carbohydr. Res.*, 71 (1979) 265–272.
30 T. S. MEYER, B. L. LAMBERTS, AND R. S. EGAN, *Carbohydr. Res.*, 66 (1978) 33–42.
31 T. A. SCOTT, N. N. HELLMAN, AND F. R. SENTI, *J. Am. Chem. Soc.*, 79 (1957) 1178–1182.
32 R. L. SIDEBOTHAM, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
33 R. H. DEIBEL AND H. W. WELLEY, JR., in B. L. BUCHANNAN AND N. E. GIBBONS (Eds.), *Bergey's Manual of Determinative Bacteriology*, 8th edn., Williams and Wilkins, Baltimore, 1974, pp. 490–517.
34 A. L. COYKENDALL, in H. M. STILES, W. J. LIESCHE, AND T. C. O'BRIAN (Eds.), "Proceedings, Microbial Aspects of Dental Caries", *Microbiol. Abstr., Spec. Suppl.*, 3 (1976) 703–712 (from Information Retrieval, Inc., Washington, DC).
35 B. PERCH, E. KJEMS, AND T. RAUN, *Acta Pathol. Microbiol. Scand., Sect. B*, 82 (1974) 357–370.
36 K. G. YOST AND P. J. VAN DEMARK, *Appl. Environ. Microbiol.*, 35 (1978) 920–924.
37 S. EBISU, A. MISAKI, K. KATO, AND S. KOTANI, *Carbohydr. Res.*, 38 (1974) 374–381.
38 M. CESKA, K. GRANATH, B. NORRMAN, AND G. GUGGENHEIM, *Acta Chem. Scand.*, 26 (1972) 2223–2230.
39 I. TVAROSKA, S. PEREZ, AND R. H. MARCHESSAULT, *Carbohydr. Res.*, 61 (1978) 97–106.

40 C. A. WILHAM, B. H. ALEXANDER, AND A. JEANES, *Arch. Biochem. Biophys.*, 59 (1955) 61–75.
41 F. R. SEYMOUR, R. D. PLATTNER, AND M. E. SLODKI, *Carbohydr. Res.*, 44 (1975) 181–198.
42 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.