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STRUCTURAL ANALYSIS OF INSOLUBLE D-GLUCANS BY FOURIER-TRANSFORM, INFRARED DIFFERENCE-SPECTROMETRY: CORRELATION BETWEEN STRUCTURES OF DEXTRANS FROM STRAINS OF *Leuconostoc mesenteroides* AND OF D-GLUCANS FROM STRAINS OF *Streptococcus mutans**

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ABSTRACT

Fourier-transform infrared (F.t.-i.r.) difference-spectra have been recorded, relative to a water-soluble dextran of low degree of branching, for (*a*) dextrans from *Leuconostoc mesenteroides* NRRL B-742 (the L fraction) and NRRL B-1149 (the A fraction), (*b*) D-glucans from *Streptococcus mutans* KR-1 and OMA 176, and (*c*) the controls of amylose, cellulose, nigeran, and pseudonigeran. Confirmation has been obtained for the presence, in the spectra of the relatively insoluble dextrans and D-glucans, of a previously recognized, characteristic absorbance at 822 cm^{-1}, and the correlation of this band with contiguous, linearly (1→3)-linked, α-D-glucopyranosyl residues, to which polymer insolubility (and cariogenic properties) has been ascribed. This analytical method allows the mole percent of the contiguously linked 3-mono-*O*-substituted α-D-glucopyranosyl residues to be quickly and non-destructively established in solid-state samples, when employing weights of polysaccharides in the microgram range. The wavenumbers and intensities of other bands observed in the F.t.-i.r. difference-spectra of D-glucans containing (1→4)-D-linkages are also discussed.

*Unusual Dextrans, Part XV. For Part XIV, see ref. 1. The opinions expressed herein are those of the authors, and are not to be construed as reflecting the views of the Navy Department or the Naval Service at large. The use of commercially available products does not imply endorsement of these products by the Navy Department, or preference over other, similar products on the market.

INTRODUCTION

As part of a series of structural studies on unusual dextrans, Fourier-transform infrared (F.t.-i.r.) difference-spectrometry has been shown to yield highly sensitive, specific information from ~80-μg samples in the solid state[2]. The F.t.-i.r. technique is convenient, as it is nondestructive and needs little preparation of the sample, and amounts weighing only a few micrograms can be studied; the 80-μg sample size employed herein was chosen for ease of handling of the sample and accuracy of weighing thereof. It is also possible spectrally to substract contributions resulting from impurities (*e.g.*, other polysaccharides) rather than physically to remove these impurities from the sample. The water-soluble dextrans used in the previous F.t.-i.r. study represented structural types in which the identity and proportion of non-(1→6)-linked α-D-glucopyranosyl residues had been established by periodate-oxidation and methylation-structural analyses and by ^{13}C-n.m.r. spectrometry, as indicated in specific references cited in ref. 2. By this F.t.-i.r. method, the difference-spectra show bands originating only from non-(1→6)-α-D-linkages, as the absorbances corresponding to the (1→6)-α-D-linkages of a reference dextran of low degree of branching have been subtracted. This reference dextran, dextran B-1254 fraction L[$], was shown by methylation-fragmentation, structural analysis[3] to contain (as a maximum) 1.8% of linear (1→3)-linkages and 4.4% of branched (1→3)-linkages. Compared to ^{13}C-n.m.r. spectrometry, the technique for F.t.-i.r. difference-spectrometry has several advantages for the quantitative, structural analysis of polysaccharides that disperse in aqueous solution with difficulty, or not at all. We report here the results obtained for a number of essentially water-insoluble α-D-glucans that we have selected as models having intrinsic significance, and for several water-soluble dextrans of related importance.

We have found the 1600–400-cm^{-1} region of the F.t.-i.r. difference-spectrum to provide the most useful information for structural analysis. Such F.t.-i.r. data are as informative as the ^{1}H-n.m.r. chemical-shift data that have been obtained from the same compounds[4], although they are somewhat less specific than ^{13}C-n.m.r. chemical-shift data[5,6]. The F.t.-i.r. difference-spectrometry technique is also complementary to the g.l.c.–m.s., structural-analysis approach that we have developed[3,7], because of the small sample-size required for both methods.

It should be recognized that the nature of the data from F.t.-i.r. spectra differs in several important respects from that of the previously obtained n.m.r. and g.l.c.–m.s. data, necessitating a somewhat different approach to the analysis of the F.t.-i.r. data. G.l.c.–m.s. data provide a variety of internal checks (*e.g.*, relative g.l.c. peak-heights and a multiplicity of *m/e* values) that aid in establishing the validity of a structural determination. Similarly, the analysis of ^{13}C-n.m.r. data is based on an anticipated one-to-one relationship between carbon-atom positions and spectral resonances. However, the bands in the F.t.-i.r. spectrum arise from relatively subtle effects, and, at present, neither the wavenumbers nor the intensities of these bands can be assigned *a priori* to structural features. In addition, relatively small features

of the total i.r. spectrum of a polysaccharide are under consideration, and therefore, care must be taken to avoid confusing bands that are associated with structural features with bands that could possibly be caused by intensely i.r.-absorbing features of minor constituents or trace impurities.

Our initial approach to the analysis of F.t.-i.r. difference-spectra employed the general assumptions that had been successfully applied to the analysis of the ^{13}C-n.m.r. spectra of dextrans; that is, (*a*) differently *O*-substituted residues will contribute unique i.r. bands, (*b*) the i.r. bands of each of these types of residue will be independent of the effects of the neighboring residues, and (*c*) each of these differently *O*-substituted types of residue will contribute, to the F.t.-i.r. difference-spectrum of the polymer, bands that are of intensity proportional to the mole percent of that residue in the polysaccharide. The general validity of the first assumption was indicated by establishing that a set of dextrans having different structural features (*e.g.*, different positions of branching from the backbone of the polysaccharide) gave F.t.-i.r. difference-spectra exhibiting different spectral-patterns. The general validity of the third assumption was demonstrated by the observation that the intensity of the bands of the F.t.-i.r. difference-spectra associated with 2,6-di-*O*-substituted α-D-glucopyranosyl residues is proportional to the mole percent of these residues present in branching dextrans. Such an observation should not be confused with the concept that differently substituted residues contribute, to the spectrum of the polymer, an amount of the total band-intensity which is proportional to the mole percent of each residue in the polymer; there does not need to be, and apparently is not, such a relationship for polysaccharides (see Fig. 4 of ref. 2).

However, due to the relatively limited number of polysaccharides that have been examined by F.t.-i.r. difference-spectrometry, it has been difficult to establish, precisely, the nature and magnitude of the changes in the spectral profile of the F.t.-i.r. difference-spectra upon comparing the effects of the same linkage when that linkage originates from differently *O*-substituted residues [*e.g.*, the (1→3)-α-D-linkage, either when resulting from a 3-mono-*O*-substituted residue, or a 3,6-di-*O*-substituted residue], or the magnitude of changes in a F.t.-i.r. difference spectrum that could result from structural changes in residues adjacent to the residues directly involved in the intra-saccharide linkage. Therefore, the present report has two objectives: (*a*) to extend our knowledge of F.t.-i.r. difference-spectra to structural relationships for α-D-glucans containing (1→3)-linkages, and (*b*) to a lesser extent, to examine similar relationships for D-glucans having a content of (1→4)-linkages.

For α-D-glucans of bacterial origin, linkages through O-3, which are of primary concern in this report, occur in several different, structural ways that may be differentiated by symbols, as follows: (*a*) (1→3)-linkage is a general term applicable to any of the linkage types, (*b*) (1→3; *b*6) is a branch-point linkage involving a 3,6-di-*O*-substituted α-D-glucopyranosyl residue, (*c*) (1→3; *l*) is a linkage in an unspecified, linear-chain situation involving a 3-mono-*O*-substituted residue, (*d*) (1→3; *l*, *c*) is a linear linkage contiguous with other (1→3)-linkages [usually (1→3; *l*)-], and (*e*) (1→3; *l*, *nc*) is a linear linkage not contiguous with other (1→3; *l*)-linkages. Likewise, such

differentiation can be made among the corresponding types of (1→2)- and (1→4)-linkages.

The terminology that sets dextrans apart from other α-D-glucans is based on the generally accepted definition that, in dextrans, the majority of the α-D-glucopyranosyl residues are linked by (1→6)-bonds. Dextrans constitute a large class of extracellular polysaccharides that are synthesized from sucrose (with one known exception[9,10]) by the dextransucrases of *Leuconostoc mesenteroides* and its congeners[9,10]. Among the dextrans known, the non-(1→6)-linkage occurring most frequently is the (1→3)-, which most commonly is the (1→3; *b*6)- (see ref. 6), but several dextrans also contain (1→3; *l*, *nc*)-linkages in the range of 24–35% (see ref. 8). The percentage of (1→3)-linkages in dextrans, as established by periodate-oxidation, structural analysis[9], constitutes a spectrum of values[9,11] lying in the range of ~5 to ~40%.

For brevity, and simplicity of expression, the dextrans may be grouped into several classes, based on operational definitions[12]. The structures of many dextrans differ from that of linear dextran by the inclusion of di-*O*-substituted residues, and are described as class I dextrans, having an *m* value to denote the position of *O*-substitution of the branch-point residue. Dextrans containing significant proportions of 3-mono-*O*-substituted α-D-glucopyranosyl residues [(1→3; *l*, *nc*)-linkages], and exhibiting diagnostic, ^{13}C-n.m.r. resonances (recorded at 90°) at ~83.3, ~99.9, and ~101.05 p.p.m., are described as class II dextrans. A third group of dextrans, also containing (1→3)-α-D-linkages, but differing in solubility and spectroscopic properties, has tentatively been described as class III dextrans, the class being operationally defined as consisting of those dextrans whose i.r. spectra contain a significant band at 822 cm^{-1}.

The α-D-glucans from various oral Streptococci, such as *Streptococcus mutans* and *S. sanguis*, also characteristically have (1→6)- and (1→3)-linkages. This series on unusual dextrans has examined[1] the soluble (S) fractions obtained from native D-glucans produced by glucosyltransferases obtained from various strains of *S. mutans*. Comparisons, based on 25-MHz, ^{13}C-n.m.r. spectra of the compounds in water, between these soluble D-glucans from *S. mutans* and the soluble fractions obtained from native, extracellular dextrans produced *in vivo* by various strains of *L. mesenteroides* indicate the essential identity of the S fractions from *S. mutans* and the fractions from *L. mesenteroides* described as class I ($m = 3$) dextrans [dextrans containing (1→3; *b*6)-linkages]. Such observations are also in agreement with the results of methylation-fragmentation, structural-analysis studies on soluble D-glucans produced by glucosyltransferases from strains of *S. mutans*[13]. These polysaccharides arising from strains of *S. mutans* have been described either as dextrans or as D-glucans, and in the following discussion, the terms will be used interchangeably, recognizing the fact that all known D-glucans produced by *S. mutans* contain both (1→3)- and (1→6)-linkages, but that many of the insoluble products can deviate extensively from the (1→6)-linked backbone concept of linear dextran.

Early investigations[9,14] of the structure of dextrans noted that the i.r. spectra of many dextrans from *L. mesenteroides* and its congeners contain weak, but distinct,

bands at ~12.6 μm (795 cm^{-1}) and ~12.2 μm (822 cm^{-1}). In general, the intensity of the band at 795 cm^{-1} could be related to the percent content of (1→3)-linkages in the dextran, and the band at 822 cm^{-1} could be related to insolubility properties; however, there was no direct correlation, in terms of simple (1→3)-linkage content, to the intensity of the 822-cm^{-1} band, or to the solubility properties. Independently, a set of D-glucans, composed of very high percentages of [presumably, contiguously linked] (1→3)-α-D-linked residues, was obtained from fungal (*Aspergillus niger*) and soil-yeast (species of *Cryptococcus* and *Schizosaccharomyces*) sources, and the i.r. spectra of these polysaccharides also contained the 822-cm^{-1} band[15]. Later, this band at 822 cm^{-1} was observed in the i.r. spectra of insoluble D-glucans that had been produced by glucosyltransferase systems obtained from strains of *S. mutans*[16], and also in those of insoluble D-glucans[17] that had been fractionated from the same source by methods similar to those employed for fractionation of the extracellular dextrans from *L. mesenteroides*[18].

The nature of the insoluble D-glucan produced by *S. mutans* was examined by Ebisu *et al.*[19], who employed products produced by glucosyltransferases from strain OMZ 176, in conjunction with methylation-fragmentation analysis, structural modification by enzymic degradation, and controlled Smith degradations, and who concluded that the principal, non-dextran-like, structural feature of this D-glucan was the presence of (1→3, *l*, *c*)-linked residues. Hare *et al.*[13] also examined D-glucans produced by glucosyltransferases, from several strains of *S. mutans*, by use of methylation-fragmentation studies and of specific, polymer degradation with (1→3)-α-D-glucanase and dextranases, and concluded that the insoluble D-glucans consisted of regions of (1→3, *l*, *c*)-linked residues and also of linear-dextran-like regions which, in addition, contained (1→3; *b*6)-linked residues. On the basis of selective, dextranase degradation, which yielded an insoluble residue containing a high proportion of (1→3)-linkages, it is possible that many of these dextran-like regions are peripheral to the (1→3; *l*, *c*)-linked regions.

Colson *et al.*[20] continued the examination of the fractionated D-glucans (from *S. mutans*), that had been studied by ^{1}H-n.m.r. and i.r.[17] spectroscopy, by the use of ^{13}C-n.m.r. spectra of solutions of high pH (pD). The anomeric regions of both the ^{1}H- and ^{13}C-n.m.r. spectra yield chemical shifts for (1→3, *l*, *c*)-linked residues that are distinct from those for (1→3; *b*6)-linked residues, although, in both cases, the chemical shifts for the (1→3, *l*, *c*)-linked residues tend to be obscured by other anomeric resonances. Such ^{13}C-n.m.r. measurements are hindered by the necessity of solubilizing the samples, and by the relatively high field-strengths which are needed in order to resolve the closely packed resonances in the anomeric spectral-regions of these polysaccharides. One of the more-insoluble D-glucan fractions, having a high content of (1→3)-linkages (73%), namely, D-glucan K-1R fraction B, was treated with a dextranase, yielding an insoluble polysaccharide containing an increased (relative to the starting material) percentage (92%) of (1→3)-linkages. Again, such results are in accord with the concept that class I ($m = 3$), dextran-like

regions exist in the insoluble D-glucans produced by *S. mutans*, and that these regions are peripheral to regions of insoluble, (1→3, *l*, *c*)-linked residues.

All of these studies[13,17,19,20] of D-glucans from *S. mutans* employed polysaccharides produced *in vitro* with glucosyltransferases obtained from the various bacterial strains. Tsumuraya and Misaki[21] described insoluble D-glucans, from *S. salivarius* (strain HHT), that were produced in two ways: (a) the *in vitro* method just described, which employs a glucosyltransferase, and (b) the *in vivo* method, from a growing culture. The structures of the D-glucans from *S. salivarius* were examined by methylation-fragmentation analysis, Smith degradations, and mild Smith-degradations. It was concluded that, in general, the structures of the D-glucans produced by both the *in vitro* and the *in vivo* method by strain HHT of *S. salivarius* were the same, and similar to those[13] of the insoluble D-glucans produced from *S. mutans* by *in vitro* methods. However, certain differences were found for the structures of the insoluble D-glucans produced *in vitro* and *in vivo* by *S. salivarius*; the D-glucan from the growth broth (*in vitro* production) was found to have a lower content of (1→3; *l*, *c*)-linkages (80% *vs.* 88%), and the linear-dextran-like region contained a higher degree of branching [and had (1→4)-, as well as (1→3)-, branching linkages].

An extensive survey[9], based on periodate-oxidation studies, of the dextrans and dextran fractions resulting from *in vivo* production by strains of *L. mesenteroides* and its congeners indicated that these bacterial strains can produce a wide variety of structures which are based on the linear dextran structure, including those D-glucans described in Table III of ref. 9. In addition, several polysaccharides (identified by superscript *b*) listed in that Table have physical properties similar (or essentially identical) to those of the insoluble D-glucans of *S. mutans* and *S. salivarius*. The relationship of structure to physical properties for the insoluble D-glucans obtained from strains of *S. mutans* and of *L. mesenteroides* has been discussed[12], although it should be stressed that no information is at present available as to the relative positions of the (1→3; *l*, *c*)-linked and the linear-dextran-like regions of the structure of the insoluble D-glucans from *L. mesenteroides*.

Therefore, as indicated in the preceding section, an accurate assessment of the actual percentage of the (1→3; *l*, *c*)-linkage content is essential to an understanding of the structure of insoluble D-glucans from the bacterial sources just described. Hare *et al.*[13] provided a succinct summary of the relationship (*a*) between the establishment and cariogenicity of strains of *S. mutans* with regard to dental plaque, and (*b*) of the insolubility properties and (1→3; *l*, *c*)-linkage content of the D-glucans produced by these bacterial strains. However, in contrast to the relative ease with which the content of (1→3, *l*)-linkages can be established, there is no simple, convenient method for establishing the percentage of (1→3; *l*, *c*)-linked residues in a given polysaccharide. As already described, the reports that identified (1→3; *l*, *c*)-linkages[13,19,21] employed multiple structural-determinations, interspaced with chemical or enzymic degradations. The structural model for the (1→3; *l*, *c*)-linked segments of the insoluble D-glucans does exist, namely, pseudonigeran, an essentially linear D-glucan, named by Horisberger *et al.*[22], which is composed of ~98% of 3-mono-*O*-substituted

α-D-glucopyranosyl residues. The relatively small proportion (~1.5%) of (1→4)-linked residues in pseudonigeran interferes relatively little with the properties of the (1→3; *l*, *c*)-linked residues, which preponderate in this biopolymer. Although the i.r. spectrum of pseudonigeran, as described by Horisberger *et al.*[22], was not studied, the essential identity of this material to the polymer reported by Johnston[23], and examined by i.r. spectrometry by Bacon *et al.*[15], assured the presence of a band at 822 cm^{-1} in the i.r. spectrum of this D-glucan. It should be noted that, in terms of the total, dispersive i.r. spectra[14,15,24] of these dextrans and D-glucans, the bands at 822 and 798 cm^{-1}, which are associated with (1→3)-linkages, are relatively minor, spectral features.

Dextran B-1149 fraction A is an additional polysaccharide of interest for the study of (1→3; *l*, *c*)-linkages. The ^{13}C-n.m.r. spectrum of this relatively insoluble D-glucan, which has been shown by periodate oxidation to contain (1→3)-linkages[9], contains no resonances that have been associated with (1→3: *b*6)-linkages or with (1→3, *l*, *nc*)-linkages, but displays diagnostic, ^{13}C-n.m.r. chemical-shifts similar to, but slightly displaced from, the aforementioned, diagnostic shifts for (1→3)-linkages. On the basis of these observations, and in conjunction with the previous[2], that the dispersive, i.r. spectrum of dextran B-1149 fraction A contains a band at 822 cm^{-1}, but not at 798 cm^{-1}, it has been concluded that the structure of this D-glucan contains (*a*) a relatively large percentage of contiguously linked, linear-dextran-like, 6-mono-*O*-substituted α-D-glucopyranosyl residues, (*b*) a smaller proportion of (1→3; *l*, *c*)-linked residues, and (*c*) no spectroscopically detectable (1→3; *l*, *nc*)-linked or (1→3: *b*6)-linked residues. Therefore, dextran B-1149 fraction A is an interesting dextran. It contains (*a*) a spectroscopically significant proportion of (1→3, *l*, *c*)-linked residues, yet it is sufficiently soluble in water to be studied in aqueous systems, and (*b*) (1→3: *l*, *c*)-linkages, but no significant proportion of (1→3; *b*6)-linkages. This makes it unique among currently known D-glucans of bacterial origin, and allows the examination of spectral features arising from (1→3: *l*, *c*)-linkages without the interference of spectral features that could arise from other (1→3)-linkages. Although dextran B-1149 fraction A does not contain (1→3: *b*6)-linkages, acetolysis data indicate small mole-percentages of (1→2)-linkages[25], and ^{13}C-n.m.r. data[12] indicate that these are (1→2; *b*6)-linkages. Some other, independent data which indicate that dextran B-1149 fraction A contains mole-percentages of (1→3)-linked residues that are significantly larger than 10% have been discussed in the context of our ^{13}C-n.m.r. studies[12].

Our secondary objective in this report, the study of the bands that can arise in F.t.-i.r. difference-spectra from the presence of (1→4)-linkages in the structures of polysaccharides, stems from both our relatively limited knowledge of these structure-to-spectra relationships, and the possibility that a number of D-glucans of oral, bacterial origin could contain these (1→4)-linkages. As previously discussed, the insoluble D-glucan produced by a growing culture of a strain of HHT of *S. salivarius* was found to contain a significant proportion of (1→4)-linkages[21]. Similarly, one of the relatively few, structural analyses of a D-glucan produced by a growing culture of *S. mutans* (strain E49), by Lewicki *et al.*[26], indicated the presence of ~8 mole-

percent of 4-mono-*O*-substituted D-glucopyranosyl residues. Furthermore, as previously discussed, pseudonigeran is known to contain ~2% of (1→4)-linked residues. Our previous study of F.t.-i.r. difference-spectra included only a single example of a D-glucan containing (1→4)-linked residues, namely, dextran B-1254 fraction S[L], a dextran that differs from linear dextran by a significant content (~20 mole-percent) of 4,6-di-*O*-substituted residues.

TABLE I

WAVENUMBERS OF THE MAJOR BANDS OBSERVED FOR THE F.T.-I.R. DIFFERENCE-ABSORBANCE PLOTS OF DEXTRANS AND COMPARISON POLYSACCHARIDES[a]

Number[c]	*Compound*								
	Pseudo-nigeran	*B-1149*	*OMZ 176*	*K-1R*	*K-1R*	*OMZ 176*	*B-742*	*Amylose*	*Nigeran*
		Fraction							
		A	*B*	*B*	*B-residue*	*S*	*L*		
	Spectra[b]								
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
1a[c]								1179.4	1179.4
1b	1175.1		1175.1		1175.2				
1		1149.3			1147.3	1147.1	1144.6	1151.4	
2a	1138.5		1142.9						
2b							1108.9	1127.7	1132.0
2	1093.3	1091.1	1091.3	1091.3	1091.3	1091.1	1078.2	1089.0	1093.3
3	1065.4		1065.3	1065.5	1065.3		1061.0		
4	1033.0		1033.0	1031.0	1031.1	1030.9		1026.6	1030.7
5a						985.7	985.7	983.5	977.0
5	970.6	970.6	970.7	970.7	970.7				
6a							948.9		
6	931.8	929.7	929.7			929.7	928.4	934.0	934.0
7a	889.5			897.5	897.5				
7b									865.1
7c	822.0	822.0	820.0	820.0	820.0				
7			798.4			798.4	789.8	789.8	791.9
9a[d]							768.8		
9b		742.2				731.6	754.2		
9[d]							739.0		
11a							604.0	604.1	600.3
11							574.5	576.6	572.3
12[d]							550.2		
13	537.9					537.9	527.4		
14							482.3	484.1	

[a]All absorbance-spectra bands are the result of subtracting the spectrum of dextran B-1254 fraction L[$] from the spectrum of interest. Unless otherwise noted, all wavenumbers refer to maxima. [b]These letters correspond to the plots in Figs. 1 and 2. [c]These numbers are referenced to the absorbance plots A through I in Figs. 1 and 2, and to the numbers in ref. 2. [d]These wavenumbers refer to minima.

RESULTS AND DISCUSSION

The wavenumbers of the major bands observed for the F.t.-i.r. difference-absorbance plots of the dextrans, α-D-glucans, and comparison substances are given in Table I. Seven new examples of α-D-glucans that contain significant percentages of

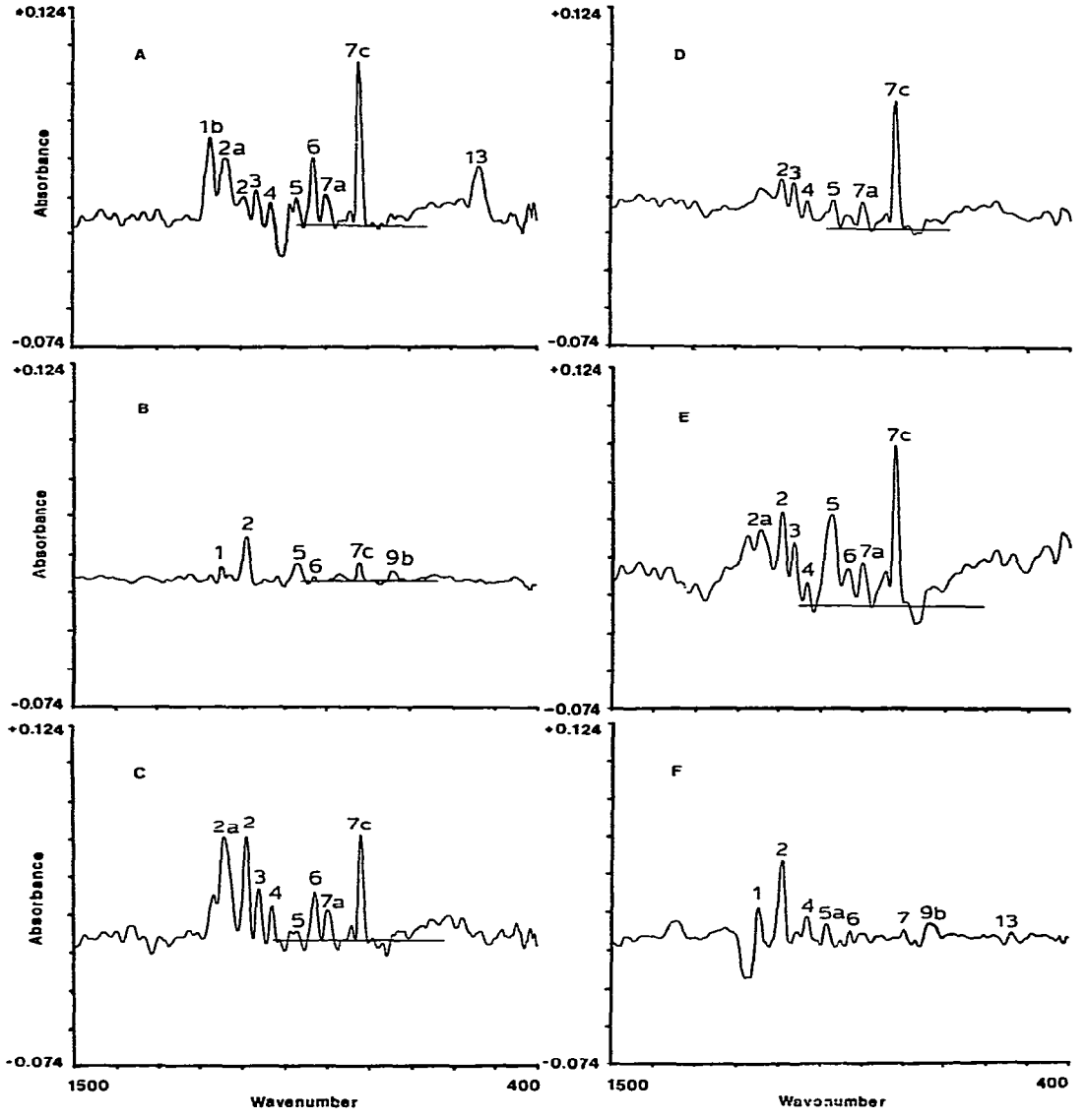

Fig. 1. The F.t.-i.r. difference-absorbance plots for A, pseudonigeran; B, dextran B-1149 fraction A; C, D-glucan OMZ 176 fraction B; D, D-glucan K-1R fraction B; E, D-glucan K-1R fraction B-residue; and F, D-glucan OMZ 176 fraction S. (All absorbance spectra are the result of subtracting the spectrum of dextran B-1254 fraction L[Ś] from the spectrum of interest. The numbered bands correspond to those identified in Table I.)

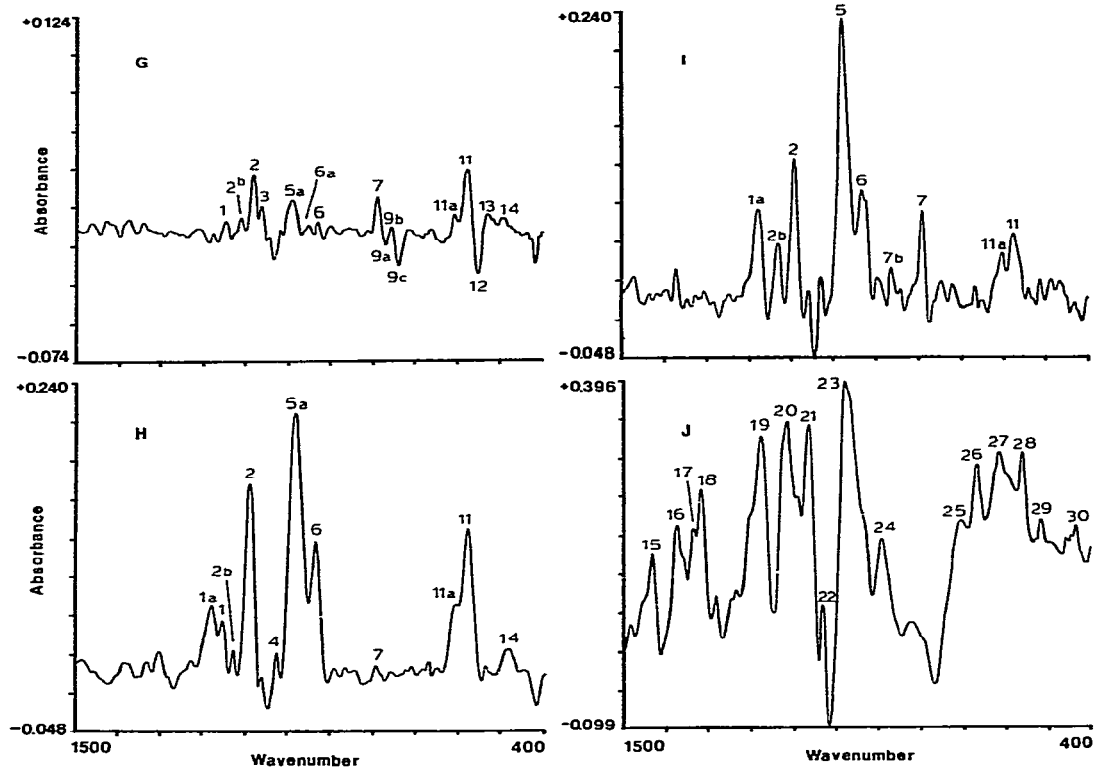

Fig. 2. The F.t.-i.r. difference-absorbance plots for G, dextran B-742 fraction L; H, amylose; I, nigeran; and J, cellulose. (Note the differences in the vertical-scale expansions. All absorbance spectra are the result of subtracting the spectrum of dextran B-1254 fraction L[S] from the spectrum of interest. The numbered bands correspond to those identified in Table I.)

(1→3)-linked residues have been examined, namely, four D-glucans produced by glucosyltransferases from strains (OMZ 176 and K-1R) of *S. mutans*; a dextran fraction arising from extracellular, *in vivo* production by *L. mesenteroides* B-1149; pseudonigeran [an essentially linear, (1→3)-α-D-linked D-glucan]; and nigeran [which differs from the D-glucans of bacterial origin by containing a large proportion of (1→4)-linkages, rather than (1→6)-linkages]; see Figs. 1 and 2.

In addition, we had previously examined the F.t.-i.r. difference-spectra of dextrans B-1142 and B-1191, which contain significant percentages of (1→3; *b*6)-linkages, and of dextran B-1355 fraction S, which contains significant percentages of (1→3; *l*, *nc*)-linkages[2]. In all examples, the difference-spectra represent the result of interactively subtracting the spectrum of dextran B-1254 fraction S[L] from that of the polysaccharide under consideration. In Table I (first column), the absorption bands are identified by small numerals that correspond to (*a*) the respective peaks in Figs. 1 and 2, and (*b*) the equivalent wavenumbers in Table II of ref. 2. Thus, direct comparison may be made between spectra reported here and those of representative dextrans having various non-(1→6)-linkages.

The difference-spectra of α-D-glucans having (1→3)-α-D-linkages. — The deflections of the F.t.-i.r. difference-spectrum of pseudonigeran are very small, compared to the difference-spectra of other polysaccharides which are exclusively composed of non-(1→6)-linked α-D-glucopyranosyl residues (*e.g.*, amylose and nigeran). The dominant feature of the pseudonigeran difference-spectrum is a sharp (half-height peak-width of ~10 cm^{-1}), symmetrical band at 822 cm^{-1} (band 7c), which corresponds to the band that had been observed in the dispersive, i.r. spectrum of this material. The band at 798 cm^{-1} (band 7), originally observed to be present in the i.r. spectra of many dextrans known to contain (1→3)-linkages[9-14], and which we have found to be very prominent in the F.t.-i.r. difference-spectra of dextrans containing (1→3; *b*6)- and (1→3; *l*, *nc*)-linkages[2], is essentially absent from the F.t.-i.r. difference-spectrum of pseudonigeran. The 822-cm^{-1} band is also present in the F.t.-i.r. difference-spectra of the following insoluble polysaccharides: D-glucan OMZ 176 fraction B, D-glucan K-1R fraction B, and D-glucan K-1R fraction B residue (after dextranase treatment).

In our earlier approach to the quantitation of the intensity of the F.t.-i.r. band for 2,6-di-*O*-substituted residues, the function $y \propto 1/(n + 1)$ (where y is the band intensity, and n, the number of backbone chain-extending residues between branch-point residues) was employed, as no i.r. standard was available for a polysaccharide completely composed of 2,6-di-*O*-substituted α-D-glucopyranosyl residues. In an attempt to quantitate the contribution of the 822-cm^{-1} band, a similar, but somewhat more direct, approach can now be taken in the following steps. (*a*) A "baseline" is established for each difference-spectrum by identifying an average absorbance-value across the 1500–400-cm^{-1} region (the baseline chosen is identified in each difference-spectrum in Fig. 1). (*b*) The peak-height of the 822-cm^{-1} peak of each difference-spectrum is expressed in an absorbance-difference value (ΔA_{822}), as listed in Table II.

TABLE II

CORRELATION OF THE MOLE-PERCENT CONTENT OF CONTIGUOUSLY LINKED, 3-MONO-*O*-SUBSTITUTED α-D-GLUCOPYRANOSYL RESIDUES IN D-GLUCANS TO THE INTENSITY OF THE 822-cm^{-1} BAND OF THEIR RESPECTIVE F.T.-I.R. DIFFERENCE-SPECTRA

D-*Glucan*	ΔA_{822}[a] × 10^2	*Normalized*[b] ΔA_{822}	*Alternative estimate of (1→3; l, c)-linkage content*
Pseudonigeran	9.41	98	98[c]
B-1149 fraction A	0.93	9.7	~10[d]
OMZ 176 fraction B	5.95	62	67[e,f]
K-1R fraction B	7.60	79	84[e]
K-1R fraction B-residue	9.01	94	91.6[e]

[a]Data taken from plots in Fig. 1. [b]Values normalized to the 98% (1→3)-linkage content established for pseudonigeran in ref. 22. [c]Data taken from ref. 22. [d]Data taken from ref. 12. [e]Data taken from ref. 20. [f]Data taken from ref. 27.

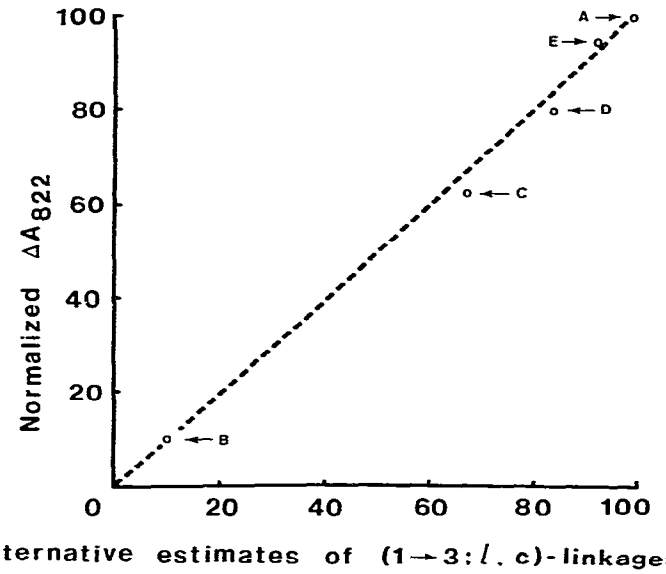

Fig. 3. The differences in absorbance between the peak height of the i.r. band at 822 cm^{-1} (with regard to the average "baseline" of the difference-spectra) for various insoluble D-glucans *vs.* alternative estimates of the (1→3; *l*, *c*)-linkage content of these D-glucans as established by methods not dependent on i.r. measurements. (The capital letters identifying the specific values correspond to the absorbance-difference plots of Fig. 1, and are referenced to the specific D-glucans in Table I.)

In addition, a second value, an estimate of the content of (1→3; *l*, *c*)-linkages as established by alternative means, is listed for each D-glucan in Table II. As pseudonigeran has been determined to contain 98% of (1→3; *l*, *c*)-linkages, this value is employed for the normalization of the ΔA_{822} values, as listed in Table II. The two sets of values from Table II, the normalized, ΔA_{822} values, and the alternative estimate of (1→3; *l*, *c*)-linkage content, were then plotted in Fig. 3. The points in Fig. 3 show a good, linear relationship; however, this relationship must be treated with caution, as the accuracy of either set of values is uncertain. Firstly, the exact accuracy of the ΔA_{822} value depends on the choice of the baseline which, with regard to these data, has not been established on a statistical basis. Secondly, it is possible that even greater error exists for the values of the (1→3; *l*, *c*)-linkage content established by alternative means. These values were taken from the literature, and determination of the (1→3; *l*, *c*)-linkage content can be difficult with regard to the total, (1→3)-linkage content, and it is especially difficult to establish whether a significant proportion of (1→3; *l*, *nc*)-linkages is present.

The D-glucans from *S. mutans* OMZ 176 and K-1R (the S and B fractions) are identical to those samples previously described and studied[17,20]. The original, insoluble D-glucan K-1R is described in ref. 20, and the proportion of (1→3)-linkages was established by ^{13}C-n.m.r. spectroscopy to be 84%. The preceding D-glucan fraction was treated with a dextranase, and the resulting product[20] contained 91.6% of (1→3)-linkages. The K-1R residue reported here is not the same material as just

described, but was prepared in the same way from the same starting fraction B of D-glucan K-1R. For Table II, it has been assumed that all (1→3)-linkages are (1→3; *l*, *c*)-linkages, a reasonable assumption, as the diagnostic, 798-cm^{-1} band associated[2] with (1→3; *b*6)- and (1→3; *l*, *nc*)-linkages is absent from the F.t.-i.r. difference-spectra of these D-glucans from K-1R. The D-glucan OMZ 176 fraction B is the same material as that reported in ref. 20, where the (1→3)-linkage content was described as 85%, and it was concluded that the ratio of (1→3, *l*)- to (1→3; *b*6)-linkages is 4:1, implying a 67% content of (1→3, *l*)-linkages, assumed to be contiguously linked.

The ^{13}C-n.m.r. data for dextran B-1149 fraction A indicate a polysaccharide containing a large proportion of linear-dextran-like structure and a much smaller percentage of (1→3)-linkages [previously described, on the basis of the chemical shifts of the ^{13}C-n.m.r. spectra, as (1→3; *l*, *c*)-linkages]. The ratio of the resonance intensities of the 90°, ^{13}C-n.m.r. spectra[12] for resonances associated with 3-mono-*O*-substituted residues (82.5 p.p.m.) and 6-mono-*O*-substituted residues (75.1 p.p.m.) is ~1: 16; however, it has been established that the intensities of the resonances of carbon atoms directly associated with intersaccharide linkages (*e.g.*, the 82.5-p.p.m. resonance) are considerably weaker than the other resonances, and a correction factor must be applied[6]. The maximum value observed for such a correction factor would be 0.3 [indicating a minimum content of (1→3)-linkages of somewhat less than 20%]: however, a more realistic, average value for the correction factor would be ~0.5, and therefore, the value of ~10% for alternatively established (1→3; *l*, *c*)-linkage content has been employed in Table II.

Within the limitations of the accuracy of the measurements, it would therefore appear than the peak-height of the 822-cm^{-1} band of the F.t.-i.r. difference-spectra is directly proportional to the (1→3; *l*, *c*)-linkage content of a D-glucan. The assumption, based on ^{13}C-n.m.r. spectrometry, that dextran B-1149 fraction A contains no (1→6; *b*6)- or (1→3; *l*, *nc*)-linkages appears to be confirmed by the absence of a band at 798 cm^{-1} in the difference-spectrum of this compound, and the presence of an intense band at 798 cm^{-1} in the difference-spectra of dextrans B-1142, B-1191, and B-1355 fraction S, all of which are dextrans that contain a significant proportion of (1→3; *b*6)- or (1→3; *l*, *nc*)-linkages. However, at present, the intensity of the 798-cm^{-1} band cannot be directly related to the percent of linkage[2], and, in the difference-spectrum of D-glucan OMZ 176 fraction B, the intensity of the 798-cm^{-1} band is very weak for a compound for which methylation-fragmentation analysis[20] indicates a (1→3; *b*6)-linkage content of ~20%.

The apparent linearity of the relation of the F.t.-i.r. band at 822 cm^{-1} to the (1→3; *l*, *c*)-linkage content of a polysaccharide (see Fig. 3), and the absence of this band from the F.t.-i.r. spectrum of any other D-glucans that have been studied, has several interesting implications. Firstly, it is implied that, for F.t.-i.r. difference-spectrometry, the nature of the residues that are *adjacent* to the specific residue under consideration can profoundly affect the i.r. bands associated with that residue. This phenomenon is in contrast to the effects observed in the ^{13}C- and ^{1}H-n.m.r. spectra of these D-glucans[17,20], and for the ^{13}C-n.m.r. spectra of D-glucans in general[6],

where the effects of structural changes in adjacent residues are normally confined to relatively modest changes in the chemical shifts of selected resonances associated with the residue under consideration.

Secondly, the apparent linearity of the plot in Fig. 3 suggests that, although adjacent, 3-mono-*O*-substituted residues are required for display of the 822-cm^{-1} band by a given 3-mono-*O*-substituted residue, the actual intensity of this band is directly proportional to the mole-percent of contiguously linked, 3-mono-*O*-substituted residues in a given D-glucan, and that long-range effects (in terms of molecular structures) are not involved. Such an observation is in accord with generally accepted approaches to the interpretation of i.r. spectra, in contrast to u.v. spectrometry, where long-range, structural features (*e.g.*, conjugated, unsaturated bonds) can result in situations where the net, spectral effect is much greater than the spectral effects of the individual linkages. The absence of interfering bands in the region of 822 cm^{-1} from the F.t.-i.r. spectra of other D-glucans [as indicated in our previous study[2] and in the following discussion on (1→4)-linked D-glucans] makes the 822-cm^{-1} band a useful spectral feature for studying the presence of (1→3; *l*, *c*)-linkages.

The foregoing discussion has dealt with analysis of the 822-cm^{-1} band, which is a well defined and prominent feature of the difference-spectra of D-glucans containing (1→3: *l*, *c*)-linkages. However, for the general analysis of difference-spectra, it is necessary to know both the accuracy and the reproducibility of these data, especially with regard to the wavenumbers. To date, the difference-spectra of 17 compounds, recorded in three short time-intervals separated by ~6 months, have been examined. The first group of difference-spectra was reported in our initial F.t.-i.r. article[2]; the second consists of all difference-spectra reported herein, except for plots C, D, and E; and the third comprises the difference-spectra of the fractions from K-1R, and D-glucan OMZ 176 fraction B. In addition, a difference-spectrum of OMZ 176 fraction B, not listed herein, was recorded with the second set of difference-spectra.

For all cases, the difference-spectra were obtained by interactively subtracting the F.t.-i.r. data-set, obtained from dextran B-1254 fraction L[$] for the original group of spectra, from the F.t.-i.r. data-sets obtained for the other polysaccharides. Although repetitive spectra of the same sample were not recorded, groups of compounds having identical, structural features (*e.g.*, 2,6-di-*O*-α-D-glycosylated α-D-glucopyranosyl residues), and presumably identical, spectral bands, have been studied. The information for three class I ($m = 2$) dextrans, namely dextran B-1402, B-1422, and dextran B-1299 fraction S, was included with the first group of data; Table II of ref. 2 shows that the maxima of many bands (5, 6, and 14) are essentially identical to within ± 0.1 cm^{-1}, the variation of the maxima of most bands being <2 cm^{-1}.

Similarly, the difference-spectra of the two class I ($m = 3$) dextrans (dextrans B-1142 and B-1191), which had been recorded in the same group of difference-spectra (see Table II of ref. 2), show even less variation, with most maxima differing by <0.3 cm^{-1}. The difference-spectrum of D-glucan OMZ 176 fraction S, a polysaccharide indicated[1] by ^{13}C-n.m.r. spectroscopy to be identical to class I ($m = 3$) dextran, was recorded in the second set of difference-spectra (see Table I), and a comparison of

these values with those of the difference-spectra of dextrans B-1142 and B-1191 indicated differences of ~ 2 cm^{-1} between the maxima of the principal bands. The B fraction of D-glucan OMZ 176 was examined twice by F.t.-i.r. difference-spectrometry, along with the second and third set of spectra, and typical changes in band maxima were by 2 cm^{-1}, or less (*e.g.*, 820.0 *vs.* 822.2, 929.8 *vs.* 929.7, 1031.0 *vs.* 1033.0, and 1191.3 *vs.* 1191.1 cm^{-1}). On the basis of these observations, it could be inferred that, for comparison of difference-spectra recorded over a relatively short interval of time (a day, or a few days), deviations of >0.5 cm^{-1} for band maxima probably indicate differences in structural features, whereas, for comparison of difference-spectra recorded at greater time-intervals (and, presumably, comparison of those difference-spectra recorded on different instruments), deviations in band maxima of >2 cm^{-1} would be needed, in order to indicate differences in structural features.

The data for the bands observed in the F.t.-i.r. difference-spectra of α-D-glucans that contain (1→3)-linkages may be summarized as follows. (*a*) The intensity of band 2 (~ 1086 cm^{-1}) may be considered to be a measure of the (1→3; *b*6)-linkage content. However, band 2 is also a general measure of the degree of branching through C-2 and C-4, and, as discussed in a following section, a possible contribution to band 2 can arise from (1→4)-linkages. (*b*) Based on the single example of the difference-spectrum for dextran B-1355 fraction S, it would appear that (1→3; *l*, *nc*)-linkages are related to band 4 (~ 1034 cm^{-1}). The band 2 of this difference-spectrum is somewhat more intense than the established percentage of (1→3, *b*6)-linkages[8] for dextran B-1355 fraction S would indicate, further suggesting that (1→3; *l*, *nc*)-linkages can make a contribution to this band. (*c*) Band 7c (822 cm^{-1}) has been associated only with (1→3; *l*, *c*)-linkages. Based on the pseudonigeran spectrum, it would appear that the (1→3; *l*, *c*)-linkages contribute to band 1b (~ 1175 cm^{-1}) and band 2a (~ 1139 cm^{-1}), and also, in lesser degree, to bands 2, 3, and 4; however, due to the relatively intense contribution of (1→3; *b*6)- and (1→3; *l*, *nc*)-linkages to band 2, the aforementioned bands would be poor diagnostic tools for the quantitation of contiguously linked (1→3)-linkages. (*d*) Several weaker bands persist in all of these difference-spectra [*e.g.*, band 5 (~ 971 cm^{-1}) and band 6 (~ 930 cm^{-1})], and it is possible that these bands will result from the subtraction of the spectrum of linear dextran from the spectrum of any other α-D-glucan. (*e*) The major, remaining, spectral feature of these difference-spectra is band 7 (~ 790 cm^{-1}), which is often followed by a minimum at band 8 (~ 764 cm^{-1}) and a second maximum at band 9 (~ 738 cm^{-1}). Band 7–band 8–band 9 was observed as a prominent feature in the difference-spectra of all previously reported, branching dextrans[2], and it can also be observed in the difference-spectrum of dextran B-742 fraction L (see plot G of Fig. 2).

It is, therefore, tempting to correlate band 7 of these difference-spectra to the absorption at 795 cm^{-1} in the dispersive spectra of dextrans, produced by strains of *L. mesenteroides*, that contain (1→3)-linkages. However, there are several problems in relating band 7 to the 795-cm^{-1} band of the dispersive spectra; these are: firstly, there does not appear to be a direct relationship of peak-height to (1→3)-linkage

content for band 7 (*e.g.*, dextran B-1142, dextran B-1191, and dextran B-1355 fraction[2] S); secondly, the difference-spectra of several dextrans that contain non-(1→3)-branching features, and little or no (1→3)-linkage, also exhibit the band 7–band 8–band 9 pattern {*e.g.*, dextran B-1402, dextran B-1424, dextran B-1254 fraction S[L] (see ref. 2), and dextran B-742 fraction L}; and thirdly, the difference-spectra of several D-glucans produced by strains of *S. mutans* (*e.g.*, D-glucans OMZ 176 fractions S and B, and D-glucan K-1R fraction B), D-glucans known to contain significant proportions of (1→3)-linkages, have no, or a very small, band 7. Therefore, the precise relationship of band 7 to polysaccharide structure remains unknown.

The difference-spectra of α-D-glucans having (1→4)-α-D-linkages. — We now have data for four α-D-glucans that contain significant mole-percentages of (1→4)-linked residues. Relatively large percentages of 4,6-di-*O*-substituted α-D-glucopyranosyl residues are incorporated[28] in dextran B-1254 fraction S[L] (19.5%) and dextran B-742 fraction L (12.4%). Additional, minor constituents, 3.4% of 4-mono-*O*-substituted residues for dextran B-1254 fraction S[L], and 0.7% of 3-mono-*O*-substituted residues for dextran B-742 fraction L, have also been detected by methylation-fragmentation analysis[28]. It is assumed that these minor, structural features do not contribute significantly to the F.t.-i.r. difference-spectra of these branched dextrans. The general features of the difference-spectrum of dextran B-742 fraction L (plot G in Fig. 2) are quite similar to those previously observed for the difference-spectrum of dextran B-1254 fraction S[L] (plot F in Fig. 2 of ref. 2); for example, band 2 is less intense for the more-linear dextran B-742 fraction L than for dextran B-1254 fraction S[L]. In addition, band 11 and the accompanying minimum (band 12), which were observed in the difference-spectrum of dextran B-1254 fraction S[L], are also observed in the difference-spectrum of dextran B-742 fraction L.

The difference-spectra of amylose and nigeran are quite distinctive (see plots H and I in Fig. 2), as both contain quite intense bands, although a general "baseline" is apparent for each spectrum. For convenience of presentation, the vertical scales of both of these difference-spectra have been compressed, relative to the plots in Fig. 1 and to the plot for dextran B-742 fraction L in Fig. 2 (compressed by a factor of 1.45). Not only are the difference-spectra of amylose and nigeran similar in band amplitude, but they are also similar in terms of their general, spectral "profile" and the wavenumbers of the maxima. Amylose is an essentially linear α-D-glucan composed of 4-mono-*O*-substituted α-D-glucopyranosyl residues, or, alternatively, of (1→4; *l*, *c*)-linkages. A convenient interpretation of these difference-spectra is as follows. (*a*) For α-D-glucans, (1→4)-linked residues contribute more-intense F.t.-i.r. bands than do (1→3)-linked residues. (*b*) The difference-spectrum of nigeran, a D-glucan composed of alternating 3-mono-*O*-substituted and 4-mono-*O*-substituted residues, is therefore dominated by the contribution of bands from the 4-mono-*O*-substituted residues. (*c*) The wavenumbers of the F.t.-i.r. bands of the (1→4; *l*, *c*)-linked residues (from amylose) are quite similar to those of the F.t.-i.r. bands from the (1→4; *l*, *nc*)-linked residues (in nigeran). Such an approach to spectral analysis would provide an explanation of the great similarities of bands 1a, 2, 5a, 6, 11a, and 11 in the difference-

spectra of amylose and nigeran. The relatively weak bands for nigeran (bands 2b, 7b, and 7) could then be explained as contributions from the less-intense bands of (1→3; *l*, *nc*)-linked residues.

The prominence of band 11 (~575 cm^{-1}) in the difference-spectra of amylose, nigeran, dextran B-1254 fraction S[L], and dextran B-742 fraction L further confirms our suggestion[2] that this band is diagnostic of (1→4)-linkages. The difference-spectra of both dextran B-1254 fraction S[L] and dextran B-742 fraction L display band 11 (a maximum) and band 12 (a minimum), and it is possible that this pattern is diagnostic of 4,6-di-*O*-substituted residues. The difference-spectra of neither amylose nor nigeran displays the band 11–band 12 pattern, but both difference-spectra do contain band 11, with a slightly weaker band 11a (~600 cm^{-1}), and it may be that the band 11a–band 11 pattern is indicative of 4-mono-*O*-substituted α-D-glucopyranosyl residues. It should be noted that the difference-spectra of both amylose and nigeran contain prominent peaks of band 2 (1091 cm^{-1}), and this is the band for which a peak-height to degree-of-branching correlation has been suggested[2]. Therefore, the use of band 2 for estimating the degree of branching is apparently useful only when the (1→4; *l*)-linkage content of a polysaccharide is minimal, or non-existent; however, the presence, or absence, of bands 11a and 11 should be useful in independently establishing the content of (1→4; *l*)-linkages in a given α-D-glucan.

The difference-spectrum of the β-D-glucan cellulose. — The F.t.-i.r. difference-spectrum of cellulose was also recorded (see plot J in Fig. 2), and was plotted at a vertical-amplitude scale-reduction of 2.50, compared to spectra A through G. Cellulose and amylose are extremely similar structurally, being linear D-glucans differing only in the anomeric configuration of the 4-mono-*O*-substituted residues (all β-linkages for cellulose, and all α-linkages for amylose). However, the F.t.-i.r. difference-spectrum of cellulose shows bands of very large amplitude, compared to those of amylose, and displays essentially no baseline. Owing to the great difference in the spectral pattern of cellulose, as contrasted to the other F.t.-i.r. difference-spectra in Figs. 1 and 2, the prominent maxima of the cellulose spectrum (see Fig. 2) are not listed in Table I, but are identified by the following numbers and corresponding wavelengths: 15, 1431.3; 16, 1375.3; 17, 1334.4; 18, 1317.2; 19, 1375.3; 20, 1117.0; 21, 1065.3; 22, 1033.0; 23, 983.5; 24, 893.1; 25, 708.0; 26, 667.0; 27, 613.2; 28, 557.3; 29, 518.5; and 30, 434.6 cm^{-1}.

Barker *et al.*[29], employing a dispersive i.r. instrument, concluded that the spectra of α-D-glucans contain a band at 844 ±8 cm^{-1}, and that the spectra of β-D-glucans contain a band at 891 ±7 cm^{-1}. However, the 910–830-cm^{-1} region of the F.t.-i.r. difference spectrum of cellulose contains no pronounced maxima or minima (a minimum at ~844 cm^{-1} could result from the subtraction of the dextran spectrum), although the relatively weak band 24 (893 cm^{-1}) might be caused by this effect. As the F.t.-i.r. difference-spectra presented here, and previously[2] (for intercomparison of the spectra of α-D-glucans), show essentially no bands in the 1500–1200-cm^{-1} spectral-region, the prominent bands in this region of the spectrum of

cellulose suggest that this region could be useful for the general identification of β-linkages in D-glucans.

General observations and conclusions. — It is difficult to make direct comparison of these F.t.-i.r. difference-spectra to the weaker signals obtained from similar compounds by dispersive i.r. techniques. For example, the 950–700-cm^{-1} region of the dispersive i.r. spectra of amylose, cellulose, dextran, and nigeran, as shown in Fig. 1 of ref. 29, hardly predict the difference-spectra of these compounds as shown herein in Fig. 2. It should be noted that the spectra of cellulose and amylose in KBr pellets, recorded with dispersive i.r. instruments, showed differences in relative intensities of various absorbances for different specimens of the same polysaccharide. The intensity of these various absorbances have been shown to (*a*) differ for certain polymorphic, crystalline forms, (*b*) be intensified by the degree of order or crystallinity, and (*c*) be diminished by an increase in the proportion of the amorphous regions of the sample[29]. It remains to be established whether such variations in the crystallinity of polysaccharide samples have a similar effect for these F.t.-i.r. difference-spectra (which, compared to the studies made with dispersive instruments, have been recorded at much higher levels of radiant energy). However, within the context of the D-glucan samples of microbial origin studied herein, which are the products of similar, ethanol–water precipitation procedures, such factors of crystallinity do not seem to have any effect on band intensity.

Although the features of these F.t.-i.r. difference-spectra lack the close-to-theoretical, structure-to-spectra relationship operative for ^{13}C-n.m.r. spectra and in g.l.c.–m.s., methylation-fragmentation analysis, a variety of structural relationships for α-D-glucans becomes apparent on studying these F.t.-i.r. data. Band 2 can indicate the presence and the degree of branching, although care must be exercised if (1→4, *l*)-linkages are present. Band 11 is apparently diagnostic for (1→4)-linkages, and it is possible that adjacent bands (11a or 12) indicate, respectively, whether these linkages are (1→4; *b*6)- or (1→4; *l*)-linkages. For (1→3)-linked α-D-glucans, it is probable that bands 2, 4, and 7 are respectively diagnostic for (1→3; *b*6)-, (1→3; *l*, *nc*)- and (1→3; *l*, *c*)-linkages. However, for the quantitative differentiation of the various types of (1→3)-linkage (and for the analysis of F.t.-i.r. difference-spectra in general), it should be recognized that some contribution to the diagnostic bands can be made from effects different from those of the principal, structural feature associated with a given band.

This approach to spectral analysis obviously depends on the availability of relatively pure polysaccharides of well defined structure. A current deficiency in our knowledge relates to the effects resulting from the (1→2; *l*)-linkages of α-D-glucans, a situation that reflects the rarity of known α-D-glucans that contain large percentages of this type of linkage.

The persistence of well defined regions of average absorbance in these difference-spectra of α-D-glucans, an effect that we have referred to as a "baseline", provides a convenient basis for spectral interpretation. The deflections of large amplitude, and the lack of an apparent baseline for the difference-spectra of levan (see plot H

in Fig. 2 of ref. 2) and cellulose, indicate the small, and relatively subtle, spectral effects discussed for the different α-D-glucans.

The 822-cm^{-1} band (band 7c) associated with the (1→3; *l*, *c*)-linkages appears, in terms of structural specificity, to be unique among the bands observed in the difference-spectra of the various α-D-glucans. This is, indeed, fortunate, because the (1→3; *l*, *c*)-linkages apparently impart insolubility to polysaccharide structures, and it is therefore difficult to assess the content of this structural feature by methods that depend on the solubility of polymers.

EXPERIMENTAL

Materials. — *Leuconostoc mesenteroides* dextran B-1149 (see ref. 9) and dextran B-742 fraction L (see refs. 3 and 9) were those previously prepared and characterized. Dextran B-1149 was the total fermentation-product, and therefore was originally not designated as a fraction[18]. Here, however, it is designated fraction A in order to indicate the comparability of its purification procedure[9,18] to that for other A-type dextran fractions we have used that were insoluble in the fermentation mixture and, in the purification procedure, sedimented along with the bacterial cells. The water-insoluble D-glucans (OMZ 176 fraction B and K-1R fraction B) were produced by glucosyltransferases from the respective bacterial strains of *S. mutans*, and were the fractions previously described[17]. The water-soluble D-glucan OMZ 176 fraction S was the sample previously studied[1] by ^{13}C-n.m.r. spectroscopy. The sample designated D-glucan K-1R fraction-B-residue was the insoluble product resulting from the treatment of D-glucan K-1R fraction B with a dextranase [*Penicillium* sp. dextranase (Worthington)][20]; this fraction was not the same material as that analyzed by ^{13}C-n.m.r. spectrometry, but was prepared in the same way, from the same batch of D-glucan K-1R fraction B. Nigeran was a gift from Dr. T. Sawai, Aichi Kyoiku University, Kariya, Japan, who prepared it by extraction from mycelial cell-walls of a suitable strain of *Aspergillus niger*, and purification in the recommended way[31]. Pseudonigeran was a gift from Dr. J. H. Nordin, University of Massachusetts, Amherst, Mass., who prepared it by extraction from mycelial cell-walls of *A. luchensis*[32]. Amylose (potato type III) was obtained from Sigma Chemical Co., St. Louis, Mo., and cellulose (CC31), from Whatman Ltd., Maidstone, Kent, England.

Methods. — Samples (~80 μg) that had been thoroughly dried (see ref. 9 for recommended procedure) were weighed to ±0.1 μg, and incorporated into 3-mm, KBr micropellets. The i.r. spectra were recorded for 350 ±0.1 s with a Nicolet 7199 Fourier-transform, infrared spectrometer capable of wide coverage of wavelength (6000–400 cm^{-1}) equipped with a mercury–cadmium–telluride detector. The resulting F.t.-i.r. data-files were then weight-normalized (in absorbance) against the essentially linear dextran B-1254 fraction L[$] (see refs. 3 and 33). The data file of dextran B-1254 fraction L[$] was then interactively subtracted from each of the other dextran data-files, and the resulting, dextran difference-absorbance data were plotted at a uniform, wavenumber scale for each polysaccharide. The same absorbance

scale (vertical axis) was used for the α-D-glucans in plots A–G, Figs. 1 and 2. However, for amylose and nigerose (plots H and I, Fig. 2), a compressed scale was required, because of the high absorbance of the (1→4)-linkages relative to the (1→3)-linkages. When multiplied by 1.45, the peak heights of spectra H and I become directly comparable with those of spectra A–G. Similarly, the compressed, vertical axis of plot J, the difference-spectrum of cellulose, must be multiplied by 2.50 for direct comparison with plots A–G.

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