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# $^{13}$C-NUCLEAR MAGNETIC RESONANCE SPECTRA OF COMPOUNDS CONTAINING β-D-FRUCTOFURANOSYL GROUPS OR RESIDUES

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## ABSTRACT

$^{13}$C-N.m.r. spectra have been recorded for sucrose, melezitose, levan, inulin, palatinose, and D-fructose. Except for the last, each compound contains a different *O*-substituted D-fructofuranose residue or group, or β-D-fructofuranosyl residue or group. On the basis of chemical-shift displacements, resulting from *O*-substitution at specific carbon atoms, resonances can be assigned to the carbon atoms of the β-D-fructofuranosyl residue. Fortuitously, the α-D-glucopyranosyl group present in some of these compounds exhibits resonances that do not obscure the β-D-fructofuranosyl resonances. *O*-Substitution of the β-D-fructofuranosyl residue causes a downfield displacement of the corresponding, linked-C resonance; however, the other major resonances of this residue are not affected by bulky substituents. Members of a series of levan fractions, the products of partial, acid hydrolysis of *Streptoccoccus salivarius* levan, were then examined for changes in relative degree of branching.

## INTRODUCTION

We have previously correlated saccharide structures with $^{13}$C-n.m.r. chemical-shifts for dextrans[1–4], and amyloses[5]. On the basis of relating $^{13}$C-n.m.r. resonances diagnostic of branching to types of branching known from permethylation-frag-

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mentation, g.l.c.–m.s. analysis[6,7], various resonances have been assigned to the specific carbon atoms of D-glucopyranosyl residues. Furthermore, $^{13}$C-n.m.r. data have provided information (*e.g.*, on the nature of specific, anomeric linkages of the polymers) that is difficult to obtain by alternative means.

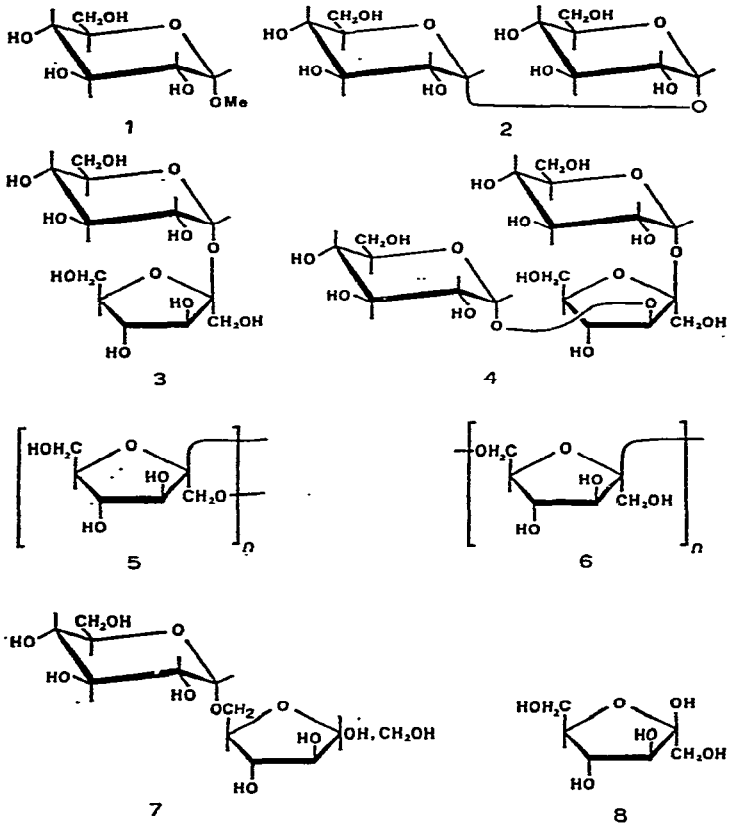
We now discuss relationships of $^{13}$C-n.m.r. spectra to structure for compounds containing β-D-fructofuranosyl residues. Members of one class of β-D-fructofuranosyl-containing polymers, the levans, are known to be produced in conjunction with some dextrans. However, although the dextrans have been shown to contain a wide variety of branching types, and to give a corresponding variety of $^{13}$C-n.m.r. spectra, less varietal evidence is available for levans and inulins, two of the most extensively studied classes of β-D-fructofuranosyl polymers.

Information on the structure of polysaccharides that is based on $^{13}$C-n.m.r. spectra is dependent on the correct assignment of resonances to specific carbon atoms of given residues, and also on a knowledge of resonance displacements resulting from *O*-substitution of these residues. The previous $^{13}$C-n.m.r. studies on α-D-glucopyranosyl polymers employed the general approach of obtaining a wide variety of differently linked D-glucans, and correlating change in the polymer structure to change in the $^{13}$C-n.m.r. spectrum. A convenient and fruitful approach was to consider each polymer as an assembly of types of *O*-alkylated residue and to assume that these spectra of the different *O*-alkylated residues would primarily differ from each other in terms of a downfield displacement (~8 p.p.m.) of the resonances of the carbon atoms associated with the *O*-alkylated (saccharide-linkage) positions. We have observed that a specifically substituted type of residue has specific $^{13}$C-n.m.r. resonances which, to a first approximation, are independent of the position of that residue in the polymer, and that these resonances have intensities proportional, in general, to the relative percentage of that residue in the polymer[3]. Subtle changes in the position of a specific type of residue can cause resonance displacements, but these are normally less than 1 p.p.m. The broad resonances and relatively complicated spectra of the D-glucans also necessitated the use of high-temperature (90°) recording-conditions that were not necessary for the compounds discussed here.

It is not immediately obvious that compounds containing β-D-fructofuranosyl residues can be treated by the approach that has proved successful for polymers composed of α-D-glucopyranosyl residues[1–5]. The D-glucopyranoside structure exists in a clearly favored chair-form [$^4C_1$(D)]; with the exception of C-1, the α-D-glucopyranosyl residue has all substituents equatorial to the ring, and therefore, extensive changes in *O*-substitution can occur without a dramatic change in the favored conformation. However, the β-D-fructofuranosyl residue has a puckered ring, and conformational analysis suggests[8] that, in solution, differences in energy between conformers are small. Therefore, the introduction of a bulky *O*-substituent onto the β-D-fructofuranosyl residue could result in a profound change in the conformation, causing major resonance-displacements for all carbon atoms of this residue. In addition, there has been a lack of agreement for the assignment of resonances to specific carbon atoms in β-D-fructofuranose. Two early $^{13}$C-n.m.r. studies on D-fructose[9,10]

made tentative resonance-assignments for β-D-fructofuranose, but the respective assignments were not in agreement. Spectral analysis for free D-fructose is complicated as, in solution, this ketose assumes both furanose and pyranose forms, with α-D-fructopyranose the favored form. A $^{13}$C-n.m.r. analysis of furanoside systems by Perlin *et al.*[11-13] provided several valuable insights into the n.m.r.-spectral behavior of the D-fructofuranoside system. It was noted that D-fructofuranose derivatives can be expected to display α-anomeric resonances *downfield* from the corresponding β-anomeric resonances[11], an effect the opposite of that observed for the anomeric resonance of D-glucopyranose and D-glucopyranosides. This anomeric-resonance relationship has been demonstrated by separating the anomers of D-fructofuranose pentaacetate, confirming the identity of these anomeric fractions by specific rotation, and then recording the $^{13}$C-n.m.r. spectrum of each fraction[14] (α anomer, 107.9 p.p.m.; β anomer, 104.9 p.p.m.). Angyal and Bethell[15] re-investigated the resonance assignments for D-fructose by the rigorous method of synthesizing D-fructose specifically deuterated at various carbon atoms (*e.g.*, 1-D, 3-D, and 4-D). The deuterium nucleus couples with the carbon atom to which it is attached, causing that carbon resonance to split and be effectively removed from the spectrum. Employment of this specific deuteration, in conjunction with several techniques to alter the furanose–pyranose equilibrium, provided resonance assignments for β-D-fructofuranose, assignments that differed from both of the previous[9,10] ones. Although a correct assignment of resonances for β-D-fructofuranose is useful for resonance assignments to the β-D-fructofuranosyl residue, anomeric substitution can be expected to cause certain resonance displacements. However, Angyal and Bethell[15] then studied the corresponding methyl β-D-fructofuranosides from each of their specifically deuterated (*i.e.*, 1-D, 3-D, and 4-D) D-fructoses. Comparison of the spectra of these three methyl glycosides allowed a resonance assignment to be made for methyl β-D-fructofuranoside, an assignment that is the same as that now obtained by an alternative method (see the following section). Additional resonance-assignments for D-fructose species have also been made for strongly basic, aqueous solutions[16] and for pyridine solutions[17]. Therefore, although $^{13}$C-n.m.r. spectra of β-D-fructofuranose have been recorded under a variety of experimental conditions, only a small part of these data bears a direct relationship to the positions of resonances of β-D-fructofuranosyl residues in D-fructofuranans in aqueous solution.

Although the polymers of β-D-fructofuranose known at present have less variation in structure than the corresponding α-D-glucopyranans, the former class has an advantage, in that a number of oligosaccharides that contain a single D-fructofuranosyl group or residue are known. The existence of these β-D-fructofuranosyl-containing saccharides, which are relatively easy to obtain, combined with the fortuitous lack of spectral overlap of the $^{13}$C-n.m.r. resonances of the α-D-glucopyranosyl and β-D-fructofuranosyl residues, allows the following spectral analysis to be made.



RESULTS AND DISCUSSION

In contrast to the major resonances of the dextrans, those in the $^{13}$C-n.m.r. spectra of levans have more widely spaced chemical-shifts, and these resonances appear in spectral regions not normally associated with the dextrans. The resonances of the anomeric carbon atoms of the $\beta$-D-fructofuranosyl residues are at $\sim$105 p.p.m., and those of the ring-carbon atoms are in the 75–83-p.p.m. region. These differences allow the use of naturally occurring, $\beta$-D-fructofuranosyl-containing compounds to correlate the various resonances to specific carbon atoms. When the $^{13}$C-n.m.r. spectrum of methyl $\alpha$-D-glucopyranoside (**1**) (see Table I for the spectra of the compounds) is compared to that of $\alpha,\alpha$-trehalose (**2**), the only major spectral-change found is in the anomeric resonance which, for the latter, is shifted $\sim$6 p.p.m. upfield. This 6-p.p.m. value of $\Delta\delta$ is apparently the normal, chemical-shift displacement found for a glucopyranoside when replacing an alcohol "aglycon" group with a hemiacetal "aglycon" group. The $^{13}$C-n.m.r. spectrum of sucrose ($\beta$-D-fructofuranosyl $\alpha$-D-glucopyranoside, **3**) shows 12 well-defined resonances, six of these being essentially identical to those in the spectrum of **2** and being given by the $\alpha$-D-glucopyranosyl carbon atoms. The remaining six resonances of sucrose correspond to the

carbon atoms of the β-D-fructofuranosyl group, and fall into three distinct categories: the resonance of the anomeric carbon atom, the carbon resonances in the 75–83-p.p.m. region, and the resonances in the 60–65-p.p.m. region which are associated with linked, primary carbon atoms. In extending the designations for the major $^{13}$C-n.m.r. peaks (A through F)[1] of dextran, the major peaks for the β-D-fructofuranosyl residue will be referred to as G through L, proceeding from downfield. Peaks H, I, and J represent the group of ring-carbon atoms (C-3, C-4, and C-5), whereas peaks K and L correspond to C-1 and C-6.

Comparison of the spectrum of melezitose (**4**; *O*-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl α-D-glucopyranoside) to that of sucrose (**3**) provided several observations. Firstly, all peaks for non-anomeric α-D-glucopyranosyl residues are doublets, clearly indicating that a change in the "aglycon" group is reflected in small displacements for all carbon resonances throughout a given pyranoside ring. Secondly, these doublet peaks confirm the assignment of these resonances to the carbon atoms of the α-D-glucopyranosyl residue. Thirdly, a new anomeric resonance appears at 101 p.p.m., a region associated with the linked carbon atoms of α-D-glucopyranosyl residues[1], and this confirms the α-D configuration of the D-glucopyranosyl group at the (1→3)-linkage[2,4]. Fourthly, peak I, at 77.5 p.p.m., has been displaced downfield to 84.15 p.p.m. ($\Delta\delta$ 6.7 p.p.m.), a displacement of magnitude comparable to, and of the same sign as, that observed for α-D-glucopyranosyl glycosylation[1], indicating that peak I corresponds to C-3 of the β-D-fructofuranosyl residue. Fifthly, peak J is displaced upfield by 0.7 p.p.m., indicating a β-effect (in terms of adjacent carbon atoms) and suggesting that peak J corresponds to C-4. Neither sucrose nor melezitose can provide a basis for the assignment of peaks K and L.

Compounds composed of D-fructofuranosyl residues provide additional data for the assignment of carbon atoms. Inulin (**5**), a (2→1)-linked β-D-fructofuranosyl polymer, displays resonances for the nonbonded C-3, C-4, and C-5 atoms of the β-D-fructofuranosyl residues (H, I, and J) that are essentially identical to those of sucrose. Peak K remains undisplaced, indicating that this peak corresponds to the free C-6 atoms. Levan (**6**), composed primarily of (2→6)-linked β-D-fructofuranosyl residues, also show undisplaced H, I, and J peaks. However, the K and L peaks of levan are both displaced from their "normal" positions found in the spectra of sucrose and melezitose. It should be emphasized that two classes of β-D-fructofuranosyl-containing compound are under consideration here: sucrose and melezitose respectively contain a β-D-fructofuranosyl group and residue anomerically linked to a hemiacetal "aglycon", whereas inulin and levan are primarily composed of β-D-fructofuranosyl residues anomerically linked to an alcoholic "aglycon". On changing from a hemiacetal to an alcoholic "aglycon", the $\Delta\delta$ effect has clearly been shown for the $^{13}$C-n.m.r. spectrum of the α-D-glucopyranosyl group, and it is surprising that little such effect is observed for the β-D-fructofuranosyl group or residue. Additional evidence for the assignments of the K and L peak resonances is available in the literature. Hough *et al.*[18] investigated the $^{13}$C-n.m.r. spectra of several sucrose derivatives that have a chlorine substituent at C-1 or C-6, or both, of D-fructose.

TABLE I

CHEMICAL SHIFTS FOR $^{13}C$-N.M.R. SPECTRA OF $\beta$-D-FRUCTOFURANOSYL COMPOUNDS AND OF REFERENCE COMPOUNDS[a] AT 34°

| *Methyl α-D-glucopyranoside* (1) | *α,α-Trehalose* (2) | *Sucrose* (3) | *Melezitose* (4) | *Inulin* (5) | *Levan* (6) | *Palatinose* (7) | *D-Fructose* (8) | *Designation of major resonances* |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 105.69 | 105.40 | |
| | | 104.61 | 104.64 | 104.14 | 105.10<br>104.54 s[b]<br>104.42 s | | | G |
| 100.13 | | | 101.13 | | | 102.71<br>99.49 | 102.47 | A |
| | | | | | | 99.29 | 99.02 | |
| | 94.20 | 93.09 | 92.69<br>84.15 | | | | | |
| | | 82.30 | 82.16 | 82.02 | 82.07 s | 82.75 | 82.97<br>82.33 | H |
| | | | | | 81.20 | 81.00<br>79.94 | 81.66 | |
| | | 77.46 | | 77.98 | 77.36 | 77.16<br>76.40 | 77.10<br>76.49 | I |
| | | 75.00 | 74.26 | 75.29 | 76.17 s | 75.59 | 75.50 | J |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 74.01 | 73.53 | 73.54 | 74.05<br>73.80 | | | 74.06 | | B |
| 72.46 | 73.10 | 73.33 | 73.25<br>73.16 | | | 72.90 | | C |
| 72.13 | 72.02 | 72.01 | 72.40<br>71.96 | | | 72.40 | | D |
| | | | | | | | 70.67 | |
| 70.50 | 70.70 | 70.20 | 70.59<br>70.48 | | | 70.58 | | E |
| | | | | | | | 70.17<br>68.61 | |
| | | | | | | 68.38<br>67.87 | | |
| | | | | | | | 64.94 | |
| | | | | | 64.33 | | 64.33<br>63.95 | |
| | | 63.30 | 63.13 | 63.10 | 63.42 s<br>63.19 s | 63.77 | 63.77<br>63.36 | K |
| | | 62.38 | 62.38<br>61.57 | 61.85 | | | 62.14 | L |
| 61.52 | 61.56 | 61.14 | 61.34 | | 61.03 | 61.61<br>58.43 | | F |
| 55.94 | | | | | | | | |

[a]Chemical shifts in p.p.m. relative to tetramethylsilane. [b]The symbol s indicates a shoulder or weak peak.

The spectra of the chloro-substituted compounds show that the 63.1-p.p.m. resonance (K) corresponds to C-6 of the D-fructofuranosyl group, and the 62.5-p.p.m. resonance (L), to C-1 thereof. In addition, Angyal and Bethell[15] reported on the spectrum of methyl β-D-fructofuranoside (which contains an alcoholic aglycon group), already discussed here in connection with resonance assignment. All furanoside resonances for this methyl D-furanoside and for sucrose have been found to agree closely (see Table II), except for that for C-1 (62.38 p.p.m. for sucrose, and 60.0 p.p.m. for methyl β-D-fructofuranoside). For the hemiacetal "aglycon", namely, the β-D-fructofuranosyl group or residue in sucrose and melezitose, the C-1 resonance is at 62.4 p.p.m., and for the β-D-fructofuranosyl group attached to an alcoholic "aglycon", the C-1 resonance is at ~61.0 p.p.m. This explains how, for inulin, a 1-linked polymer, the anomeric-carbon resonance may be displaced downfield by ~1 p.p.m., and still exhibit a resonance upfield from the C-1 resonance of sucrose and melezitose. Compared to the $\Delta\delta$ value of C-1 of the α-D-glucopyranosyl group, of ~7 p.p.m. for hemiacetal and alcoholic "aglycon" groups, the anomeric resonance of the β-D-fructofuranosyl group is insensitive ($\Delta\delta$ value of ~1 p.p.m.) to this change. On the basis of limited data, it would therefore appear that C-1, C-2, and C-6 of β-D-fructofuranosides display $^{13}$C-n.m.r. resonances that are less sensitive to substitution displacements than are the corresponding carbon atoms of α-D-glucopyranosyl residues.

Levans (**6**) contain a moderate proportion of 1,6-di-*O*-substituted β-D-fructofuranosyl residues (~10%) at branching points, and terminal (nonreducing) β-D-fructofuranosyl groups (~10%). These residues contribute minor peaks (designated s in Table I), and provide evidence regarding the magnitude of $\Delta\delta$ on *O*-substitution of various carbon atoms. In the 60–65-p.p.m. region of the spectrum of levan, a minor doublet appears, centered at 63.4 p.p.m. On the basis of display of a downfield $\Delta\delta$ on glycosylation, this resonance is the expected C-1 resonance of the 1,6-di-*O*-substituted β-D-fructofuranosyl residue. Similarly, the partially obscured peak on the downfield side of the major, 61.03-p.p.m. peak may represent the resonance of the nonlinked C-6 atom of the (nonreducing) terminal β-D-fructofuranosyl group.

TABLE II

COMPARISON OF $^{13}$C-N.M.R. CHEMICAL-SHIFTS[a] OF β-D-FRUCTOFURANOSIDES

| *Designation of resonance* | *Carbon atom* | *Compound* | | |
|---|---|---|---|---|
| | | *Sucrose*[b] | *Melezitose*[b] | *Methyl β-D-fructofuranoside*[c] |
| G | C-2 | 104.61 | 104.64 | 104.7 |
| H | C-5 | 82.30 | 82.16 | 82.1 |
| I | C-3 | 77.46 | [d] | 77.7 |
| J | C-4 | 75.00 | 74.26 | 75.9 |
| K | C-6 | 63.30 | 63.13 | 64.7 |
| L | C-1 | 62.38 | 62.88 | 60.0 |

[a]Chemical shifts in p.p.m. relative to tetramethylsilane. [b]Data taken from Table I. [c]Data taken from ref. 15. [d]The C-3 atom is *O*-substituted, and the resonance is displaced to 84.15 p.p.m.

These data also indicate that the value of $\Delta\delta$ displayed on glycosylation is relatively small (~2 p.p.m.) for both C-1 and C-6. In the anomeric region of the spectrum of levan, a minor doublet, centered at 104.5 p.p.m., represents the minor, anomeric resonances of the branching and terminal β-D-fructofuranosyl groups; again, the $\Delta\delta$ values are smaller than for analogous, anomeric resonances of the α-D-glucopyranosyl residues in dextrans.

In summary, all $^{13}$C-n.m.r. data are in accord with the accepted structures of sucrose, melezitose, inulin, and levan. No evidence has been found to indicate that the D-fructose is present in any configuration other than β-D. An assignment of $^{13}$C-n.m.r. resonances to carbon atoms of β-D-fructofuranosyl residues has been proposed: peak L (C-1), peak G (C-2), peak I (C-3), peak J (C-4), peak H (C-5), and peak K (C-6). All data recorded for these compounds are in agreement with these assignments.

The spectrum of palatinose (**7**) may be interpreted by employing the known resonance assignments[15] for D-fructose (**8**). Superficially, the spectra of palatinose and D-fructose are similar; however, the major (pyranoside) resonances in each spectrum result from different effects. D-Fructose is known to favor the β-D configuration in solution, and this preponderant species contributes six prominent resonances. The remaining 12 D-fructose resonances are grouped into two sets of six resonances of equal intensity. The intermediate and weak sets of D-fructose resonances have been respectively assigned[9] to β- and α-D-fructofuranose, with each resonance assigned to a specific carbon atom[15].

Palatinose (**7**, 6-*O*-α-D-glucopyranosyl-D-fructofuranose) contains a 6-*O*-substituted D-fructofuranose residue and, as C-6 is blocked, the D-fructopyranose ring cannot be formed. However, the 6-*O*-substituent is an α-D-glucopyranosyl group, which contributes six prominent resonances (A, B, C, D, E, and F) to the spectrum. The D-fructofuranose residue can assume either anomeric configuration, and, therefore, two disaccharide species exist in equilibrium in solution with a possible maximum of 24 resonances. Actually, the 6-*O*-α-D-glucopyranosyl group is relatively insensitive to the anomeric configuration of the D-fructose residue, and, therefore, only the C-1 resonance of the D-glucopyranosyl group is split (with a minor, but resolved, 99.49-p.p.m. resonance near the major, 99.29-p.p.m. resonance). The remaining resonances of the spectrum of palatinose are grouped into two, equal-intensity sets of six resonances, an intermediate set, and a weak set, which are very similar to the D-fructofuranose resonances, and are, therefore, assigned, respectively, to the β-D-fructofuranose and the α-D-fructofuranose residues. In general, these D-fructofuranose resonances of **7** have chemical shifts so similar (within ~0.2 p.p.m.) to those of D-fructose that the assignment of these resonances in the spectrum of **7** can be based directly on these D-fructose assignments. For both anomers, 6-*O*-glycosylation resulted in a major, downfield displacement (~6 p.p.m.) of the C-6 resonances, and a lesser β (in terms of adjacent carbon atoms) upfield displacement (~1.5 p.p.m.) of the C-5 resonance. The latter displacement is not unexpected[19], but it contrasts with the lack of an observable, upfield displacement of the C-5 resonance for 6-*O*-

substitution of the α-D-glucopyranosyl residue[1-4]. The C-1 resonance of the α-D-fructofuranose residue of palatinose is displaced upfield (58.43 p.p.m.) from the corresponding resonance (63.95 p.p.m.) of D-fructose, and is an effect not currently understood. Therefore, the resonance assignments for 7 are as follows; β-D-fructofuranose residue: C-1, 63.77; C-2, 102.71; C-3, 76.40; C-4, 75.60; C-5, 79.94; and C-6, 68.83 p.p.m., and α-D-fructofuranose residue: C-1, 58.43; C-2, 105.69; C-3, 82.75; C-4, 77.16; C-5, 81.00; and C-6, 67.87 p.p.m. In terms of resonance peak-heights, the equilibrium ratio for the anomers of 7 is ~1:4 (favoring β), which is similar to the corresponding ratio for D-fructose.

The foregoing data strongly support the concept that there is a set of six $^{13}$C-n.m.r. resonances associated with the β-D-fructofuranosyl group or residue which persists through a wide variation in *O*-substitution. For each carbon atom studied (C-1, C-2, C-3, and C-6), *O*-substitution results in a downfield resonance-displacement relative to the corresponding free resonance of that carbon atom. In addition, based on data for melezitose, inulin, and levan, the C-1, C-2, and C-6 resonances of the β-D-fructofuranosyl residue are, in terms of magnitude of resonance displacement, less sensitive to *O*-substitution (or substituent effect) than the resonances of corresponding carbon atoms of the α-D-glucopyranosyl residue. The data also demonstrate that the same type of residue in chemically different environments (*e.g.*, the 2- and the 3-*O*-α-D-glucopyranosyl substituents of melezitose) can have different chemical shifts for each corresponding carbon atom. However, these minor differences in chemical shifts are relatively small, but are distinguishable because of the sharp line-width of the oligomer resonances. Such minor resonance-displacements support the general approach of considering such steric effects to be negligible when the broader resonances associated with polysaccharides are dealt with.

The origin and nature of the specific fractions of levan that were studied requires comment. Levans of microbial origin have been isolated from several sources, and the materials studied here are the products[20] of hydrolysis of native levan prepared from a culture of *Streptococcus salivarius* ATCC 13419 with 0.01M hydrochloric acid for 1 h at 35°. The resulting hydrolyzate was then subjected to fractional precipitation with ethanol, yielding ~30 levan fractions. These fractions were then examined by light-scattering, ultracentrifugation, viscometry, end-group analysis, and high-pressure liquid-chromatography[21]; two conclusions were as follows. Firstly, the levan fractions differ in molecular weight ($\overline{M}_w$), which ranges from $10^4$ to $10^7$. Secondly, various physical measurements, including intrinsic viscosity *vs.* $\overline{M}_w$, indicate that the levans of high molecular weight have physical properties different from those of fractions of lower molecular weight, and that the change occurs at $\overline{M}_w \sim 10^5$. These changes in physical properties *vs.* molecular weight are most readily rationalized by assuming that, at $\overline{M}_w \sim 10^5$, the effective volume of the levan fraction changes, presumably by a difference in volume of solvent (water) included. Furthermore, a change in effective volume for the levan fractions suggests that there is some structural difference between the levans of high and low molecular weight. If it is assumed that, structurally, the native levan is essentially homogeneous, it is probable that structural

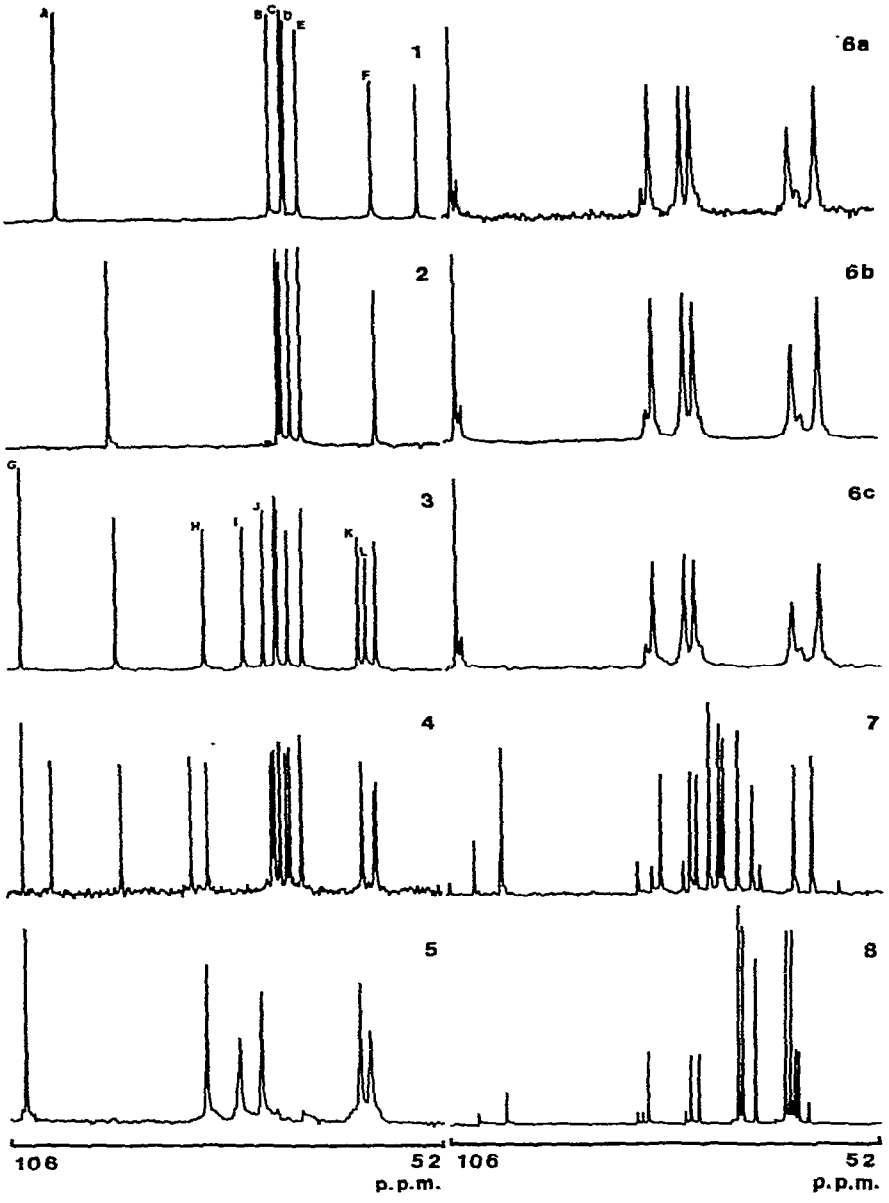



Fig. 1. $^{13}$C-N.m.r. spectra at 34° for: **1**, methyl α-D-glucopyranoside; **2**, α,α-trehalose; **3**, sucrose; **4**, melezitose; **5**, inulin; **6a**, levan of high molecular weight; **6b**, levan of intermediate molecular weight; **6c**, levan of low molecular weight; **7**, palatinose; and **8**, D-fructose.

differences arise in the levan fractions of differing molecular weight as a result of the favored hydrolysis of specific residues in levan. Although levan is known to be mainly linear, extending through 6-*O*-substituted β-D-fructofuranosyl residues, approximately one residue in every ten is branched through 1,6-di-*O*-substituted β-D-fructofuranosyl residues; these branch points give rise to corresponding, terminal β-D-fructofuranosyl

groups. Should the intersaccharide linkages associated with these three residues differ in relative ease of hydrolysis by acid, an extensively hydrolyzed fraction of levan (low molecular weight) would be expected to differ in degree of branching from a less extensively hydrolyzed fraction of levan (high molecular weight). The minor resonances of D-glucans are diagnostic of the branching residues present, and we have shown[3] that the intensity of these minor resonances, relative to those of the major resonances, is proportional to the degree of polymer branching. The $^{13}$C-n.m.r. spectra of three representative examples of the levan fractions were recorded under essentially identical conditions (see Fig. 1). These levan fractions[21] were: **6a** [low molecular weight (designated E6) $\overline{M}_w \sim 2 \times 10^4$], **6b** [intermediate molecular weight (designated F14), $\overline{M}_w \sim 3 \times 10^5$], and **6c** [high molecular weight (designated F2), $\overline{M}_w \sim 8 \times 10^6$]. Expanded plots of these levan spectra allowed comparisons of resonance intensity of about one part in 20 to be made. However, no significant differences in position or relative intensity of these chemical shifts could be observed on correlating spectra of these three levan fractions. Therefore, if the differences observed for physical measurements on levan fractions of high and low molecular weight obtained by acid hydrolysis of *S. salivarius* (ATCC 13419) levan are dependent on polymeric structure, these differences either are not due to the *average* frequency of branching, or are attributable to subtle differences in branching whose detection lies below our current, $^{13}$C-n.m.r. analytical capability.

## EXPERIMENTAL

The $^{13}$C-n.m.r. conditions and methods of preparation of samples have been described[12]. In general, a Varian XL-100-15 spectrometer equipped with a Nicolet TT-100 system was employed in the Fourier-transform mode. Samples (~200 mg) were dissolved in deuterium oxide (5 mL). Inulin was obtained from Pfanstiehl Laboratories Inc., Waukegan, Ill. The production of the levan fractions has been described[21]. The rest of the compounds were obtained from Sigma Chemical Co., St. Louis, Mo. All compounds were employed without further purification.

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