Carbohydrate Research, 76 (1979) 45–57

MANIFESTATION OF ANOMERIC FORM, RING STRUCTURE, AND LINKAGE IN THE ^{13}C-N.M.R. SPECTRA OF OLIGOMERS AND POLYMERS CONTAINING D-FRUCTOSE: MALTULOSE, ISOMALTULOSE, SUCROSE, LEUCROSE, 1-KESTOSE, NYSTOSE, INULIN, AND GRASS LEVAN*

HAROLD C. JARRELL*, THOMAS F. CONWAY†, PATRICK MOYNA*,‡, AND IAN C. P. SMITH*

*Division of Biological Sciences, National Research Council of Canada, Ottawa K1A OR6 (Canada); †Moffett Technical Center, CPC International Inc., Argo, Illinois 60501 (U.S.A.)

(Received February 24th, 1979; accepted for publication, April 4th, 1979)

ABSTRACT

^{13}C-N.m.r. spectroscopy has been used to determine the equilibrium composition of solutions of maltulose and isomaltulose in deuterium oxide. Resonance assignments have been made for maltulose, isomaltulose, sucrose, leucrose, 1-kestose, nystose, inulin, and grass levan. Some earlier assignments for sucrose and grass levan are corrected. The resonances of the D-glucopyranosyl group in maltulose and isomaltulose have been observed to be sensitive to the ring and anomeric forms of the adjacent D-fructose residue. Spin–lattice relaxation-times (T_1) and nuclear Overhauser enhancement factors (n.O.e.f.) for the carbon atoms of the D-fructofuranosyl residues of inulin have been measured, and used in conjunction with deuteration, to aid in resonance and linkage assignments.

INTRODUCTION

Carbon-13 n.m.r. spectroscopy is now an established method for the determination of the composition, linkage, and, in a few cases, the sequence in complex carbohydrates[1]. There are, however, only a few scattered reports on the interpretation of ^{13}C-n.m.r. spectra of oligomers and polymers containing D-fructose. Although the ^{13}C-n.m.r. spectra of sucrose[2–5], 1-kestose, nystose, stachyose, raffinose, and planteose[2,3], and inulin and a levan[6] have been reported, in only one instance[6] has a definitive assignment of the D-fructose resonances been made. Angyal and Bethell[7] facilitated the interpretation of the spectra of D-fructose-containing compounds by their unequivocal assignment of the ^{13}C resonances of D-fructose. We now report our assignments for three disaccharides containing D-fructose, namely, maltulose (4-*O*-α-D-glucopyranosyl-D-fructose) (**1**–**3**), isomaltulose (6-*O*-α-D-gluco-

*Issued as NRCC Publication No. 17607.

‡Visiting Research Officer under the Exchange Programme of the Canadian International Development Agency; present address, Universidad de la Republica, Facultad de Química, Montevideo, Uruguay.

pyranosyl-D-fructose) (4,5), and leucrose (5-*O*-α-D-glucopyranosyl-D-fructopyranose) (6), and an analysis of the ^{13}C-n.m.r. spectra of sucrose, 1-kestose (7), nystose (8), inulin (9), and a levan.

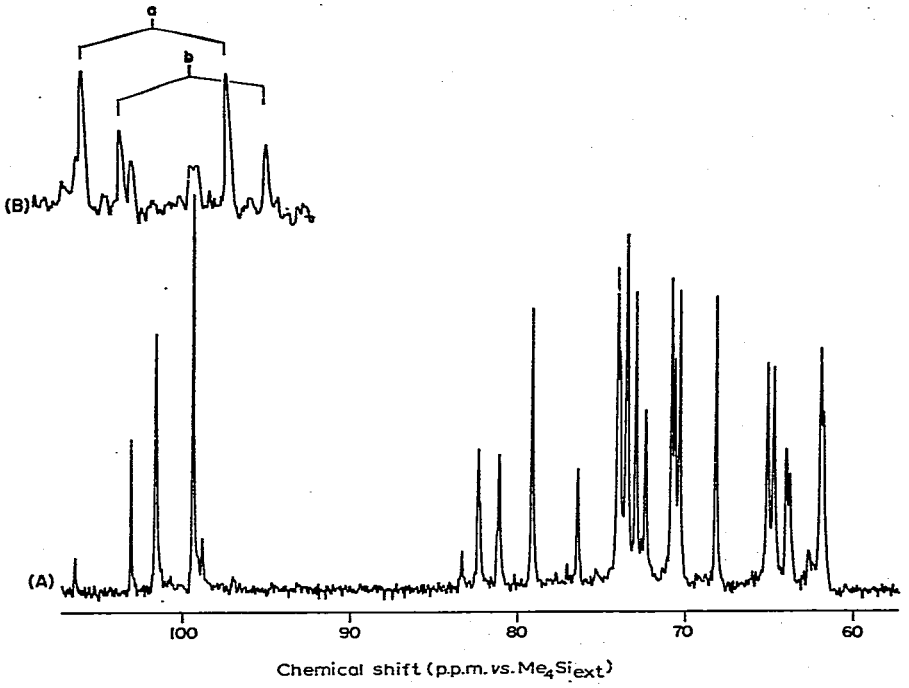


Fig. 1. (A) ^{13}C-N.m.r. spectrum of maltulose in deuterium oxide (100 mg/mL) at 30°; 28,000 transients, pulse angle 45°, digital resolution 1 Hz, 1.000-kHz plot-width, no line broadening applied. (B) Partial, gated-decoupled spectrum; 46,000 transients, pulse angle 45°, digital resolution 2.5 Hz, 1.000-kHz plot-width, line broadening 1.6 Hz. [a and b are doublets (^1J = 171 and 173 Hz, respectively) assigned to C-1 of D-glucose in structures 3 and 2, respectively.]

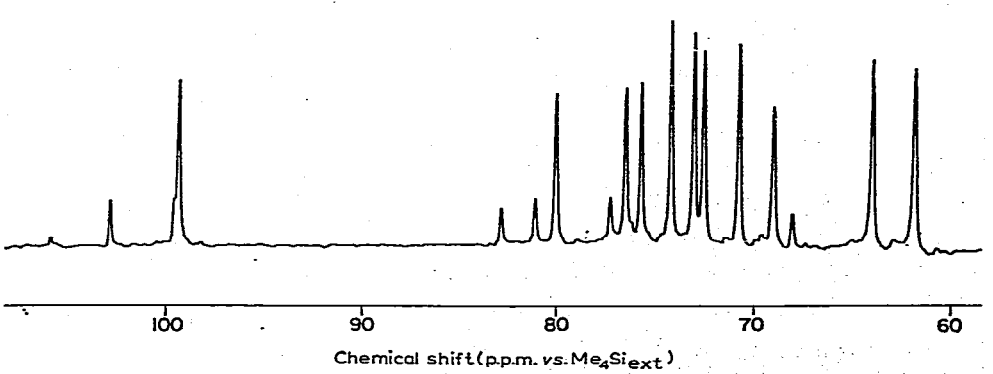


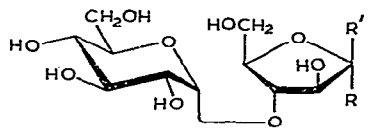
Fig. 2. ^{13}C-N.m.r. spectrum of isomaltulose in deuterium oxide (100 mg/mL) at 30°; 4800 transients, pulse angle 45°, digital resolution 2 Hz, 1.000-kHz plot-width, line broadening 1.1 Hz.

TABLE I

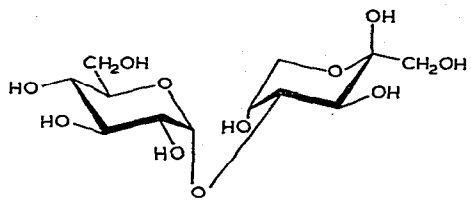
CARBON-13, CHEMICAL-SHIFT DATA[a] FOR D-FRUCTOSE AND STRUCTURES 1–6

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1′*	*C-2′*	*C-3′*	*C-4′*	*C-5′*	*C-6′*
α-D-Fructofuranose[b]	63.8	105.5	82.9	77.0	82.2	61.9						
β-D-Fructofuranose[b]	63.6	102.6	76.4	75.4	81.6	63.2						
β-D-Fructopyranose[b]	64.7	99.1	68.4	70.5	70.0	64.1						
Maltulose [α-Fru*f*](1)	64.6	106.4	83.4	81.3	82.3	62.5	98.9	72.4	73.9	70.6	73.5	61.6
Maltulose [β-Fru*f*](2)	63.7[c]	103.1	76.5	82.4	81.2	63.9[c]	99.4	72.4	73.9	70.6	73.5	61.6
Maltulose [β-Fru*p*](3)	65.0[c]	99.4	68.1	79.2	70.3	64.6[c]	101.6	73.0	74.0	70.8	73.5	61.8
Isomaltulose [α-Fru*f*](4)	63.9	105.9	82.9	77.3	81.2	68.0	99.7	72.6	74.2	70.8	73.1	61.8
Isomaltulose [β-Fru*f*](5)	63.9	102.9	76.5	75.8	80.1	69.0	99.4	72.6	74.2	70.8	73.1	61.8
Leucrose(6)	65.1	99.2	69.2	71.2	80.2	63.4	101.5	73.2	74.2	70.9	73.3	61.9

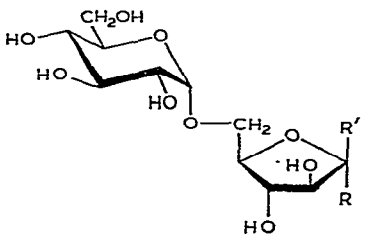
[a]In p.p.m. from external tetramethylsilane, for deuterium oxide solutions. [b]Data taken from ref. 7. [c]Assignments may be reversed.



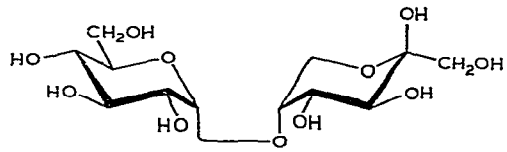
1. R = OH, R′ = CH_2OH
2. R = CH_2OH, R′ = OH



3



4. R = OH, R′ = CH_2OH
5. R = CH_2OH, R′ = OH



6

RESULTS AND DISCUSSION

The ^{13}C-n.m.r. spectra of maltulose and isomaltulose in deuterium oxide are respectively shown in Figs. 1 and 2. Table I records chemical-shift data for structures **1**–**3** (maltulose), **4** and **5** (isomaltulose), and D-fructose.

Maltulose. — It is apparent from the spectrum of maltulose that, in addition to form **3**, containing a D-fructopyranose residue, two forms, **1** and **2**, having a D-fructofuranose residue, were present. Resonances for the α-D-glucopyranosyl group were readily assigned by comparison of the data in Table I with those reported for D-glucopyranosides[5,8]. Assignment of the resonances at δ_c 101.6, 99.4, and 98.9 to C-1 of the D-glucopyranosyl group was confirmed by the observation that these signals appeared as doublets in a coupled spectrum of maltulose. Linkage of the D-glucopyranosyl group to O-4 of D-fructose was indicated by a deshielding (4–9 p.p.m.) of C-4 of **1**–**3**, relative to the corresponding carbon atom of D-fructose; for maltose[9] and nigerose[10], the corresponding carbon atoms are deshielded with respect to those of D-glucose by 7.4 and 7.1 p.p.m., respectively. The sensitivity of the D-glucose resonances to the ring form and anomeric configuration of the adjacent, D-fructose residue is suggested by three resonances attributable to C-1 and two sets of resonances for C-2 to C-6 of the D-glucopyranosyl group. Taking into account the relative intensities and peak areas, the resonances of C-1 of the D-glucosyl group may be assigned as follows: 101.6 p.p.m., attached to β-D-fructopyranose (see **3**); 99.4 p.p.m., attached to β-D-fructofuranose (see **2**); 98.9 p.p.m., attached to α-D-fructofuranose (see **1**). Thus, the C-1 D-glucosyl group resonance is sensitive to the

anomeric configuration of the adjacent D-fructose residue, a phenomenon that we have also observed for isomaltulose (see later). Interestingly, the other D-glucosyl resonances of maltulose can be divided into two sets, respectively attributable to a D-glucosyl group attached to a β-D-fructopyranose residue (as in **3**) and to a D-fructofuranose residue (as in **1** and **2**).

Finally, using peak areas (C-3 to C-6 of D-fructose), the composition of the maltulose solution at equilibrium was estimated to be 1:4:8 at 32°, and 1:3:3 at 60°, for the ratios of **1**:**2**:**3**. D-Fructose has been reported[7] to give, in deuterium oxide at 27°, an equilibrium mixture of α- and β-D-fructofuranose and β-D-fructopyranose in the ratios of 1:5:19; at 55°, the corresponding ratios were 1:5:11. It is known[11] that 3-*O*-alkylation of D-fructose destabilizes the β-pyranose form, and shifts the equilibrium towards the α-pyranose and the furanose forms. It would appear that 4-*O*-glucosylation of D-fructose results in a similar shift of the equilibrium towards the furanose forms. The observed decrease in population of the pyranose form of the D-fructose residue in maltulose on going from 32 to 60° provides confirmation[7] of the aforementioned resonance-assignments to the various ring forms.

Isomaltulose. — The spectrum of isomaltulose exhibited signals (see Table I) that were attributable to structures **4** and **5**. Signal assignments were readily made by considerations similar to those used for maltulose. A (1→6)-linkage was readily concluded from the observations that (*a*) only furanose forms of the D-fructose residue were present, and (*b*) the resonances of the C-6 atom of the D-fructose residue of **4** and **5**, at δ_c 68.0 and 70.0, respectively, were shifted downfield by 6–7 p.p.m. relative to the corresponding resonances of D-fructose. Taking into account the relative intensities, the signals at δ_c 99.7 and 99.4 were respectively assigned to C-1 of the D-glucopyranosyl group in **4** and **5**. The presence of two C-1 (D-glucosyl) resonances reflects a sensitivity to the anomeric configuration of the D-fructose residue. Colson *et al.*[10] reported a similar sensitivity of a D-glucosyl C-1 resonance to the linkage at the anomeric center of the adjacent D-glucose residue in maltose. The ratio of **4** to **5** was determined, from peak areas (C-3 to C-6), to be 1:4; at 65°, the corresponding ratio was found to be 1:2.5. These values do not differ greatly from the ratios of 1:5 at 27° and 1:3 at 85° that had been determined by Angyal and Bethell[7] for the α and β anomers of D-fructofuranose.

Leucrose. — Leucrose (**6**) was shown by Stodola *et al.*[12] to be 5-*O*-α-D-glucopyranosyl-D-fructose. Chemical-shift data for **6** in deuterium oxide solution are given in Table I. Resonances attributed to the D-glucopyranosyl group were readily assigned by comparison with those of the corresponding moiety in maltulose. A (1→5)-linkage is evident from the observation that only one ring form of the D-fructose residue could be detected. The resonance at δ_c 99.2 was assigned to C-2 of a β-D-fructofuranose residue; the corresponding carbon atom of maltulose and D-fructose had chemical shifts of δ_c 99.4 and 99.1, respectively. Resonance assignments for C-1, and C-3 to C-6, of the D-fructose residue were made by comparison with the corresponding resonances of β-D-fructopyranose and maltulose. Confirmation of the presence of a (1→5) intersaccharide linkage was obtained from a 10.2-p.p.m., down-

TABLE II

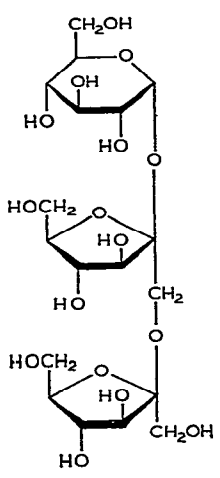
CARBON-13, CHEMICAL-SHIFT DATA[a] FOR INULIN AND RELATED COMPOUNDS

Carbon atom[b]	*Sucrose*		*1-Kestose* (7)		*Nystose* (8)		*Inulin* (9)		*Grass Levan*
	Me_2SO-d_6[c,d]	D_2O[d,e]	Me_2SO-d_6[c,f]	D_2O[e,g]	Me_2SO-d_6[c,f]	D_2O[e,g]	Me_2SO-d_6[c,d]	D_2O[d,e]	D_2O[d,e]
1	62.0	62.6	61.0	62.2	61.1	62.05			62.6
1′			60.9	61.7	61.2	62.20	61.6	62.2	61.3
1″					61.0	61.53			
2	103.9	104.9	103.7	104.9	103.7	104.90	103.7		104.9
2′			103.2	104.5	103.2	104.26	103.0	104.4	105.4
2″					102.8	104.43			
3	77.1	77.7	76.9	77.9	77.7	77.93[h]			77.6
3′			76.4	77.9	77.5	78.70	76.9	78.3	
3″					76.5	77.89[h]			
4	74.3	75.3	73.8	75.1	74.6	75.05			76.5
4′			74.7	75.7	74.6	75.50	74.4	75.6	
4″					74.0	75.83			
5	82.4	82.5	82.9	82.4	81.9	82.44			81.5
5′			82.9	82.4	81.9	82.30	81.7	82.3	82.4
5″					82.1	82.30			
6	62.0	63.5	62.0	63.5[h]	61.9	63.46			64.6
6′			61.6	63.4[h]	61.9	63.46	61.6	63.4	
6″					61.7	63.46			
1+	91.7	93.3	91.9	93.7	92.0	93.74	91.9	93.7	93.3
2+	71.5	72.2	71.5	72.4	71.6	72.40	71.5	72.8	72.2
3+	72.8	73.8	72.8	73.8	72.8	73.80	—	74.3	73.8
4+	69.8	70.4	69.7	70.5	69.8	70.40	69.7	70.6	70.4
5+	72.7	73.6	72.7	73.6	72.8	73.65	72.4	73.5	
6+	60.4	61.7	60.3	61.4	60.5	61.28	—	—	

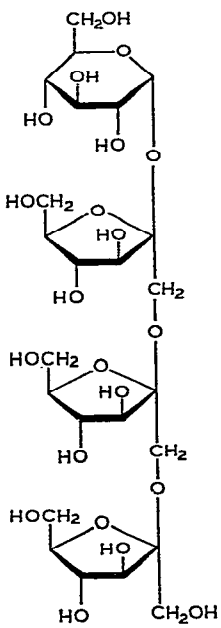
[a]In p.p.m. [b]Primed numbers are associated with a D-fructofuranosyl unit attached to sucrose, and doubly primed numbers refer to a D-fructofuranosyl unit attached to 1-kestose; numbers having a superscript "+" refer to the D-glucopyranosyl group. [c]Reference, internal tetramethylsilane in dimethyl sulfoxide-d_6. [d]Measured at 20 MHz. [e]Reference, external tetramethylsilane, for solutions in deuterium oxide. [f]Data from ref. 3 (25 MHz). [g]Measured at 68 MHz. [h]Assignments may be reversed.

field shift of the C-5 (D-fructose) resonance relative to the corresponding resonance of D-fructose, a result reminiscent of an observed, 8.8-p.p.m., downfield shift of the C-4 resonance of maltulose relative to C-4 of β-D-fructopyranose. Interestingly, although 3- and 4-*O*-alkylation (see maltulose) destabilizes the β-pyranose tautomer of D-fructose, and increases the relative amount of α-pyranose form present at equilibrium, it appears that the (1→5)-glycosidic bond in leucrose obviates the presence of any appreciable proportion of the α-D-fructopyranose form. The presence of only one detectable ring-form of the D-fructose residue at equilibrium accounts for the small degree of mutarotation observed by optical rotation[12] for leucrose.

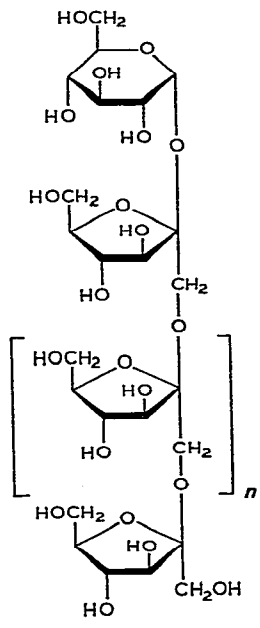
Sucrose. — Chemical-shift data for sucrose are given in Table II. Although the spectrum of sucrose has been documented[2–5], the signal assignments were tentative. Hough *et al.*[4] assigned the resonances of the hydroxymethyl group by chlorination. Recently, Koch *et al.*[5,13] reported resonance assignments for sucrose that were based upon *C*- and *O*-deuteration studies. Pfeffer and Valentine[14] used a differential, isotopic-shift technique to assign the resonance of lactose, methyl lactoside, sucrose, and D-fructose. Using the assignments for β-D-fructofuranose and methyl β-D-fructofuranoside[7], and the results from chlorination studies[4], an unambiguous assignment of the ^{13}C resonances for sucrose may be made (see Table II). It should be noted that the data in Table II interchange a recent[5] assignment of the C-1 and C-6 resonances for the D-fructosyl group of sucrose in deuterium oxide; this is based on the study by Hough *et al.*[4], who specifically chlorinated C-6 of the D-fructosyl group, in order to distinguish between the C-1 and C-6 resonances for sucrose.



7



8



9

1-Kestose and nystose. — The chemical-shift data for 1-kestose (**7**), nystose (**8**), inulin (**9**), and a grass levan are given in Table II. The data for nystose in deuterium oxide were obtained at 68 MHz, in order to afford greater spectral dispersion; the data for these compounds in dimethyl sulfoxide are those measured by Horton *et al.*[3] at 25 MHz. Although these authors[3] attempted tentative, partial assignments for 1-kestose and nystose, no attempt was made to assign resonances to specific D-fructosyl groups or residues. The spectrum of **7** was noted to resemble the sum of the spectra of sucrose and methyl β-D-fructofuranoside. In order to avoid ambiguities from comparison of data obtained in different solvents, and to facilitate assignment of resonances to specific residues, spectra of all compounds were obtained in the same solvent.

The general assignment of resonances in the spectrum of **7** in deuterium oxide was made by comparison of the observed chemical shifts with the data for sucrose and methyl β-D-fructofuranoside[7]. The resonance at δ_c 61.7 was assigned to C-1 of the (terminal) D-fructosyl group for the following reasons. In the spectrum of grass levan, the C-1 resonance of the β-D-fructofuranosyl residues (except for that in the sucrose end-group) occurs at δ_c 61.3, and may be used as an indication of the chemical shift to be expected for C-1 of a β-D-fructofuranosyl group linked glycosidically to an O-CH_2- group. In addition, it might be expected that C-1 of the D-fructosyl residue in the sucrose end-group of 1-kestose (**7**) would exhibit a chemical shift (δ_c 62.2) which resembles that observed for the C-1 atoms of inulin (δ_c 62.2). The resonance at δ_c 104.9 was assigned to C-2 of the D-fructosyl residue of the sucrose end-group, because sucrose, nystose (**8**), an oligomer containing three β-D-fructofuranosyl residues attached to a sucrose end-group*, and grass levan all exhibit a resonance at δ_c 104.9. Finally, the resonance at δ_c 75.1 was attributed to C-4 of the D-fructosyl residue of the sucrose end-group on the basis of the following observations. Methyl β-D-fructofuranoside gives rise to a C-4 resonance at δ_c 75.9, whereas C-4 of sucrose has a chemical shift of 75.3 p.p.m. Thus, C-4 of the (terminal) β-D-fructofuranosyl group may be expected to have a resonance at lower field than that of C-4 in the sucrose end-group. In addition, oligomers containing only β-D-fructofuranose linked (2→1) exhibit[15] a resonance at δ_c 75.6, but not at δ_c 75.1.

A complete interpretation of the ^{13}C-n.m.r. spectrum of 1-kestose (**7**) in deuterium oxide facilitated the assignment of resonances in the spectrum of nystose (**8**). Addition of a β-D-fructofuranosyl group to **7** gives rise to additional resonances. Resonances at δ_c 104.90 and 104.43 may be assigned to the sucrose end-group and the terminal β-D-fructofuranosyl group, respectively, by comparison with the corresponding resonances of **7**. Thus, the internal D-fructosyl residue has a C-2 resonance at δ_c 104.26; for a pentasaccharide* containing one β-D-fructofuranosyl residue more than **8**, the relative intensities of the resonances at δ_c 104.9, 104.4, and 104.3 were 1:1:2, which supports the resonance assignments for C-2 of **8**. Resonances at

*A pentasaccharide having the structure β-D-Fru*f*-[(2→1)-β-D-Fru*f*]$_2$-β-D-Fru*f*-(2↔1)-α-D-Glc*p*, unpublished results.

δ_c 62.05 and 61.53 were assigned to C-1 of the sucrose end-group and (terminal) D-fructosyl group by analogy with the observed data for the C-2 resonances of **7**. The C-2 resonance of the internal D-fructofuranosyl residues was concluded to be at δ_c 62.20 by comparison with data for the C-2 resonance (δ_c 62.2) of inulin; in addition, the pentasaccharide* gave rise to a resonance at δ_c 62.2 that was attributable to the two internal D-fructosyl residues. Chemical shifts of δ_c 77.93 and 77.89 corresponded closely to that of the C-3 resonance (δ_c 77.9) in the spectrum of **7**, and were therefore assigned to C-3 of the D-fructosyl residue of the sucrose end-group and of the (terminal) D-fructosyl group. The chemical shift (δ_c 78.70) of C-3 of the (internal) D-fructosyl residue correlates less exactly with the value (δ_c 78.3) of the corresponding resonance of inulin. Similar arguments enable assignments for resonances at δ_c 75.05 and 82.44 to be made for C-4 and C-5, respectively, of the sucrose end-group, and those at δ_c 75.83 and 82.30, respectively, to the (terminal) D-fructofuranosyl group. Signals at δ_c 75.50 and 82.30, attributed to C-4 and C-5, respectively, of the internal D-fructofuranosyl residue, correlate well with the corresponding C-4 and C-5 resonances of inulin at δ_c 75.6 and 82.3, respectively.

Having assigned in detail the ^{13}C-n.m.r. spectra of 1-kestose (**7**) and nystose (**8**) in solution in deuterium oxide, assignment of the spectra of **7** and **8** in dimethyl sulfoxide-d_6 were made by using similar reasoning. However, inspection of Table II reveals that solvent effects are not identical for each carbon resonance and, therefore, make less straightforward the use of assignments that had already been made for spectra obtained in deuterium oxide. In particular, the C-3 resonances of the D-fructosyl residues in **7** and **8** do not appear to follow the trends that had been observed for aqueous solutions. Thus, whereas, in deuterium oxide solution, **7** gives a single resonance for C-3 of both D-fructose units, two resonances were observed in dimethyl sulfoxide-d_6 solution. The signal at δ_c 76.9 was assigned to C-3 of the sucrose end-group by comparison with values of δ_c 77.1 and 76.9 observed for the corresponding resonances in the spectra of sucrose and inulin, respectively. Interestingly, **8** exhibits three resonances that are attributable to C-3 of the D-fructose units, of which two are shifted downfield relative to the corresponding resonances of **7**. Although the resonance at δ_c 75.6 may be assigned to C-3 of the (terminal) D-fructofuranosyl group by comparison with the corresponding resonance of **7**, resonances at δ_c 77.7 and 77.5 can not be assigned definitively to specific residues.

Grass levan. — The resonance assignments for grass levan, given in Table II, have been discussed[6] in detail. However, we have revised several of the earlier assignments; specifically, the C-2, C-3, and C-5 resonances of the D-glucosyl group have been reassigned, to agree with those reported for D-glucosides[5,8] and sucrose.

Inulin. — The ^{13}C-n.m.r. spectrum of inulin (**9**) in dimethyl sulfoxide-d_6 shown in Fig. 3, exhibits a resonance attributable to the D-fructosyl residue in the sucrose residue, as well as that due to the backbone β-D-fructofuranosyl residues. In addition, the C-1 and C-6 atoms of the D-fructofuranosyl units gave a single, broad resonance. Resonance assignments for inulin were readily made by comparison of the data with those observed for 1-kestose (**7**) and nystose (**8**). A close examination of the ^{13}C-n.m.r.

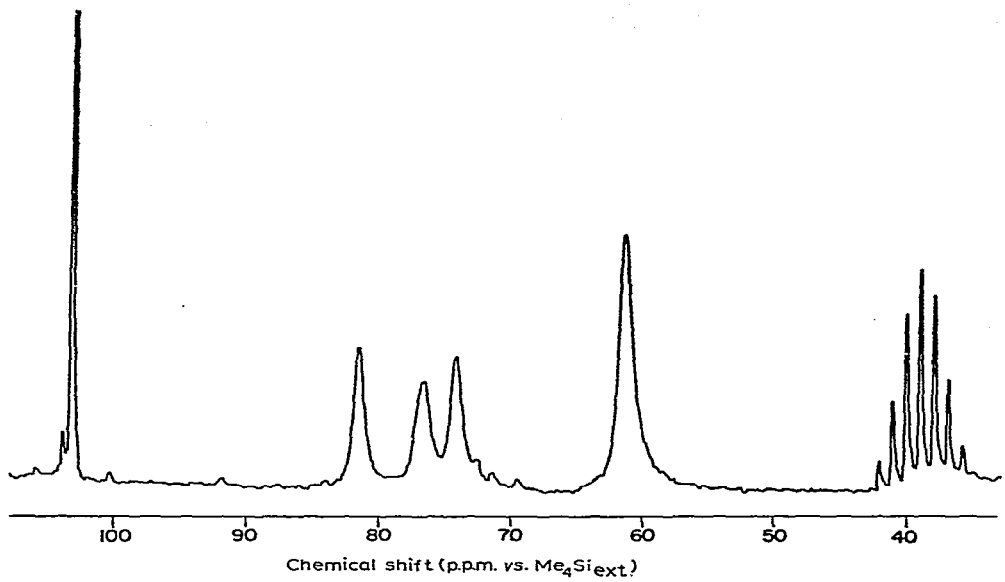


Fig. 3. ^{13}C-N.m.r. spectrum of inulin (9) in dimethyl sulfoxide-d_6 (100 mg/mL) at 30°; 50,000 transients, pulse angle 75°, digital resolution 3.3 Hz, 1.500-kHz plot-width, line broadening 1.6 Hz.

spectrum of inulin revealed less-intense signals which are attributable to the sucrose end-group. Integration of the C-2 signal of the D-fructosyl residue of the sucrose end-group (δ_c 103.7) and C-2 of the D-fructofuranosyl units of the linear chain (δ_c 103.0) indicated that there is an average of 16 D-fructofuranosyl units linked to the terminal sucrose group; inulin had been reported[16] to contain 20–24 D-fructosyl units.

The ^{13}C-n.m.r. spectrum of **9** in deuterium oxide differs from that in dimethyl sulfoxide-d_6; in addition to the expected, solvent-induced differences in chemical shifts, only one resonance attributable to C-2 of D-fructofuranose units and two resonances due to C-1 and C-6 were observed. Resonance assignments were made by comparison of the data for **9** in deuterium oxide with those for **7** and **8** in the same solvent. Close examination of the spectrum also revealed resonances attributable to the D-glucopyranosyl group of the terminal sucrose residue.

It is evident from the data given in Table II that attachment of a β-D-fructofuranosyl group to C-1 of another D-fructofuranose unit does not lead to a significant, downfield shift of the C-1 resonance. In addition, because the C-1 and C-6 chemical-shifts do not differ greatly, either for inulin or for grass levan, the position of the intersaccharide linkage in these and other D-fructose-containing oligo- and poly-saccharides may be difficult to assign unambiguously by using comparative techniques only. Koch and Stuart[12] reported that treatment of mono- and poly-saccharides with deuterated Raney nickel and deuterium oxide causes deuterium exchange with carbon-bound hydrogen atoms geminal to the hydroxyl group (primary and secondary); sucrose and inulin were reported to be deuterated at C-3, C-4, and C-1 or C-6, or both, of the D-fructosyl units. Inulin was deuterated by their procedure, and the re-

TABLE III

RELAXATION TIMES (T_1), N.O.E.F. VALUES, AND LINEWIDTHS ($\Delta\nu$) FOR INULIN (9)

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
T_1 (ms)	28	357	49	50	55	41
NT_1[a] (ms)	56	[b]	49	50	55	82
N.O.e.f.[c]	0.80	0.69	0.68	0.68	0.52	1.0
$\Delta\nu$ (Hz)[d]	—	—	14.0	9.3	8.5	—

[a]N is the number of directly attached protons. [b]No contiguous proton; relaxation of C-2 is due to dipole–dipole interactions with distant protons, and T_1 is therefore considerably longer than those of proton-bearing carbon atoms. [c]Determined from peak areas, except for C-1 and C-6, for which, because of signal overlap, peak heights were used. [d]The linewidth of the C-2 resonance (4.0 Hz) was used to correct for field inhomogeneity.

sulting incorporation of deuterium was estimated to be 50, 30, and 70% at C-3, C-4, and C-6, respectively. Substantial loss of intensity of the C-6 signal confirmed our resonance assignments for C-1 and C-6, as well as the presence of a (2→1) intersaccharide linkage.

An alternative method of determining the position of intersaccharide linkage in D-fructose-containing oligo- and poly-saccharides is the use of spin–lattice relaxation-times (T_1). Allerhand and Doddrell[17] used a partially relaxed spectrum of stachyose to differentiate between the resonances of the terminal and nonterminal D-galactopyranosyl units. A similar approach was adopted by Neszmélyi *et al.*[18], who used T_1 values measured for *k*-strophanthoside to assign the carbohydrate sequence. Presented in Table III are the T_1 values of inulin measured in 1 : 1 dimethyl sulfoxide-d_6–deuterium oxide, the corresponding NT_1 values (N is the number of directly-attached protons), the nuclear Overhauser enhancement factors (n.O.e.f. values), and the observed linewidth ($\Delta\nu$). In order to perform measurements of T_1 in a reasonable time, a solvent mixture was chosen in which a suitable concentration of inulin could be obtained and in which the resonances for C-1 and C-6 were resolved. The higher viscosity due to the presence of dimethyl sulfoxide serves to discriminate to a greater extent between T_1 values determined by overall or segmental motion.

The n.O.e.f. values differ significantly from the maximum that is possible (1.988), although they are greater than the theoretical minimum (0.0385). Before interpreting the T_1 data, it was therefore necessary to establish whether or not the ^{13}C spin-system is in the region of motional narrowing. By using the standard, spectral-density function for isotropic motion[19], and a T_1 value of 50 ms (for C-3 or C-4, which were not expected to undergo internal motion as readily as either C-1 or C-6), we obtained the two possible solutions; effective correlation time 1.2 or 20 ns.rad^{-1}, n.O.e.f. 1.70 or 0.176, and linewidth 6.7 or 30.8 Hz. Our n.O.e.f. and linewidth data lie between these two extremes, probably due to the influence of a distribution of correlation times for overall motion [20]. For a molecular weight of

3060, we estimate, from the Stokes–Einstein equation, a correlation time of 1.5 ns.rad^{-1}, in much better agreement with the value of 1.2 ns.rad^{-1} predicted from the T_1 value of 50 ms in the motional-narrowing region. In addition, observed linewidths can easily be greater than those predicted from correlation times, because of chemical-shift heterogeneity and incomplete ^{1}H-decoupling, but they are rarely less. Our data are, in fact, very similar to those observed by Schaefer[20] for isotactic polystyrene in *o*-dichlorobenzene, where it was shown that the correlation time lies in the motional-narrowing region, but must have a distribution of values. We thus interpret our T_1 data in terms of the motional-narrowing formalism: long T_1 values are indicative of a greater rate or amplitude of motion, and *vice versa*.

Inspection of the NT_1 values indicated that C-6 undergoes a faster internal reorientation than C-1, as might be expected if C-1 is involved in an intersaccharide linkage. Hence, the (2→1)-linkage in inulin is also evident from the NT_1 values. In addition, it is interesting to note that the NT_1 value of C-1 is similar to those of the ring-carbon atoms of D-fructofuranose. The similarity of the NT_1 values for C-1 and the ring-carbon atoms suggests that the furanose ring undergoes reorientation at approximately the same rate as C-1, indicating that, under these conditions, there is no significant segmental motion, other than for C-6, within the monomer units. It should be noted that it is not necessary to determine the T_1 values of C-1 and C-6 in order to make assignments, as comparison of intensities within a single, partially relaxed spectrum[17,21] would be sufficient.

EXPERIMENTAL

Maltulose was a gift of Dr. J. E. Hodge, USDA Northern Regional Research Center, Peoria, Illinois; 1-kestose (**7**) and nystose (**8**) were supplied by Dr. C. P. J. Glaudemans, National Institutes of Health, Bethesda, Maryland. Isomaltulose (palatinose) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, and leucrose (**6**) was available at Moffett Technical Center, as originally prepared[12]. Inulin (**9**) was purchased from BDH Chemicals, Toronto, Ontario, Canada.

Nuclear magnetic resonance spectra were recorded with Varian CFT-20 and FT-80 (10-mm tubes, 30°), Varian XL-100-15 (12-mm tubes, variable temperature), and Bruker HX-270 (68 MHz, 10-mm tube, 20°) instruments, with complete proton-decoupling. The solvent deuterium resonance was used as a field-frequency lock. Chemical shifts were measured, relative to internal tetramethylsilane, for solutions in dimethyl sulfoxide-d_6, and, relative to tetramethylsilane in a sealed concentric tube of outside diameter 5 mm, for solutions in deuterium oxide. The concentration of samples was ~100 mg/mL, and, where appropriate, aqueous solutions were prepared 24 h in advance, in order to ensure that equilibrium had been reached.

Spin–lattice relaxation-times (T_1) were measured by the inversion-recovery procedure[22] for solutions of inulin in 1:1 (v/v) dimethyl sulfoxide-d_6–deuterium oxide containing 100 mg of the sample per mL. The results are the average of two measurements, and have an estimated accuracy of ±10%.

ACKNOWLEDGMENTS

We are grateful to Dr. W. Egan, National Institutes of Health, Bethesda, Maryland, for measuring the 68-MHz, ^{13}C-n.m.r. spectra, and to Dr. Fred R. Seymour for sending us copies of his relevant manuscripts. This work was supported, in part, by the National Research Council of Canada.

NOTE ADDED IN PROOF

After this manuscript had been submitted for publication, an Editor brought to our attention two papers in press in which the ^{13}C-n.m.r. spectra of D-fructose-containing compounds were discussed[23,24]. Our assignments are in general agreement with those reported for sucrose, isomaltulose, and inulin. However, in the spectrum of isomaltulose, Seymour and co-workers assigned a resonance at 58.43 p.p.m. to C-1 of the α-D-fructofuranose residue. We did not observe a resonance at this position, and have assigned a chemical shift of 63.9 p.p.m. to this carbon atom.

REFERENCES

1 H. J. Jennings and I. C. P. Smith, *Methods Enzymol., Part C*, 50 (1978) 39–50.
2 A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, 93 (1971) 2779–2781.
3 W. W. Binkley, D. Horton, N. S. Bhacca, and J. D. Wander, *Carbohydr. Res.*, 23 (1972) 301–306.
4 L. Hough, S. P. Phadnis, E. Tarelli, and R. Price, *Carbohydr. Res.*, 47 (1976) 151–154.
5 S. Ho, H. J. Koch, and R. S. Stuart, *Carbohydr. Res.*, 64 (1978) 251–256.
6 J. Tomašić, H. J. Jennings, and C. P. J. Glaudemans, *Carbohydr. Res.*, 62 (1978) 127–133.
7 S. J. Angyal and G. S. Bethell, *Aust. J. Chem.*, 29 (1976) 1249–1265.
8 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
9 P. Colson, K. N. Slessor, H. J. Jennings, and I. C. P. Smith, *Can. J. Chem.*, 53 (1975) 1030–1037.
10 P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
11 T. A. W. Koerner, Jr., R. J. Voll, L. W. Cary, and E. S. Younathan, *Biochem. Biophys. Res. Commun.*, 82 (1978) 1273–1278.
12 F. H. Stodola, E. S. Sharpe, and H. J. Koepsell, *J. Am. Chem. Soc.*, 78 (1956) 2514–2518.
13 H. J. Koch and R. S. Stuart, *Carbohydr. Res.*, 59 (1977) C1–C6.
14 P. E. Pfeffer and K. M. Valentine, *Abstr. Int. Symp. Carbohydr. Chem., London*, D9 (1978) 285–286.
15 Unpublished results.
16 H. D. K. Drew and W. N. Haworth, *J. Chem. Soc.*, (1928) 2690–2697.
17 A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, 93 (1971) 2777–2778.
18 A. Neszmélyi, K. Tori, and G. Lukacs, *Chem. Commun.*, (1977) 613–614.
19 D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, 56 (1972) 3683–3689.
20 J. Schaefer, *Macromolecules*, 6 (1973) 882–888.
21 K. Nakanishi, V. P. Gullo, I. Miura, T. R. Govindachari, and N. Viswanathan, *J. Am. Chem. Soc.*, 95 (1973) 6473–6475; K. Nakanishi, R. Crouch, I. Miura, X. Dominguez, A. Zamudio, and R. Villareal, *ibid.*, 96 (1974) 609–611.
22 R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, 51 (1969) 3140–3141.
23 F. R. Seymour, R. D. Knapp, and A. Jeanes, *Carbohydr. Res.*, 72 (1979) 222–228.
24 F. R. Seymour, R. D. Knapp, J. E. Zweig, and S. H. Bishop, *Carbohydr. Res.*, 72 (1979) 57–69.