Carbohydrate Research, 74 (1979) 63–75

FOURIER-TRANSFORM, INFRARED DIFFERENCE-SPECTROMETRY FOR STRUCTURAL ANALYSIS OF DEXTRANS*

FRED R. SEYMOUR

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

AND ROBERT L. JULIAN

Nicolet Instrument Corporation, Madison, Wisconsin 57311 (U.S.A.)

(Received December 20th, 1978; accepted for publication, January 8th, 1979)

ABSTRACT

The Fourier-transform (F.t.), infrared (i.r.) spectra of a series of branched dextrans were examined. The dextrans studied were those from the NRRL collection designated *Leuconostoc mesenteroides* B-1142, B-1191, B-1299 fraction S, B-1355 fraction S, B-1402, and B-1422, and *Streptobacterium dextranicum* B-1254 fractions S[Ł] and L[$]. The spectrum of a levan, NRRL *L. mesenteroides* B-523 fraction M, was also examined, for comparison with the spectra of the dextrans. Meaningful results were obtained by "weight-normalizing" the spectral absorbance to that of the dextran of very low degree of branching (dextran B-1254 fraction L[$]), and then subtracting this spectrum of linear dextran from each of the other polysaccharide spectra. The resulting i.r.-absorbance difference-spectra were plotted, at uniform scale-expansion across the 1800–400-cm^{-1} region, resulting in difference-absorbance features at ~1100 and ~800 cm^{-1} for all branched dextrans. These absorbance differences could be correlated to the type and degree of dextran branching, which had previously been established by permethylation analysis. It was concluded that such F.t.-i.r. difference-spectra have general application for the structural analysis of polysaccharides.

INTRODUCTION

Infrared (i.r.) spectrometry has been available for structural analysis for over forty years. However, early designs for dispersive infrared instruments suffered from weak emission-sources and the lack of data-manipulation capabilities. These problems have traditionally limited the use of i.r. spectroscopy to transmittance studies requiring relatively large amounts of sample. Developments of dispersive instrumentation have included the addition of data systems for spectral manipulation and even a small amount of signal-averaging capability. The ability to store spectra makes intercom-

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

parison of similar spectra more straightforward. In the past few years, however, infrared spectrometry has seen the advent of a new generation of i.r. instrumentation, namely, the Fourier-transform, infrared (F.t.-i.r.) spectrometer, which has inherently much greater sensitivity and ease of spectral manipulation than dispersive infrared instruments. Efficient signal-averaging capabilities allow very precise measurement of signal intensities on much smaller quantities of sample.

The general principles of F.t.-i.r. data-acquisition and processing have been described[2]. In essence, F.t.-i.r. spectrometry involves the use of an interferometric technique to multiplex the infrared signal; this means that all of the i.r. frequencies are received by the sample at the same time, and therefore, a typical F.t.-i.r. scan may be acquired in less than one second. The mathematical, Fourier transformation then transforms the time domain of the acquired data into the frequency domain, whereby the conventional spectrum of transmitted radiant-energy is presented as a function of wavelength. A major advantage of the F.t.-i.r. method lies in its signal-averaging capabilities. By scanning the sample repeatedly, and coadding the resulting interferograms, the signal-to-noise ratio increases by the square root of the total number of scans taken. Therefore, noise-free spectra can be obtained in a very short time. With use of the sophisticated data-system, the resulting spectra can readily be presented in transmittance or, more conveniently for spectral comparison, in absorbance. The absorbance is then linearly related to the amount of absorbing material in accordance with the Beer–Lambert law.

In addition to the previous instrumental limitations, a second structural-analysis problem involves the similarity of all i.r. spectra recorded for polysaccharides of greatly diverse structural character, a similarity not unexpected, as i.r. spectra report information on molecular bond-vibrations, and the total ratio of such bonds (*e.g.*, C–C, C–O, and O–H bonds) is very similar for each polysaccharide. Certain vibrational differences are to be expected, but these differences are small, and difficult to detect when combined with the total vibrational-modes arising from the polysaccharide. However, this similarity of polysaccharide spectra, in conjunction with the ease of manipulation of F.t.-i.r. data, suggested an alternative approach to spectral analysis, in which the spectrum of a simple polysaccharide is employed as a "base line", and then spectral changes observed for other polysaccharides are expressed as deviations from this "standard" spectrum (in terms of a *difference spectrum*). The dextrans discussed herein have been shown[5,9] to be branching variants of linear dextran (a polysaccharide composed exclusively of 6-mono-*O*-substituted α-D-glucopyranosyl residues). Therefore, the spectrum of dextran B-1254 fraction L[$], which has a low degree of branching, was employed as the reference spectrum.

Most i.r.-spectral studies of saccharides have depended on the examination of prominent bands introduced into the i.r. spectrum by intensely absorbing functional-groups present in the saccharide (*e.g.*, the amine bands of hexosamines[3]), and such analyses are not dependent on a F.t.-i.r.-spectral approach. Other functional groups (*e.g.*, sulfate) introduce specific, but weak, bands into the normal spectrum of a polysaccharide. We have previously employed the spectral-subtractive abilities of F.t.-i.r.

spectrometry to subtract the i.r. spectrum of cellulose from that of a 6-mono-*O*-sulfated, cellulose-like polymer, thereby enhancing the diagnostic, *O*-sulfate bands in the 1300–700-cm^{-1} region[1]. The data reported here extend this approach by treating branching residues as functional groups, and establishing the type and degree of dextran branching in terms of the position and intensity of diagnostic bands present in these F.t.-i.r. difference-spectra.

The dextrans studied by F.t.-i.r. spectrometry were chosen on the basis of structures previously established by g.l.c.–m.s. permethylation analysis[5–7], ^{13}C-n.m.r. spectroscopy[7–9], and periodate oxidation[10,11] data. The structure for a hypothetical, average repeating-unit of a dextran is shown in **1**, where Glc is a D-glucopyranosyl group or residue, *m* is the position of branching in the branching residue, and *n* is the

$$-[(1\rightarrow6)\text{-}\alpha\text{-Glc-}\{(1\rightarrow6)\text{-}\alpha\text{-Glc-}\}_n\text{-}]_x\text{-}$$
$$\begin{matrix} m \\ \uparrow \\ 1 \\ \alpha\text{-Glc} \end{matrix} \quad \mathbf{1}$$

number of 6-mono-*O*-substituted α-D-glucopyranosyl residues between branching residues. The type of branching and relative ratios of residue types present in the repeating unit of each dextran have been established by g.l.c.–m.s., permethylation analysis, and confirmed by periodate-oxidation and ^{13}C-n.m.r.- and ^{1}H-n.m.r.-spectral studies. ^{13}C-N.m.r. data show that all observable residues have the α-anomeric configuration, the ^{13}C-n.m.r. resonance-width suggests that the comb-like structure depicted is correct (rather than the alternative, dendritic structure[12]), and ^{13}C-n.m.r. resonance-relaxation (T_1) studies suggest that these side-chains are only one residue long[13]. Previous ^{13}C-n.m.r. spectral analyses of dextrans employed the concept that, to a first approximation, each type of residue in a polysaccharide acts independently of adjacent residues[7–9]. In the application of such a concept in this situation, it could be presumed that the branch-point and terminal residues act as a functional group that contributes specific bands to the total i.r. spectrum. As *n* (the measure of the percentage of linear residue types) increases, the spectral contribution of the branch-point residues can be "diluted out" of the total i.r. spectrum. Therefore, an inverse proportionality between *n* and branching-resonance intensity could be expected. From a quantitative approach, the hypothetical, branching functional-group actually consists of two saccharide residues of a size equal to that of the chain-extending residues designated by *n*. Therefore, it could be expected that an absorbance peak-height (y) of the difference spectrum would follow the relationship $y \propto 1/(n + 2)$, where the numeral 2 results from the two non-linear residues involved in the branching position.

The dextrans employed were selected, on the basis of diversity of branch type and degree of branching, from a large number of dextrans that had previously been prepared and characterized[10]. Dispersive i.r. spectroscopy was one of the methods originally employed to aid in this initial, dextran characterization[10]. The dextrans are

designated by the NRRL strain number of the bacterium that produced them extracellularly, as follows: *Leuconostoc mesenteroides* B-1142, B-1191, B-1299, B-1355, B-1402, B-1422, and *Streptobacterium dextranicum* B-1254. A levan[14] (fraction M from *L. mesenteroides* B-523) was also employed. In addition to the dextrans branching through 2,6-, 3,6-, and 4,6-di-*O*-substituted α-D-glucopyranosyl residues, two other polysaccharides were examined, namely, dextran B-1355 fraction S (which contains ~35% of 3-mono-*O*-substituted α-D-glucopyranosyl residues) and the levan B-523 fraction M. Both of these additional polysaccharides are of interest, as they are produced by bacterial strains that can also produce dextrans, and they also allow comparison of the magnitude of difference-spectra peak-heights for biopolymers of moderate (dextran B-1355 fraction S) and great (levan B-523 fraction M) structural deviations from the structure of linear dextran. Levans, composed primarily of 6-mono-*O*-substituted β-D-fructofuranosyl residues, are structurally very different from dextrans (which are composed primarily of 6-mono-*O*-substituted α-D-glucopyranosyl residues), and these classes of biopolymer have radically different ^{13}C-n.m.r. spectra[11].

RESULTS AND DISCUSSION

The dextrans studied are listed in column 1 of Table I. Samples of polysaccharide of known weight were incorporated into KBr micropellets (3 mm), the i.r. spectrum (4000–400 cm^{-1}) of each sample was measured for a specific duration of time (350 ± 0.1 sec), and the resulting data were stored in a computer. The data file for each sample was then adjusted (in absorbance) relative to the weight amount of the linear-dextran[5,9] reference-sample (dextran B-1254 fraction L[$]). The normalized, i.r.-

TABLE I

CORRELATION OF F.T.-I.R. DIFFERENCE-SPECTRA DATA AND PERMETHYLATION–FRAGMENTATION ANALYSIS DATA FOR BRANCHING OF DEXTRANS

NRRL strain number[a]	*Dextran fraction*	m[b]	n[c]	*Spectrum reference letter*[d]	y[e]	$1/(n+1)$
B-1299	S	2	0.67[f]	A	0.101	0.60
B-1402		2	1.98[g]	B	0.048	0.34
B-1424		2	3.00[g]	C	0.030	0.25
B-1142		3	1.05[h]	D	0.093	0.49
B-1191		3	2.7[h]	E	0.53	0.27
B-1254	S[Ł]	4	2.5[h]	F	0.56	0.29

[a]The strain number of the producing organism. [b]The position of substitution by the branching residue, as defined in **1**. [c]The average number of residues between branch points, as defined in **1**. Values obtained from permethylation analysis as the amount of total tri-*O*-methyl derivatives divided by the amount of the tetra-*O*-methyl derivative, as observed in the hydrogen-flame chromatogram of the PAAN derivatives. [d]The reference letters for F.t.-i.r. difference-absorbance plot, designated as minuscules for Figs. 1 and 4, and as capitals for Figs. 2 and 3. [e]The F.t.-i.r. difference-absorbance peak-height for the ~1090-cm^{-1} band less the average base-line height (1250–800 cm^{-1}) expressed in Δ absorbance units. [f]Data taken from ref. 5. [g]Data taken from ref. 6. [h]Data taken from ref. 11.

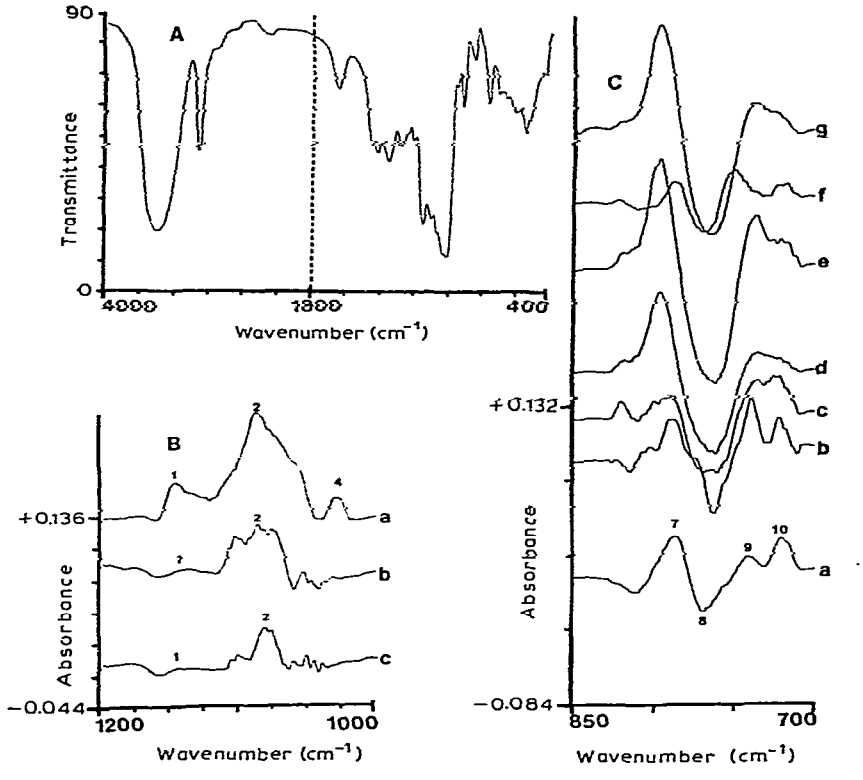

Fig. 1. A, the transmittance spectrum of a dextran of low degree of branching, that is, dextran B-1254 fraction L[S]. B, the expanded-scale stacked-plot of the 1200–1000-cm^{-1} region of the difference–absorbance plots from Fig. 2. C, the expanded-scale stacked-plots of the 850–700-cm^{-1} region of the difference-absorbance plots of Figs. 2 and 3. [The traces are identified by minuscule letters which correspond to the plots identified by the same capital letters. All difference plots are in absorbance, and are the result of subtracting the spectrum of the dextran of low degree of branching (dextran B-1254 fraction L[S]) from the spectrum of interest. The numbered bands correspond to those identified in Table II.]

spectrum file of each sample could then be expressed as a conventional, transmittance, i.r. spectrum, a representative example (dextran B-1254 fraction L[$]) being shown in Fig. 1, A. However, all of the transmittance spectra for these polysaccharides are very similar, and differences between the various spectra are difficult to observe, even when comparing overlays of large-format spectral-plots. Consequently, the spectral file of the essentially linear dextran B-1254 fraction L[$] was then interactively subtracted from the weight-adjusted spectral-files of all other samples, and the resulting difference-spectra were plotted on a uniform absorbance-scale (see Figs. 2 and 3), with only the region of interest (1800–400 cm^{-1}) shown. These operations, conducted in a uniform way for each sample, result in a series of "spectral windows", with meaningful wavenumbers and comparable ordinate-values representing absorbance deviations. The vertical scale for these dextran difference-plots was selected to correspond to the maximum change in ΔA, which was similar ($\sim$0.15) for dextrans B-1142,

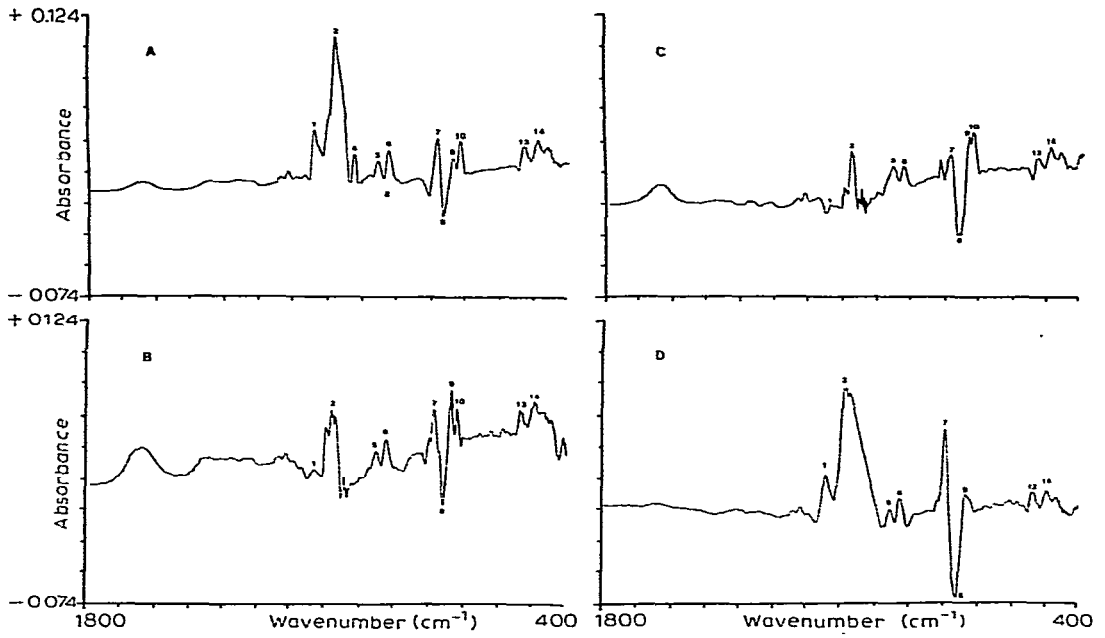

Fig. 2. The F.t.-i.r. difference-absorbance plots for: A, dextran B-1299 fraction S; B, dextran B-1402; C, dextran B-1424; and D, dextran B-1142. (All absorbance spectra are the result of subtracting the spectrum of dextran B-1254 fraction L[S] from the spectrum of interest. The numbered bands correspond to those identified in Table II.)

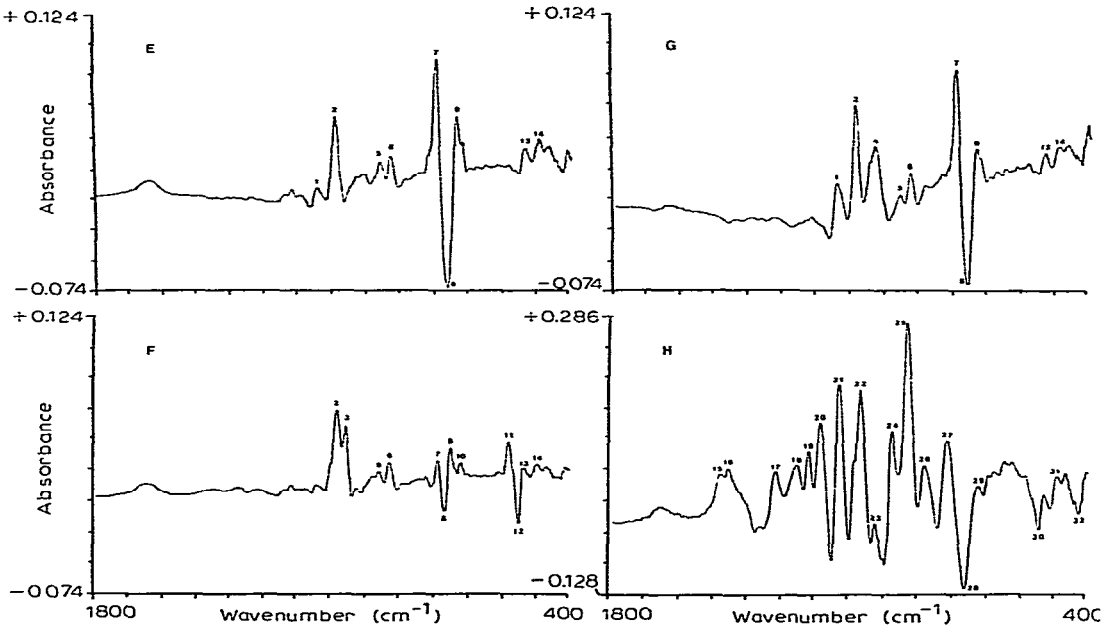

Fig. 3. The F.t.-i.r. difference-absorbance plots for: E, dextran B-1191; F, dextran B-1254 fraction S[L]; G, dextran B-1355 fraction S; and H, levan B-523 fraction M. (All absorbance spectra are the result of subtracting the spectrum of dextran B-1254 fraction L[S] from the spectrum of interest. Plot H differs in vertical scale from the other absorbance-difference plots. The numbered bands correspond to those identified in Table II.).

B-1191, and B-1355 fraction S. For these plots, A through G of Figs. 2 and 3, the ordinate scale is the same (0.022 per scale division), and direct peak-height comparisons can be made. For plot H of Fig. 3, which is the difference spectrum of levan B-523 fraction M, the maximum vertical deflections (ΔA) are much greater ($\sim$0.4), and this plot is more compressed, by a factor of $\sim$2 (0.046 per scale division), than the dextran plots.

The dextran absorbance-plots are listed in Table II. Compared to those of the dextrans, the levan absorbance-plot (Fig. 3, H) presents more-complicated spectral-features, summarized with the following numbered maxima (which are referenced to Fig. 3, H, the values in parentheses indicating the corresponding wavenumbers): 15 (1480.7), 16 (1457.1), 17 (1319.3), 18 (1256.9), 19 (1222.5), 20 (1188.0), 21 (1132.0), 22 (1069.6), 23 (1030.9), 24 (977.0), 25 (931.8), 26 (884.5), 27 (815.6), 28 (768.2, minimum), 29 (727.3), 30 (550.8, minimum), 31 (492.7), and 32 (430.2, minimum).

Inspection of the plots of Figs. 2 and 3 reveals three effects. Firstly, the dextran ΔA plots have pronounced maxima at only two values of wavenumber, a positive deflection at $\sim$1090 cm^{-1}, and a negative one at $\sim$800 cm^{-1}. Secondly, the $\sim$1090-cm^{-1} deflection is, in general, inversely proportional to n, whereas that at $\sim$800 cm^{-1} is not. Thirdly, although the levan difference-plot differs from the dextran difference-plots, the magnitude of these deflections is not dramatically greater.

TABLE II

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

Dextran Fraction Number	*B-1299* *S*	*B-1402*	*B-1422*	*B-1142*	*B-1191*	*B-1254* *S*[Ł]	*B-1355* *S*
	Linkage types [other than (1→6)]						
	(1→2)	*(1→2)*	*(1→2)*	*(1→3)*	*(1→3)*	*(1→4)*	*(1→3)*
1[b]	1146.5	1139.4	1144.7	1146.5	1146.5		1146.5
2	1086.6	1086.6	1083.1	1088.4	1095.4	1086.6	1093.7
3						1058.5	
4	1030.3						1033.8
5	961.7	961.6	961.6	961.6	961.6	961.6	961.7
6	930.0	930.0	930.0	930.0	930.0	930.0	931.7
7	787.4	789.1	790.1	797.9	797.9	787.4	797.9
8[c]	771.5	764.5	776.3	762.8	762.7	768.0	764.5
9	741.6	741.6	736.3	736.3	738.1	750.4	736.3
10	722.2	724.0	724.0			718.7	
11						579.7	
12[c]						551.5	
13	537.4	539.2	533.9	535.6	533.9	536.0	532.1
14	496.9	496.9	496.9	495.2	496.9	495.2	491.6

[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 numbers are referenced to the absorbance plots A through G in Figs. 2 and 3. [c]These wavenumbers refer to minima.

From the limited number of dextrans studied, it is difficult to establish the exact capabilities of this system. However, examination of the difference-plots A through G indicated that both the degree and the type of branching of a dextran can be determined for the individual polysaccharides. The determination of this degree of branching from the amplitude of the difference-absorbance peak at $\sim 1090\ cm^{-1}$ is a promising method. However, several questions needed to be resolved before accurate correlations could be made; for example, (*a*) should peak height or peak area be employed, (*b*) what base-line should be used, (*c*) what inverse relationship between peak height and n should be used, (*d*) how accurately are the actual values of n known for the various dextrans, and (*e*) what differences exist between resonances from dextrans branching through 2,6-, 3,6-, or 4,6-di-*O*-substituted α-D-glucopyranosyl residues?

On the basis of an examination of the difference plots, the following general assumptions and conventions have been employed: (*a*) the peak height will be used, (*b*) the base-line will be the average ΔA between 1250 and 800 cm^{-1} (excluding the peak studied), (*c*) the relationship $y \propto (1/n) + 1$ will be examined (where y is the difference in ΔA between the absorption band at $\sim 1090\ cm^{-1}$ and the associated base-line), (*d*) the values of n employed will be those of permethylation–g.l.c. analysis, as defined by the ratio of tri-*O*-methyl derivatives to the tetra-*O*-methyl derivative that is given by the uncorrected, hydrogen-flame-chromatogram integrals, and (*e*) to a first approximation, no difference exists between the various types of branching of dextran with regard to the relationships of the intensity of the $\sim 1090\ cm^{-1}$ resonance.

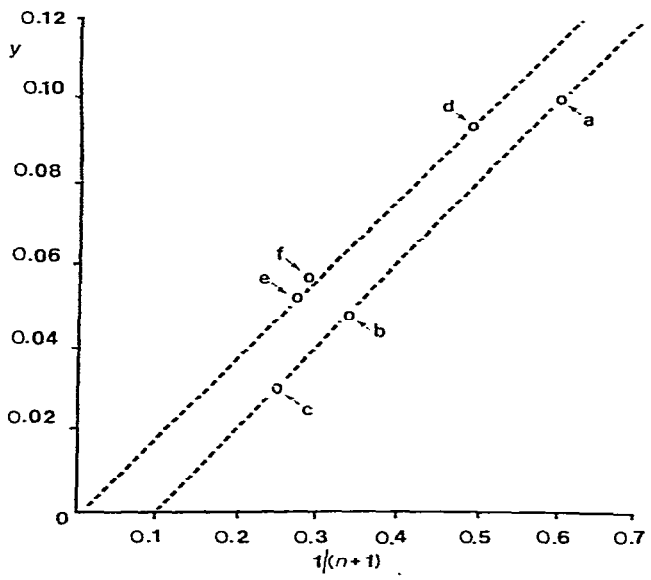

Fig. 4. The F.t.-i.r., difference-absorbance peak-height y (at $\sim 1080\ cm^{-1}$), in absorbance units, *vs.* $1/(n + 1)$, where n is the degree of linearity of the dextran in terms of the average number of D-glucopyranosyl residues between branching residues. (The letters identifying the specific values correspond to the absorbance-difference plots of Figs. 2 and 3, and are referenced to the specific dextrans in Table I.)

These operations and assumptions were applied to each of the difference-absorbance plots, A through F, of Figs. 2 and 3. The resulting peak-heights, y, obtained from these difference plots (see Table I, column 6) were then plotted against the previously known, permethylation values of n (see Table I, column 4) as shown in Fig. 4. The three dextrans branching through 2,6-di-*O*-substituted α-D-glucopyranosyl residues (dextran B-1299 fraction S, dextran B-1402, and dextran B-1422 (a, b, and c) fall in a straight line on this plot — in fact, this provided the rationale for choosing the function $1/(n + 1)$. The dextrans branching through the 3,6-di-*O*-substituted α-D-glucopyranosyl residues, namely, dextrans B-1142 and B-1191 (d and e), form a line parallel to points a, b, and c, and the single example of a dextran branching through the 4,6-di-*O*-substituted α-D-glucopyranosyl residue, dextran B-1254 fraction S[L], yields a value in general agreement with those for the other dextrans. A convenient paradigm is afforded by consideration of the branched dextran in terms of **1**, which contains $(n + 2)$ D-glucopyranosyl residues. If it is assumed that the unusual, i.r. absorbances due to branching result from the interaction of both the branch-point and the terminal residues, then, as n increases, the resonance intensity will decrease proportionally to $1/(n + 2)$. However, if it is considered that this unusual i.r. absorbance caused by branching is associated with only one residue (*e.g.*, the terminal residue), then, as n increases, the absorbance intensity will decrease proportionally to $1/(n + 1)$. There is no *a priori* reason to favor one approach over the other, but the current data favor the $1/(n + 1)$ relationship, even though any relationship between $1/(n + 1)$ and $1/(n + 2)$ provides a reasonably linear plot. For convenience of correlation, both to relative peak-height and to general peak-profile, the 1200–1000-cm^{-1} region of the spectrum of the three dextrans branching through 2,6-di-*O*-substituted α-D-glucopyranosyl residues (dextran B-1299 fraction S, dextran B-1402, and dextran B-1422) is shown in a stacked plot in Fig. 1, B. Difference-absorbance spectra of a variety of dextrans of different degrees of branching, and more-precise determination of the n values for these dextrans, will be required before definitive relationships between absorbance-difference peak-heights and degree of branching of a dextran can be established. However, the data from these selected, dextran examples indicate the general nature of these relationships.

Table II shows that a number of bands are present, although in various degrees of intensity, for the absorbance-difference plots of each of the dextrans. For example, bands 5, 6, 9, and 14 are present in most of the difference-spectra A through G, and the maxima of these peaks vary by only ~ 1 cm^{-1} from the average value for each set. The following discussion, dealing with the presence, absence, or displacement of these bands, is framed in the context that the majority of these dextran difference-absorbance bands are quite consistent. The difference feature at ~ 800 cm^{-1} differs from the foregoing phenomenon at ~ 1090 cm^{-1}, as the latter feature shows no obvious correlation to degree of branching. This ~ 800-cm^{-1} band is, apparently, caused by a C–O–C bending-mode, as opposed to the stretching mode at 1090 cm^{-1}. In the linear dextran, this band has a particular shape, and, if different bending-modes, due to branching residues, are present, these extra contributions at ~ 800 cm^{-1} will make

the band broader. Thus, subtraction of the narrower reference-band causes a double, differential appearance in the result of subtraction. Of interest are the frequencies of the "wings" of this function, specifically the higher-frequency wing (designated 7 in Table II).

For dextrans branching through 2,6-di-*O*-substituted α-D-glucopyranosyl residues the higher-frequency maxima occur at ~789 cm^{-1} for all three values of *n* (a, b, and c in Fig. 1, C). However, for the dextrans branching through 3,6-di-*O*-substituted residues, this value becomes distinctly higher, at 798 cm^{-1} (d and e in Fig. 1, C). For the dextran branching through 4,6-di-*O*-substituted α-D-glucopyranosyl residues, this value is the same, namely 787 cm^{-1} (f in Fig. 1, C), as those for the dextrans branching through 2,6-di-*O*-substituted residues. In addition, for dextran B-1355 fraction S, a polysaccharide possessed of a large extent of non-dextran character (in the form of 3-mono-*O*-substituted α-D-glucopyranosyl residues[1]), this higher-frequency wing is at 797 cm^{-1} (g in Fig. 1,C), a value identical to that of dextrans branching through 3,6-di-*O*-substituted α-D-glucopyranosyl residues. On this basis, it is concluded that the higher-frequency wing-resonance is dependent on the type of linkage rather than the type of residue, and is capable of differentiating α-D-(1→2)- and -(1→4)- from α-D-(1→3)-glucopyranosyl linkages. The difference spectra of dextrans containing 2,6- or 4,6-di-*O*-substituted α-D-glucopyranosyl residues also display a weak band at ~722 cm^{-1} (band 10), a feature absent from the difference spectra of dextrans containing (1→3)-linked α-D-glucopyranosyl residues. However, the absorbance spectrum of dextran B-1355 fraction S shows a prominent band at 1034 cm^{-1} (band 4), strongly suggesting that this feature is diagnostic for 3-mono-*O*-substituted α-D-glucopyranosyl residues.

The presence or absence of small proportions of (1→3)-linkages in dextrans branching mainly through 2,6- or 4,6-di-*O*-substituted α-D-glucopyranosyl residues has been of continuing interest[9]. These data indicate that dextran B-1299 fraction S contains a small proportion of 3-mono-*O*-substituted α-D-glucopyranosyl residues (see band 4 in Table II, and Fig. 2,B), and that the other dextrans do not contain a discernible proportion of this residue.

The absorbance difference-spectrum of the single example of a dextran branching through 4,6-di-*O*-substituted α-D-glucopyranosyl residues shows, at both ~1090 and ~800 cm^{-1}, major bands which, in general, are identical, or similar, to those bands observed for dextrans branching through the 2,6- or 3,6-di-*O*-substituted α-D-glucopyranosyl residues. However, a number of other features distinguish this difference spectrum: for example, bands 1, 4, and 10 are absent, band 9 has been displaced (by ~10 cm^{-1}) to 750 cm^{-1}, and two new bands are present, at 1058 cm^{-1} (band 3) and 580 cm^{-1} (band 11). This single example does not establish whether these resonances are specifically diagnostic for the 4,6-di-*O*-substituted α-D-glucopyranosyl residue, or for α-D-(1→4)-glucopyranosyl linkages in general.

Therefore, on the basis of resonances at ~1100, ~800, and ~600 cm^{-1}, dextrans can be differentiated by degree of branching and type of linkages present. The difference spectra shown in Figs. 2 and 3 suggest two general methods of approach for

the application of such data to determination of the structure of polysaccharides. Firstly, although levan and dextran differ fundamentally in the nature of the types of residue and linkage present, their difference-spectrum (Fig. 3,H) provides a series of usable, subtraction features which demonstrate that the spectra of the two polysaccharides are, in general, quite similar. This spectral similarity indicates that, as an analytical technique for the comparison of a series of spectra of polysaccharides, it would be much more meaningful to compare the difference-absorbance spectra of these compounds subtracted from that of *any* given polysaccharide than to attempt a direct comparison either of transmittance or absorbance i.r. spectra. Of course, when the structure of the subtractive reference chosen is close to the average structures of the polysaccharides to be compared, spectral comparison will be easier. Secondly, the nature of the difference feature observed at 1090 cm^{-1}, and of the subtractive and data-handling processes of F.t.-i.r. spectrometry, suggest that this technique can be profitably extended to the determination of the degree of branching of quite linear polysaccharides. The absolute magnitude of the absorbances involved is essentially dependent on the stability of the emission source and the accuracy of the length of data-acquisition, and both are well defined. Although the peak height of the resonance of interest will steadily diminish as n increases, the spectra of the dextrans studied will rapidly approach that of linear dextran, allowing an easier subtractive process and a concomitant increase in base-line stability and peak-height determination. It could be expected that the best results will be obtained by referencing against a totally linear dextran, and such synthetically produced polysaccharides are available[16,17].

The strengths of this i.r. method fortuitously complement our methods previously developed for the structural determination of dextrans. It is obvious that the F.t.-i.r., difference-absorbance, spectral method can be applied to small samples in the solid state. The amount of material needed is small, the current measurements indicating that comparable results could be obtained on ~5-μg samples with a data-acquisition time of 1 h; this contrasts strongly with ^{13}C-n.m.r. spectroscopy which yields maximum information when 100-mg samples are available, and with ^{1}H-n.m.r. spectroscopy, which requires several mg of material for similar results, but compares very favorably with g.l.c.–m.s., which requires at least 10 to 50 μg of material for successful determination of structure. The amount and the precision of the data provided by these experiments would appear not to be so diagnostic as the data available from ^{13}C-n.m.r. spectra, but to compare favorably to the amount of information obtainable from ^{1}H-n.m.r. spectra. In addition, when compared to permethylation–g.l.c.–m.s. structural determination, which is the only other physical method for the determination of structure that is capable of dealing with such small samples, F.t.-i.r. spectrometry requires much less time for the preparation of samples. Quite possibly, the greatest advantage of the F.t.-i.r. technique is the ability to operate in the solid state. Both ^{13}C-n.m.r. and ^{1}H-n.m.r. measurements are currently highly dependent on the total solubility and solvation of the polysaccharides under study. In like manner, the permethylation–g.l.c.–m.s. techniques require the polysaccharide to be soluble to the extent that permethylation, and the subsequent hydrolysis, can occur. The

accuracy of the final results of ^{13}C-n.m.r., ^{1}H-n.m.r., and permethylation–g.l.c.–m.s. structural analyses depends on the ultimate solubility of all component residues present in a given polysaccharide, a situation that is normally difficult to demonstrate conclusively. Therefore, F.t.-i.r. spectrometry provides a welcome, alternative check on structural determination, as it is not dependent on such considerations of solubility.

Two applications of the F.t.-i.r. method to dextrans immediately become apparent. Firstly, it is well known that the dental plaque covering teeth is mainly composed of a mixture of dextrans and levans[18]. Structural analysis of this material has been inhibited by the insolubility of the plaque and by the relatively small sizes of sample available. The data presented here indicate that a F.t.-i.r.-spectral approach should be directly applicable to a number of problems of plaque structure. For example, the difference spectrum of pure levan from the linear-dextran reference (see Fig. 3,H) indicates an absorbance deflection of $\sim$0.31 in the 1000–900-cm^{-1} region. As peaks having ΔA deflections of 0.003 are easy to identify in the difference-absorbance plots for relatively linear dextran (see Fig. 2,B and C), it is probable that, in addition to structural information on the dextran, a dextran–levan mixture could be quantitated to approximately the 1% level of levan present.

A second, possible, F.t.-i.r., subtractive-absorbance application to dextran is the identification of dextran in a solution of sucrose. Most dextran-producing microorganisms employ sucrose as the substrate, and the problems involving the inhibition of the crystallization of industrial sucrose solutions due to unwanted dextrans are well known[19]. It is possible that F.t.-i.r. subtractive spectra, by subtracting the spectra of dextran–sucrose from the spectrum of pure sucrose, could be calibrated to the level of acceptable content of dextran. The ease of handling of samples and the rapidity of the i.r. analysis could make this a feasible approach for process monitoring.

In conclusion, we point out that F.t.-i.r. absorbance-difference plots can be very useful for identifying and quantitating the structural differences of dextrans. The solid-state conditions, the small size of the sample required, and the ease of preparation of the sample make this a welcome complement to current physical techniques for structural determination of these D-glucans. The relatively large, and informative, absorbance-difference values obtained for the spectral comparisons of these structurally similar dextrans indicate that such a spectral-analysis approach may have wide application to structural determinations of polysaccharides in general.

EXPERIMENTAL

The preparation of the dextrans[10], dextran fractions[20], and levan[14] has been reported. Samples ($\sim$80 μg) were weighed to 0.1 μg, and incorporated into 3-mm, KBr micropellets. The i.r. spectra were then recorded for 350 $\pm$0.1 sec with a Nicolet 7199 Fourier-transform, infrared spectrometer equipped with a cadmium–mercury–telluride detector capable of wide coverage of wave-length (6000–400-cm^{-1}). The resulting F.t.-i.r. data-files were then weight-normalized (in absorbance) against the essentially linear dextran (dextran B-1254 fraction L[$]). The linear dextran B-1254

fraction L[$] data-file was then interactively subtracted from each of the branched-dextran data-files, and the resulting dextran difference-absorbance data were plotted at a uniform scale for each polysaccharide.

ACKNOWLEDGMENT

We thank Dr. A. Jeanes of the Northern Regional Research Center, ARS, USDA, Peoria, Ill., for providing the samples of dextran and levan.

REFERENCES

1 F. R. Seymour, R. D. Knapp, E. C. M. Chen, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 74 (1979) 41–62.
2 P. R. Griffiths, *Chemical Infrared Fourier Transform Spectroscopy*, Wiley, New York, 1975.
3 F. R. Seymour and P. H. Gross, *J. Org. Chem.*, 36 (1971) 1079–1084.
4 T. A. Bunde, F. R. Seymour, and S. H. Bishop, in R. W. Jeanloz (Ed.), *Proceedings of the Fourth International Symposium on Glycoconjugates*, Academic Press, New York, in press.
5 F. R. Seymour, M. E. Slodki, R. D. Plattner, and A. Jeanes, *Carbohydr. Res.*, 53 (1977) 153–166.
6 F. R. Seymour, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, 68 (1979) 113–121.
7 F. R. Seymour, R. D. Knapp, E. C. M. Chen, A. Jeanes, and S. H. Bishop, *Carbohydr. Res.*, 71 (1979) 231–250.
8 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 51 (1976) 179–194.
9 F. R. Seymour, R. D. Knapp, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 68 (1979) 123–140.
10 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.
11 A. Jeanes and F. R. Seymour, *Carbohydr. Res.*, 74 (1979) 31–40.
12 F. R. Seymour, R. D. Knapp, T. E. Nelson, and B. Pfannemüller, *Carbohydr. Res.*, 70 (1979) 125–133.
13 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 72 (1979) 229–234.
14 F. R. Seymour, R. D. Knapp, and A. Jeanes, *Carbohydr. Res.*, 72 (1979) 222–228.
15 F. R. Seymour, R. D. Knapp, J. Zweig, and S. H. Bishop, *Carbohydr. Res.*, 72 (1979) 57–69.
16 E. R. Ruckel and C. Schuerch, *J. Org. Chem.*, 31 (1966) 2233–2239.
17 E. R. Ruckel and C. Schuerch, *Biopolymers*, 5 (1967) 515–523.
18 R. L. Sidebotham, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
19 A. Jeanes, in P. A. Sandford and A. Laskin (Eds.), *Extracellular Microbial Polysaccharides*, ACS Symp. Ser. No. 45, American Chemical Society, Washington, D. C., 1977, pp. 284–298.
20 C. A. Wilham, B. H. Alexander, and A. Jeanes, *Arch. Biochem. Biophys.*, 59 (1955) 61–75.