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THE ASSIGNMENT OF CARBONYL RESONANCES IN ^{13}C-N.M.R. SPECTRA OF PERACETYLATED MONO- AND OLIGO-SACCHARIDES CONTAINING D-GLUCOSE AND D-MANNOSE: AN ALTERNATIVE METHOD FOR STRUCTURAL DETERMINATION OF COMPLEX CARBOHYDRATES

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ABSTRACT

In the present study, proton homonuclear (COSY) and ^{13}C–^{1}H heteronuclear shift-correlation, n.m.r. spectroscopies have been used to assign the carbonyl carbon resonances of peracetylated D-gluco- and D-mannopyranose monosaccharides and oligosaccharides containing residues of parent D-glucopyranose monomers. Chemical shifts of these assigned resonances, particularly those arising from acetyl groups near to aglycon substitution sites, were found to be sensitive to the position and configuration of glycosidic linkages present. In addition, evidence is presented that indicates that the shifts of these carbonyl carbon resonances depend on long-range interactions with other peracetylated pyranose monomers resulting from folding of the oligosaccharide chain. These results suggest that carbonyl carbon resonances of peracetylated carbohydrates may be useful probes of oligosaccharide structure.

INTRODUCTION

In the past few years, natural-abundance ^{13}C-n.m.r. spectroscopy has been shown to be a valuable technique for the identification of monosaccharide structures present in complex oligosaccharides, glycopeptides, and even glycoproteins[1–9]. The chemical shifts of pyranose-ring carbon atoms are quite sensitive to glycosidic substitution, and can be used to establish the position and configuration of linkages. Similar analyses by ^{1}H-N.m.r. can be conducted with significantly increased sensitivity[10] (>400 ×). This increased sensitivity is most important when charac-

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terizing complex carbohydrates isolated in small quantities from biological systems. On the other hand, the low abundance of the ^{13}C isotope and the large chemical-shift range of carbon resonances permit the acquisition of well resolved, ^{13}C-n.m.r. spectra of oligosaccharides, that are uncomplicated by spin–spin coupling.

The goal of the present study was to develop, for oligosaccharides, a form of n.m.r. spectroscopy having some of the advantages both of ^{1}H-n.m.r. and natural-abundance ^{13}C-n.m.r. spectroscopy. An obvious alternative is a chemical modification of the carbohydrate structure which would allow specific, ^{13}C enrichment. ^{13}C-N.m.r. spectra of permethylated carbohydrates have already been investigated, and their methoxyl carbon resonances appear to be sensitive to the position of the methoxyl substituent and the position and configuration of glycosidic linkages to neighboring, permethylated residues[11,12]. Although peracetylated D-glucoses and cellobioses have also been investigated by ^{13}C-n.m.r. spectroscopy[2,13–19], the chemical shifts of the carbonyl and methyl carbon atoms have not been assigned. In the present study, we have found that the carbonyl carbon chemical-shifts of peracetylated oligosaccharides are sensitive to carbohydrate structure. A library of various peracetylated carbohydrate structures and their corresponding carbonyl carbon chemical-shifts may ultimately be useful in attempts to evaluate the structures of unknown oligosaccharides.

EXPERIMENTAL

Materials. — α- and β-D-Glucopyranose pentaacetates, methyl α- and β-D-glucopyranosides, methyl α-D-mannopyranoside, β-cellobiose, β-maltobiose, isomaltobiose, isomaltotriose, and β-gentiobiose octaacetate were obtained from Sigma Chemical Co. (St. Louis, MO), and were used without further purification. D-[1-^{13}C]Mannose and D-[1-^{13}C]glucose were prepared from potassium [^{13}C]cyanide and D-arabinose, according to the procedure of Serianni *et al.*[20]. D-[2-^{13}C]Glucose and D-[2-^{13}C]mannose were prepared from D-[1-^{13}C]mannose and D-[1-^{13}C]glucose according to the procedure of Hayes *et al.*[21]. The *O*-methylation of α-D-[2-^{13}C]mannopyranose and the peracetylation of methyl manno- and gluco-pyranosides, β-cellobiose, β-maltose, isomaltose, and isomaltotriose were performed by standard procedures[22,23].

N.m.r.-spectral conditions. — Peracetylated sugars (1–1.5 g) were dissolved in $CDCl_3$ (4 mL) prior to spectral acquisition. ^{13}C-N.m.r. spectra were recorded at 4.7 or 9.4 T, using Bruker AM-200 or AM-400 spectrometers. All experiments were carried out at ambient probe-temperature (30 ±2°). Chemical shifts are relative to internal Me_4Si (1% in $CDCl_3$). Normal F.t., ^{13}C-n.m.r. spectra were acquired in the presence of WALTZ-16 decoupling[24].

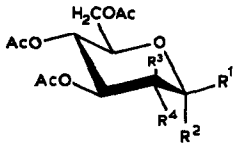
Proton-correlated shift (COSY) spectra[25–29] were acquired by using a sweep width of ±450 Hz (at 200 MHz) or ±900 Hz (at 400 MHz) in both frequency dimensions. A 45° mixing pulse was employed in order to emphasize crosspeaks arising from directly connected transitions. A relaxation delay of 2 s was used at both

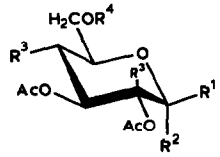
fields. Each spectrum was collected by using 2 dummy and 4 normal acquisitions. ^{13}C–^{1}H Shift-correlation spectra[25] were acquired by using a sweepwidth of ±150 Hz (at 50 MHz) or ±300 Hz (at 100 MHz) in the F_2 dimension, and ±450 Hz (at 200 MHz) or ±900 Hz (at 400 MHz) in the F_1 dimension. Broadband decoupling was applied during acquisition. The fixed delay times before (Δ_1) and following (Δ_2), the final mixing pulse, were 70 ms and 100 ms, in order to emphasize long-range couplings of carbonyl carbons to pyranose-ring protons. Each spectrum was an average of 16 acquisitions. A relaxation delay of 2 s or 4 s was included at the lower and higher magnetic-field strengths used.

All n.m.r.-spectral experiments were conducted at Los Alamos National Laboratory, Los Alamos, NM.

RESULTS

Chemical shifts (^{13}C and ^{1}H) of the acetyl groups of the following acetylated mono-, di-, and tri-saccharides were determined: methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**1a**), methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**1b**), 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**2a**), 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**2b**), methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**3a**), 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose (**4a**), 1,2,3,4,6-penta-*O*-acetyl-β-D-mannopyranose (**4b**), 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (β-cellobiose octaacetate) (**5**), 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranose (β-maltose octaacetate) (**6**), 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (β-gentiobiose octaacetate) (**7**), 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranose (α-isomaltose octaacetate) (**8a**), and 1,2,3,4-tetra-*O*-acetyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-α-D-glucoopyranosyl)-α-D-glucopyranosyl]-α-D-glucopyranose (α-isomaltotriose undecaacetate) (**9a**). Chemical shifts of the β-forms (**8a** and





1a $R^1 = R^4 = H, R^2 = OMe, R^3 = OAc$
1b $R^1 = OMe, R^2 = R^4 = H, R^3 = OAc$
2a $R^1 = R^4 = H, R^2 = R^3 = OAc$
2b $R^1 = R^3 = OAc, R^2 = R^4 = H$
3a $R^1 = R^3 = H, R^2 = OMe, R^4 = OAc$
4a $R^1 = R^3 = H, R^2 = R^4 = OAc$
4b $R^1 = R^4 = OAc, R^2 = R^3 = H$

5 $R^1 = R^4 = OAc, R^2 = H, R^3 = \beta\text{-Glc}$
6 $R^1 = R^4 = OAc, R^2 = H, R^4 = \alpha\text{-Glc}$
7 $R^1 = R^3 = OAc, R^2 = H, R^4 = \beta\text{-Glc}$
8a $R^1 = H, R^2 = R^3 = OAc, R^4 = \alpha\text{-Glc}$
8b $R^1 = R^3 = OAc, R^2 = H, R^4 = \alpha\text{-Glc}$
9a $R^1 = H, R^2 = R^3 = OAc, R^4 = \alpha\text{-Glc-(1}\rightarrow\text{6)-}\alpha\text{-Glc}$
9b $R^1 = R^3 = OAc, R^2 = H, R^4 = \alpha\text{-Glc-(1}\rightarrow\text{6)-}\alpha\text{-Glc}$

9b) of **8a** and **9a**, available as byproducts of the peracetylation reaction, are reported. For the purpose of assigning ^{13}C chemical-shifts, compounds **1–4** enriched in ^{13}C at C-1 or C-2 were also studied. These compounds will be referred to by a numerical prefix designating the specific, ^{13}C-enriched carbon atom. For example, **3a** enriched with ^{13}C at C-1 will be designated **1–3a**. For di- and tri-saccharide derivatives, ring A is defined as the pyranosyl residue at the (now protected) reducing terminus, and ring B and rings B and C are those residues at the non-reducing end of the di- and tri-saccharides, respectively. Carbonyl carbon atoms are designated by the pyranosyl carbon atom at which they are substituted, being differentiated from the latter group by having the suffix "Ac" (*e.g.*, C-1Ac). Chem-

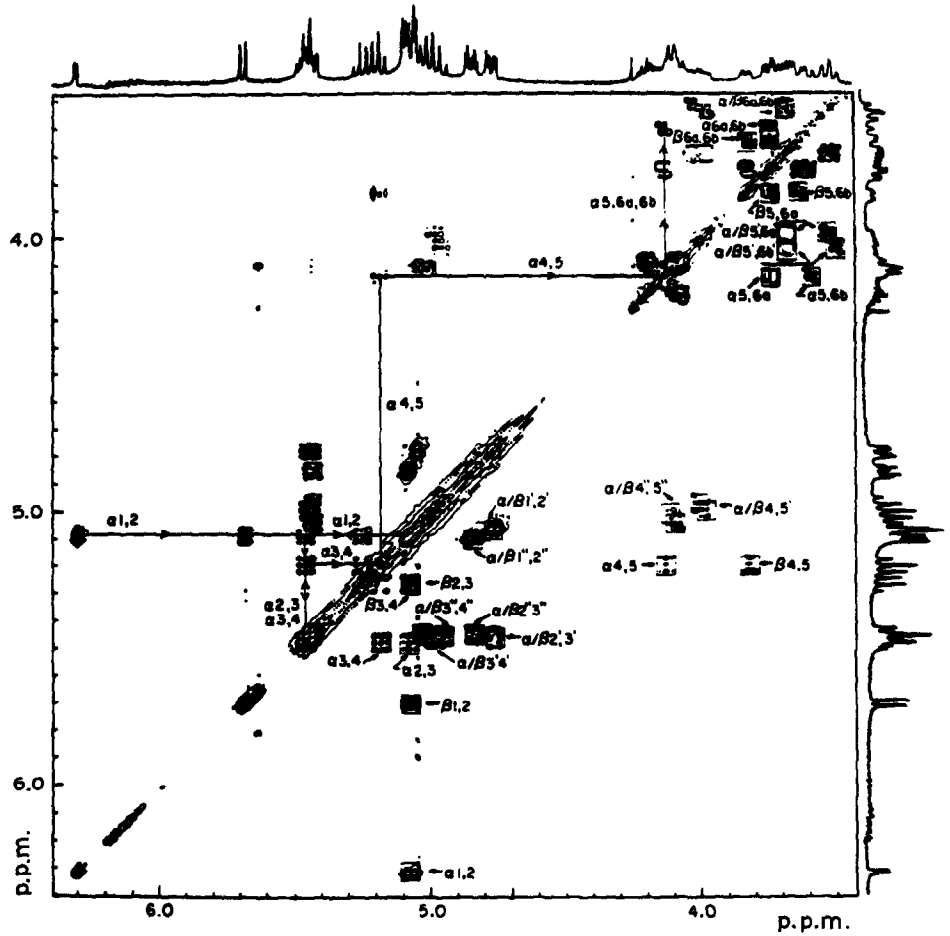


Fig. 1. COSY spectrum (contour plot) of peracetylated isomaltotriose acquired at 9.4 T using a sweep-width in both dimensions of ±900 Hz. [The plot was constructed from 512 real data points in both dimensions. A sine-bell weighting fraction was used prior to transformation in both dimensions. The normal F.t. ^1H-n.m.r. spectrum of the mixture of **9a** and **9b** is shown along each axis of the plot. Cross-peaks shown in boxes and connected with lines are coupling networks of pyranose ring A protons of **9a**.]

ical-shift data for the peracetylated furanosyl forms of all compounds studied are not reported.

Proton resonance assignments of peracetylated derivatives of D-*glucose and* D-*glucose-containing di- and tri-saccharides.* — ^{1}H-N.m.r. signals of compounds **1a–9** were assigned to specific pyranosyl-ring protons using chemical shift correlation

TABLE I

CHEMICAL SHIFTS OF RING PROTONS OF PERACETYLATED SACCHARIDES[a]

Assignment	*Compound*				
	1 *Me α-glucoside*	**2** *Me β-glucoside*	**3** *α-Glucose*	**4** *β-Glucose*	
H-1	4.94(4.96)	4.45(4.50)	6.32(6.31)	5.75(5.70)	
H-2	4.88(4.89)	4.98(5.01)	5.09(5.08)	5.13(5.13)	
H-3	5.47(5.49)	5.22(5.25)	5.47(5.49)	5.28(5.22)	
H-4	5.06(5.07)	5.09(5.04)	5.14(5.13)	5.13(5.09)	
H-5	3.94(3.92)	3.79(3.79)	4.13(4.12)	3.90(3.86)	
H-6a	4.28(4.29)	4.29(4.33)	4.28(4.28)	4.31(4.27)	
H-6b	4.12(4.09)	4.14(4.17)	4.10(4.12)	4.12(4.07)	
	5 *β-Cellobiose*	**6** *β-Maltose*	**7** *β-Gentiobiose*	**8a,b** *α,β*-Isomaltose	**9a,b** *α,β-Isomaltotriose*
H-1	5.68(5.68)	5.75(5.75)	5.69(5.70)	6.30,5.70(5.68)	6.33,5.71
H-2	5.03[b](5.23)	4.97(4.97)	5.07(5.07)	5.03,5.06(5.07)	5.09,5.08
H-3	5.23[b](5.03)	5.30(5.31)	5.23(5.24)	5.46,5.29(5.26)	5.47,5.26
H-4	3.84(3.84)	4.02(4.04)	4.99(4.99)	5.14,5.11(5.13)	5.19,5.19
H-5	3.78(3.78)	3.87(3.87)	3.81(3.81)	4.10,3.83(3.82)	4.14,3.84
H-6a	4.49(4.48)	4.45(4.49)	3.93(3.95)	3.74,3.74(3.75)	3.59,3.75
H-6b	4.13(4.13)	4.23(4.23)	3.58(3.59)	3.61,3.67(3.62)	3.74,3.63
H-1′	4.54(4.53)	5.40(4.49)	4.55(4.56)	5.10,5.10(5.11)	5.05,5.05
H-2′	4.92(4.91)	4.86(4.86)	4.96(4.95)	4.87,4.87(4.85)	4.76,4.76
H-3′	5.15(5.14)	5.36(5.38)	5.19(5.19)	5.44,5.44(5.46)	5.46,5.44
H-4′	5.06(5.05)	5.05(5.05)	5.05(5.05)	5.03,5.03(5.05)	5.03,5.01
H-5′	3.70(3.69)	3.95(3.97)	3.69(3.69)	4.03,4.03(4.03)	4.02,3.99
H-6′a	4.37(4.37)	4.24(4.23)	4.26(4.27)	4.24,4.24(4.23)	3.68,3.68
H-6′b	4.05(4.06)	4.04(4.08)	4.12(4.13)	4.05,4.05(4.03)	3.52,3.52
H-1″					5.09,5.09
H-2″					4.85,4.85
H-3″					5.44,5.44
H-4″					4.97,4.98
H-5″					4.11,4.09
H-6″a					4.12,4.12
H-6″b					4.20,4.20

[a]All chemical shifts were measured digitally from 1% Me_4Si in deuteriochloroform. Assignments for monosaccharide derivatives were determined from COSY spectra acquired at 4.7 T, using a sweep-width of 900 Hz. Assignments for di- and tri-saccharides were determined from COSY spectra acquired at 9.4 T, using a sweep-width of 1800 Hz and ^{13}C–^{1}H shift-correlation spectra, as described in the caption to Tables II and III. Numbers in parentheses are chemical shifts for the assigned protons, taken from ref. 13. [b]Assignments of H-2 and H-3 in compound **5** are the reverse of those previously made in ref. 13.

(COSY) spectra[29]. An example is given in Fig. 1, which shows a COSY spectrum (contour plot) acquired for a mixture of α- and β-isomaltotriose undecaacetate (**9a,b**). A complete assignment of protons is possible from the COSY spectrum if some information is available on the chemical shifts of corresponding protons in structurally related compounds. For example, comparison of proton resonances in the spectrum of the **9a,b** mixture with previously assigned[13] resonances of **2a**, **2b**, and **8b** (see Table I) shows that the doublet lying farthest downfield (6.33 p.p.m.) can be assigned to H-1 of **9a**. There is only a single off-diagonal contour for the H-1 resonance in the COSY spectrum at (δ 6.33, δ 5.11). Because H-1 has only one neighboring proton, H-2 of **9a** can be assigned a chemical shift of 5.11 p.p.m. The resonance at 5.11 p.p.m. on the diagonal has three apparent off-diagonal contours, at 5.27, 5.47, and 5.73 p.p.m. From the assignment data for compounds **2b** and **8b** (see Table I), H-1 and H-3 of **9b** are expected to have chemical shifts of ~5.7 and 5.3 p.p.m. The remaining off-diagonal contour, at 5.47 p.p.m., must arise from the coupling of H-2 and H-3 of **9a**. The only off-diagonal contour corresponding to a diagonal chemical shift of 5.47 p.p.m. is at (δ 5.47, δ 5.20), so that H-4 of **9a** may be assigned a chemical shift of 5.20 p.p.m. The 6-(hydroxymethyl) and H-5 protons of **9a** may be assigned in a similar manner by tracing out the coupling network.

Most of the protons on ring C are expected to have chemical shifts similar to those of the corresponding protons on ring B of **8a** and **8b**. This expected correlation aids in assigning off-diagonal contours of Fig. 1 to ring-C protons of **9a** and **9b**. Finally, because the anomeric configuration of ring A of peracetylated isomaltotriose has a small effect[4] on the chemical shifts of protons contained on rings B and C, most of the overlapping off-diagonal contours in the COSY spectrum that arise from these protons could not be interpreted. However, it is often possible to assign these near-neighbor ^{1}H-n.m.r. resonances on the basis of the chemical shift and intensities of resonances in the ^{13}C-n.m.r. spectrum to which they are coupled (see later).

Carbonyl carbon resonance assignments of peracetylated glucoses and mannoses. — Fig. 2 shows proton-decoupled, ^{13}C-n.m.r. spectra of compounds **1a**–**2b** between 168 and 171 p.p.m. The resolution of the carbonyl resonances observed in this region is somewhat unexpected, as these carbon atoms are rather isolated from the pyranosid ring-structure, and because the ring-carbon atoms (C-1–C-5) all have chemical shifts within ~5 p.p.m. of one another[13]. With the exception of the most downfield resonance and those lying between 169.4 and 169.6 p.p.m., these resonances appear to be sensitive to the anomeric form and the type of substituent attached at C-1.

Fig. 3 shows a ^{13}C–^{1}H shift-correlation map of methyl α-D-glucopyranoside tetraacetate (**1a**). It may be seen that each of the four carbonyl resonances has two sets of crosspeaks in the ^{1}H shift dimension, one lying between 4.2 and 5.5 p.p.m. and the other between 2.0 and 2.1 p.p.m. These two sets of crosspeaks arise from the coupling of the carbonyl carbon atom with its nearest ring proton ($^3J_{COCH}$ ~3

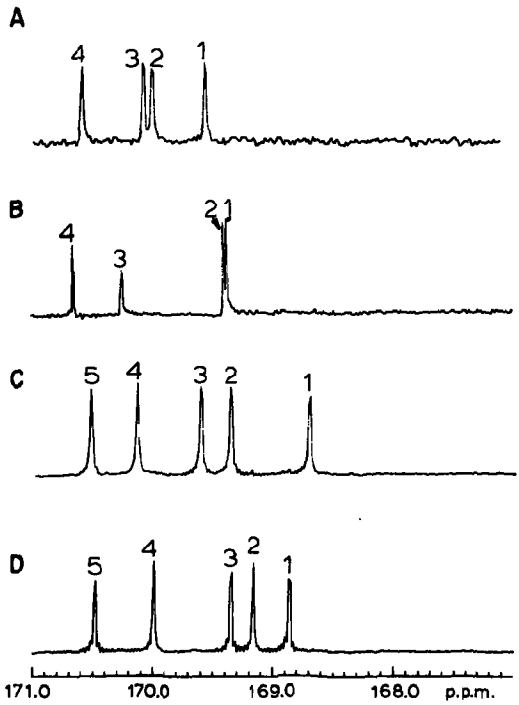


Fig. 2. The proton-decoupled, ^{13}C-n.m.r. spectra of compounds **1a–2b** (a–d).

Hz) and from its coupling to the acetyl methyl protons ($^{2}J_{CCH}$ ~7 Hz)*. Carbonyl carbon resonances of compounds **1a** and **2a** can be assigned to a particular substituent by comparing the chemical shift of the crosspeak in the ^{1}H shift dimension with the chemical shift previously assigned to ring-proton resonances (see Table I). These ^{13}C chemical shifts and assignments are summarized in Table II.

Column 8 of Table II shows that resonances 2 and 3 in the ^{13}C-n.m.r. spectrum of β-D-glucose pentaacetate (**2b**) are coupled to nearby ring-protons H-2 and H-4, both of which have identical chemical shifts (5.14 p.p.m.). In order to assign the carbon resonances on a one-to-one basis, we prepared β-D-[2-^{13}C]glucose pentaacetate (**2–2b**). In the ^{13}C-n.m.r. spectrum of this compound (not shown), peak 3 is split by spin–spin coupling to the ^{13}C nucleus at C-2 ($^{3}J_{CC}$ 2.5 Hz), while peak 2 is not broadened. Using these data and those obtained previously from ^{13}C–^{1}H shift-correlation spectroscopy, peaks 2 and 3 were assigned to C-2Ac and C-4Ac, respectively.

Fig. 4a shows the ^{13}C-n.m.r. spectrum of peracetylated methyl α-D-manno-

*The spectrum was acquired under conditions in which cross-peaks arising from coupling between a carbonyl carbon atom and a pyranose-ring proton appear more intense than those arising from coupling between C-6Ac and the two hydroxymethyl protons (see Experimental section).

TABLE II

ASSIGNMENTS AND CHEMICAL SHIFTS OF CARBONYL CARBON ATOMS AND NEAREST PYRANOSYL-RING PROTONS OF PERACETYLATED GLUCOSE AND MANNOSE DERIVATES[a]

Assign-ment	*Compound*										
	1a		**1b**		**2a**		**2b**		**3a**	**4a**	**4b**
	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$
C-1Ac					168.73(1)	6.32	168.86(1)	5.75		168.05(1)	168.34(2)
C-2Ac	170.13(3)	4.89	169.37(1)	4.95	169.61(3)	5.10	169.15(2)	5.14	169.96(3)	169.96(5)	170.16(8)
C-3Ac	170.06(2)	5.47	170.25(3)	5.23	170.15(4)	5.47	169.98(4)	5.29	169.83(2)	169.94(7)	169.74(6)
C-4Ac	169.61(1)	5.07	169.40(2)	5.05	169.36(2)	5.13	169.33(3)	5.14	169.74(1)	169.52(3)	169.58(4)
C-6Ac	170.6584)	4.32,—	170.65(4)	4.25,—	170.52(5)	4.29,—	170.47(5)	4.27,—	170.56(4)	170.56(9)	170.58(10)

[a]Data for **1a–2b** were taken from ^{13}C–^{1}H shift-correlation spectra acquired at 4.7 T, using sweep-widths in the ^{13}C shift dimension of 100 and 150 Hz for the peracetylated methyl glucosides and glucoses, respectively. The corresponding sweep-widths in the ^{1}H shift dimension were 800 and 900 Hz. The total data matrix following transformation consisted of 512 × 512 real data points. Assignments for **3a–4b** were made from ^{13}C-n.m.r. spectra of **1–3a, 2–3a**, and **1–4a,b**. Numbers in parentheses refer to peak numbers in Fig. 2.

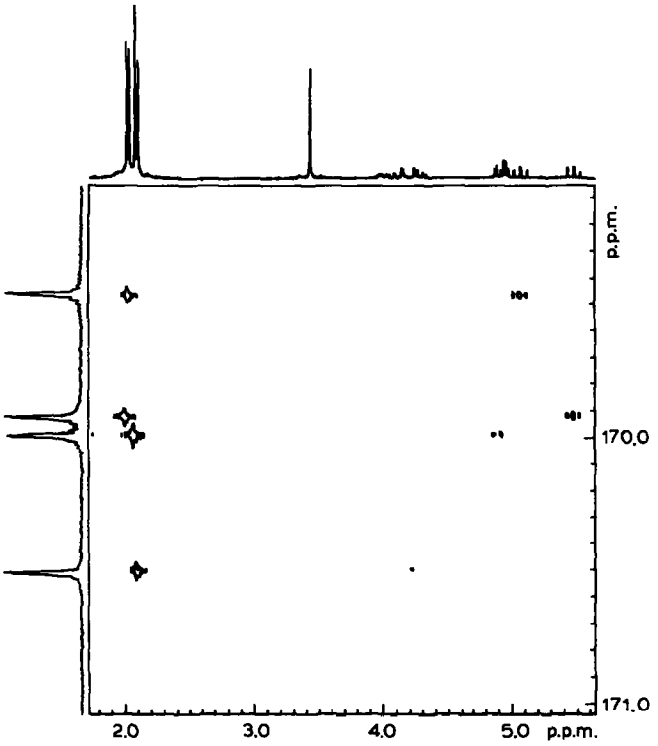


Fig. 3. ^{13}C–^{1}H shift-correlation spectrum of **1a** acquired at 47.01 T. [Sweepwidths of ±100 Hz and ±450 Hz were used in the ^{13}C and ^{1}H shift dimensions, respectively. The data were transformed by using 512 real data points in both dimensions. The proton-decoupled ^{13}C-n.m.r. and ^{1}H-n.m.r. spectra of the compound are displayed along the two axes.

pyranoside (**3a**). The resolution of carbonyl resonances is similar to that observed in the ^{13}C-n.m.r. spectrum of peracetylated methyl α-D-glucopyranoside (**1a**). A ^{13}C–^{1}H shift-correlation spectrum reveals that resonance 4 is correlated to a resonance in the ^{1}H shift dimension assigned previously to one of the 6-(hydroxymethyl) protons[30,31], and, on this basis, is assigned to C-6Ac. Resonances 1–3 are correlated to a set of strongly coupled protons between 5.15 and 5.40 p.p.m., and cannot be assigned solely on the basis of shift correlation data. These remaining resonances can be assigned by observing spin–spin coupling patterns in ^{13}C-n.m.r. spectra of methyl α-D-[1-^{13}C]- or -[2-^{13}C]mannopyranoside (**1–3a** or **2–3a**). For example, resonance 3 is clearly split into a doublet in the spectrum of **1–3a** (see Fig. 4b) as a result of coupling with the ^{13}C-enriched anomeric carbon atom, and, on this basis, may be assigned to C-2Ac. Consistent with this assignment is the observable splitting of this same resonance in the spectrum of **2–3a** (see Fig. 4c). Resonance 2 is also split into a doublet in the spectrum of **2–3a**, and is assigned to C-3Ac, since, with the exception of C-2Ac, it is the only remaining carbonyl carbon less

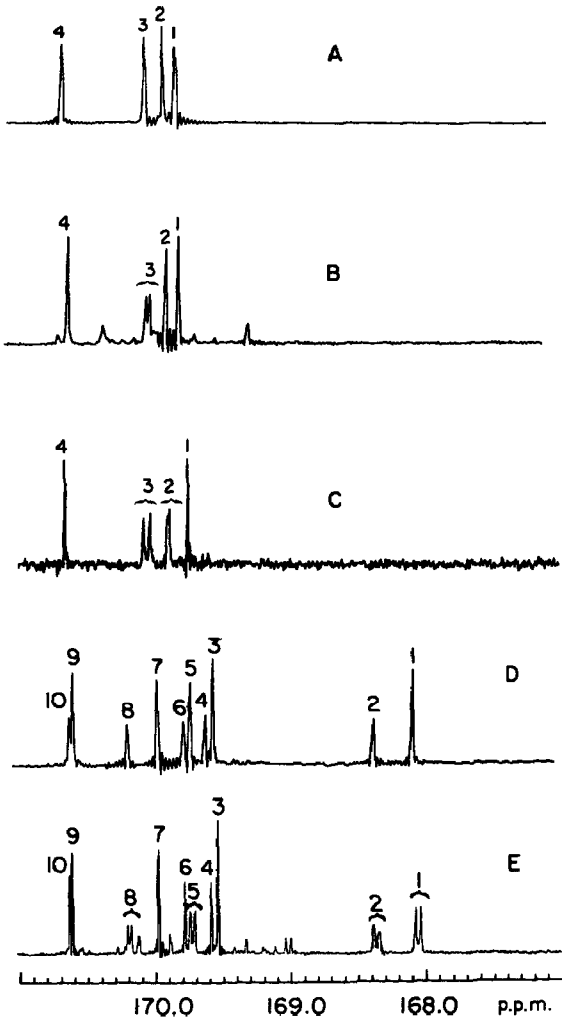


Fig. 4. Proton-decoupled, ^{13}C-n.m.r. spectra of (a) **3a**, (b) **1–3a**, and (c) **2–3a**. (Resonances at ~169.1 and 170.15 p.p.m. arise from unidentified impurities in the sample.) (d) A mixture of **4a** and **4b**. (e) A mixture of **1–4a** and **1–4b**.

than three chemical bonds away from the ^{13}C-enriched C-2 of the pyranosyl ring. By elimination, resonance 1 in the spectrum of **3a** can be assigned to C-4Ac.

Fig. 4d shows the ^{13}C-n.m.r. spectrum of a mixture of peracetylated D-mannopyranose anomers (**4a** and **4b**). The high-resolution, ^{1}H-n.m.r. spectrum of this mixture shows two downfield sets of resonances, at 6.05 and 5.90 p.p.m., having a relative intensity ratio of ~2:1. These two sets of resonances had previously been assigned to the anomeric protons in compounds **4a** and **4b**, respectively[31]. Hence, the more intense carbonyl carbon resonances in spectrum 4d (peaks 1, 3, 5, 7, and 9) must arise from carbonyl substituents of the α anomer of peracetyl-

ated D-mannopyranose, while the less intense resonances (peaks 2, 4, 6, 8, and 10) can be assigned to carbonyl carbons of the β anomer. In the spectrum of a similar anomeric mixture of compounds ^{13}C-enriched at C-1 (see Fig. 4e), resonances 1, 2, 5, and 8 are split into doublets as a result of ^{13}C–^{13}C coupling. Carbonyl resonances lying farthest upfield were assigned to C-1Ac in spectra of peracetylated D-glucopyranoses (**2a** and **2b**). On this basis, and on the basis of the splitting patterns observed in spectrum 4e, we assign resonances 1 and 2 to C-1Ac of **4a** and **4b**, respectively. On the basis of their relative intensities, resonances 5 and 8 can be assigned to C-2Ac of compounds **4a** and **4b**. The remaining unassigned resonances in the ^{13}C-n.m.r. spectrum of the mixture of **4a** and **4b** can be assigned on the basis of their chemical shifts. Resonances 9 and 10 have chemical shifts nearly identical to shifts of resonances arising from C-6Ac of compounds **1–3**, and, on this basis, can be assigned to C-6Ac of **4a** and **4b**. Resonances 3 and 4 have chemical shifts within ±0.2 p.p.m. of the C-4Ac resonances of compounds **1–3**, and, on this basis, are assigned to C-4Ac of **4a** and **4b**. By elimination, resonances 6 and 7 in spectrum 4d are assigned to C-3Ac of compounds **4a** and **4b**.

Carbon resonance assignments for peracetylated di- and tri-saccharides containing D-*glucose.* — ^{13}C-N.m.r. spectra between 168 and 171 p.p.m. of peracetylated di- and tri-saccharides **5–9** are shown in Fig. 5. The resolution of carbonyl carbon resonances in these spectra is remarkable. For examples, the ^{13}C-n.m.r. spectra of a mixture of both anomers of peracetylated isomaltotriose should contain signals for 22 carbonyl carbons. A total of 20 resonances are observed (see Fig. 5e). This resolution arises, in part, from the narrow line-widths expected for non-protonated carbons*. Chemical-shift data for these carbonyl carbon resonances and for the nearest-neighbor ring-protons to which they are coupled are summarized in Table III. For compounds **5–7**, the ring-proton chemical shifts taken from the ^{13}C–^{1}H shift-correlation data of Table III agree, within ±0.01 p.p.m., with those shifts listed in Table I. Carbonyl carbon resonances of these compounds are assigned solely on the basis of this agreement.

The ^{13}C-n.m.r. spectrum of isomaltose octaacetate, shown in Fig. 5d, contains 15 of the 16 resonances expected from a mixture of α and β anomers. Unfortunately, not all of these can be assigned from the data in Table III because some of the protons to which they are coupled have nearly identical chemical shifts. On the basis of their weaker intensities, peaks 1, 5, 10, 12, 13, and 15 most probably arise from the less favored, α anomer (**8a**). Two more resonances arising from this compound must comprise a portion of the intensity contained in peaks 6, 7, and 8. Solely on the basis of the chemical shifts of ring protons to which they are coupled, resonances 1, 13, and 15 may be assigned to C-1Ac, C-2′Ac, and C-6′Ac of **8a**,

*Preliminary spin–lattice relaxation-time (T_1) measurements of these resonances yield values between 13 and 16 s. Assuming rotational motion to be in the extreme narrowing limit ($T_1 = T_2$), we calculate a line-width at half-height of less than 0.025 Hz ($W_{1/2} = 1/DT_2$). In practice, line-widths of less than 0.03–0.05 Hz were measured for these resonances.

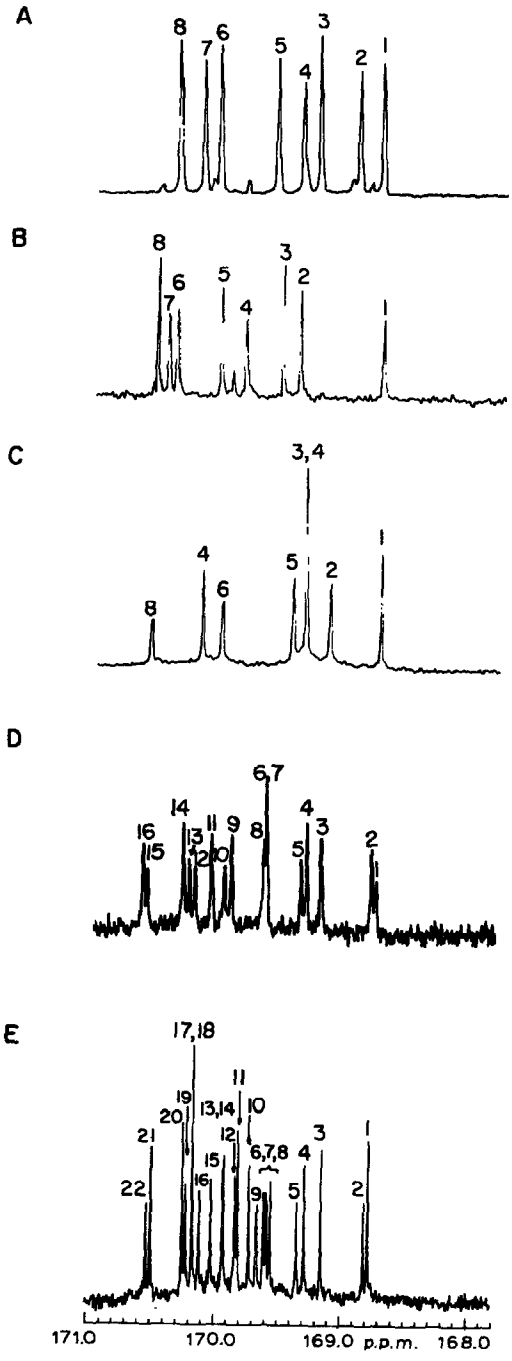


Fig. 5. The proton-decoupled, ^{13}C-n.m.r. spectra of compounds **5–9** (a–e) acquired at 9.4 T.

TABLE III

ASSIGNMENTS AND CHEMICAL SHIFTS OF CARBONYL CARBON ATOMS AND NEAREST PYRANOSYL-RING PROTONS, TAKEN FROM ^{13}C–^{1}H SHIFT-CORRELATION SPECTRA OF PERACETYLATED DI- AND TRI-SACCHARIDES[a]

Assignment	*Compound*					
	5		**6**		**7**	
	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
C-1Ac	168.78(1)	5.68	168.74(1)	5.75	168.79(1)	5.70
C-2Ac	169.40(4)	5.04	169.54(3)	4.96	169.19(2)	5.08
C-3Ac	169.61(5)	5.23	170.02(5)	5.30	170.04(6)	5.24
C-4Ac					169.38(3)	5.00
C-6Ac	170.19(7)	4.48, 4.13	170.37(6)	4.25,—		
C-2'Ac	168.96(2)	4.91	170.52(8)	4.86	169.48(5)	4.97
C-3'Ac	170.06(6)	5.14	169.82(4)	5.36	170.19(7)	5.20
C-4'Ac	169.27(3)	5.06	169.40(2)	5.05	169.38(4)	5.06
C-6'Ac	170.36(8)	4.38, 4.06	170.43(7)	—	170.59(8)	4.25,—

	8a		**8b**		**9a**		**9b**	
	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
C-1Ac	168.79(1)	6.30	168.82(2)	5.69	168.96(2)	6.33	168.78(1)	5.73
C-2Ac	169.65(6)[b]	5.05	169.22(3)	5.07	169.55(6)	5.11	169.16(3)	5.09
C-3Ac	170.21(12)	5.47	170.08(11)	5.26	170.17(18)	5.47	170.02(15)	5.27
C-4Ac	169.38(5)	5.13	169.33(4)	5.13	169.34(5)	5.20	169.28(4)	5.20
C-6Ac								
C-2'Ac	170.26(13)	4.85	170.31(14)	4.85	170.11(16)[b]	4.87	170.17(17)[b]	4.80
C-3'Ac	169.98(10)	5.45	169.92(9)	5.46	169.81(12)	5.47	169.81(11)	5.45
C-4'Ac	169.68(8)[b]	5.05	169.65(7)[b]	5.05	169.66(9)	5.04	169.72(10)	5.02
C-6'Ac	170.58(15)	4.27, 4.07	170.61(16)	4.23 4.07				
C-2''Ac					170.23(19)	4.85	170.25 (20)	4.80
C-3''Ac					169.92(13)[b]	5.45	169.92(14)[b]	5.46
C-4''Ac					169.60(8)	4.97	169.58(7)	4.99
C-6''Ac					170.53(22)	4.21,—	170.50(21)	—

[a]Data for peracetylated β-maltose were acquired at 4.7 T, using spectral conditions described in the caption to Table II. Data for other peracetylated disaccharides were acquired at 9.4 T, using sweep-widths of 320 hz and 1800 Hz in the ^{13}C and ^{1}H shift dimensions. The transformed matrix contained 512 × 512 real data points, leading to a spectral resolution of ~0.01 p.p.m. in both frequency dimensions. All shifts are reported with respect to 1% Me_4Si in deuteriochloroform. Numbers in parentheses refer to peak numbers in Fig. 5. [b]Chemical shifts for peaks 6, 7, and 8 of compounds **8a** and **8b**, and peaks 16 and 17, and 13 and 14, of compounds **9a** and **9b** have not been assigned to carbonyl carbon atoms on a one-to-one basis.

while resonances 2, 11, 14, and 16 may be assigned to C-1Ac, C-3Ac, C-2'Ac, and C-6'Ac of **8b**. On the basis of their intensities and the shifts of protons to which they are coupled, resonances 4 and 5 may be assigned to C-4Ac of **8b** and **8a**, respectively. Resonances 6, 7, and 8 are all coupled to protons having shifts of 5.05

p.p.m. and, from the data of Table I, may be assigned to C-2Ac of **8a** and C-4′Ac of **8a** and **8b**, but not on a one-to-one basis. Resonances 9, 10, and 12 are all coupled to protons having chemical shifts lying between 5.45 and 5.47 p.p.m.; H-3 of **8a** and H-3′ of **8a** and **8b** have chemical shifts within ±0.01 p.p.m. of this value (see Table I). Because of the small effect which we expect anomerization at C-1 to have on the shifts of carbon atoms in the B ring, we assign those resonances closest in chemical shift, peaks 9 and 10, to C-3′Ac of **8b** and **8a**, while peak 12 is assigned to C-3Ac of **8a**. Resonance 3, by elimination, is assigned to C-2Ac of **8b**.

Next, we considered the ^{13}C-n.m.r. spectrum of peracetylated isomaltotriose (see Fig. 5e). As in the case of isomaltose, the peracetylation created a mixture of two anomers. An inspection of resonance intensities and chemical shifts in the ^{1}H-n.m.r. spectrum of the purified products indicated that the β anomer is favored, by a ratio of ~3:2. However, it is clear that assigning resonances in the spectrum to either of the anomers on the basis of their intensities may not be nearly as straightforward as it was in the case of peracetylated isomaltose, owing to differences in n.O.e. values and relaxation times of corresponding ^{13}C nuclei in either of the two anomers. From the ^{13}C–^{1}H shift-correlated spectrum, however, peaks (13, 14) and (17, 18) are recognizable as two-carbon resonances, as these carbon resonances show two cross-peaks in the ^{1}H shift dimension.

Because of the small effect which the intervening pyranosyl B ring is expected to have on ^{13}C chemical-shifts in rings A and C, it would be expected that many of the resonances appearing in Fig. 5e could be assigned by comparing their chemical shifts with those of the previously assigned carbonyl resonances of peracetylated isomaltose. For example, the group of three resonances between about 169.20 and 169.40 p.p.m. in spectrum 5d is easily recognized as being present in spectrum 5e. Based on the assignments previously made for these resonances in the spectrum of the **8a,b** mixture, we assign peaks 3, 4, and 5 in spectrum 5e to C-2Ac and C-4Ac of **9b** and C-4Ac of **9a**. Likewise, the chemical shifts and intensities of peaks 13, 14, 15, 19, and 20 in the spectrum of **9a,b** agree quite closely with the shifts and intensities of peaks 9, 10, 11, 13, and 14 in the spectrum of **8a,b**. On the basis of this agreement, we assign peaks 13 and 14 to C-3″Ac of **9a** and **9b**, peak 15 to C-3Ac of **9b**, and peaks 19 and 20 to C-2″Ac of **9a** and **9b**, respectively. The chemical shift of the two-carbon resonance (17, 18) in spectrum 5e is quite near to that of peak 12 in spectrum 5d (170.17 p.p.m., compared to 170.21 p.p.m.), a resonance which was previously assigned to C-3Ac of compound **8a**. Inspection of the ^{13}C–^{1}H shift-correlation spectrum reveals that one component of this two-carbon resonance is coupled to a proton with a shift near to that of H-3 of **9a**, and, therefore, one-half of resonance (17, 18) may be assigned to C-3Ac of **9a**. On the basis of their intensities, and the shifts of protons to which they are coupled, peaks 11 and 12 are assigned to C-3′Ac of **9b** and **9a**, while peaks 21 and 22 may be similarly assigned to C-6″Ac of **9b** and **9a**. Carbon resonances 1, 2, and 6–10 have been assigned solely on the basis of the shifts of protons to which they are coupled. Resonance 16 and one-half of the two-carbon resonance (17, 18) may be assigned to C-2′Ac of **9a** and **9b**, but not on a one-to-one basis.

Methyl carbon and proton assignments for peracetylated D-*glucose mono-, di-, and tri-saccharides.* — As illustrated by the ^{13}C–^{1}H shift-correlation spectrum of **1a** (see Fig. 2), acetyl methyl proton resonances may be correlated with assigned carbonyl carbon resonances. We were unable to make meaningful assignments to ^{1}H methyl resonances arising from compound **9**, due to the extensive overlap of methyl proton resonances and the limited resolution of our ^{13}C–^{1}H shift-correlation spectra in the ^{1}H shift dimension. Assignments for methyl proton resonances in compounds **1–8** could, however, be made from previously assigned carbonyl carbon resonances to which they are coupled. These are summarized in Table IV.

With the possible exception of acetyl methyl proton substituents at C-1 and C-2′, most of the methyl proton shifts are quite insensitive to the different configurations of glycosidic linkages which may be present in these compounds. For example, none of the assigned chemical shifts of methyl protons in peracetylated

TABLE IV

CHEMICAL SHIFTS OF ACETYL METHYL PROTONS DETERMINED FROM ^{13}C–^{1}H SHIFT-CORRELATION SPECTRA OF PERACETYLATED MONO- AND DI-SACCHARIDES[a]

Assignment	*Compound*				
	1a	**1b**	**2a**	**2b**	**5**
C-1Ac			2.17	2.12	2.09
C-2Ac			2.00	2.04	2.02
C-3Ac			2.02	2.02	2.03
C-4Ac			2.03	2.04	—
C-6Ac			2.08	2.08	2.12
C-2Ac	2.07	2.05			2.03
C-3Ac	2.01	2.00			1.98
C-4Ac	2.03	2.03			2.01
C-6Ac	2.09	2.09			2.09

	6	**7**[b]	**8a**	**8b**
C-1Ac	2.10	2.11	2.20	2.11
C-2Ac	2.01	2.03	2.04	2.04
C-3Ac	2.01	2.01	2.03	2.02
C-4Ac	—	2.04[c]	2.07	2.06
C-6Ac	2.13	—	—	—
C-2′Ac	2.05	2.07[c]	2.10	2.13
C-3′Ac	2.00	2.01	2.01	2.01
C-4′Ac	2.02	2.02[c]	2.04	2.02
C-6′Ac	2.09	2.09	2.08	2.09

[a]Assignments of the acetyl methyl protons were made by correlating them with assigned carbonyl carbon atoms using ^{13}C–^{1}H shift-correlation data. Spectra were acquired as described in footnotes to Tables II and III. [b]The assignments reported for **1a** are identical to those reported previously[35,36]. [c]Acetyl methyl protons attached to pyranose-ring positions 4, 2′, and 4′ could not be assigned on a one-to-one basis.

β-cellobiose [β-(1→4) linkage] differ by more than ±0.02 p.p.m. from the corresponding shifts of peracetylated β-maltose [α-(1→4) linkage].

Our initial attempts to assign ^{13}C resonances arising from the acetyl methyl carbons were even more disappointing. In general, these resonances lie in a narrow chemical-shift range between 20.5 and 20.9 p.p.m. Not even in the case of the peracetylated methyl D-glucopyranosides were all methyl resonances resolved. At the other extreme, no single methyl carbon resonances were resolved in the ^{13}C-n.m.r. spectrum of peracetylated isomaltotriose.

DISCUSSION

Although the number of compounds studied is certainly a limiting factor, a few trends appear evident from the carbonyl carbon chemical-shift assignments of peracetylated monosaccharides listed in Table II and from the chemical-shift differences in Table V. For example, it appears that, at least in the case of peracetylated D-mannoses and D-glucoses, an equatorially disposed C-1Ac (as in **2b** and **4b**) gives rise to a resonance lying downfield of a corresponding resonance arising from a carbonyl substituted axially (as in **2a** and **4a**). This is in contrast to the chemical shifts of anomeric ring-carbon resonances, where anomeric carbon atoms with equatorial substituents give rise to resonances lying farther upfield than resonances from corresponding carbon atoms with axial substituents[2]. It also appears that these same resonances are reasonably good indicators of the configuration of C-2, as illustrated by the marked difference between chemical shifts of resonances arising from C-1Ac of **2a** and **4a**. This may be of potentially great value in the determination of the type of reducing monosaccharide contained in a complex carbohydrate, should the shift of the C-1Ac resonance be unique and characteristic of other types of peracetylated monosaccharides.

The configuration of C-1, bearing the anomeric carbonyl substituent, also causes significant changes in the shifts of C-1Ac, C-2Ac, and C-3Ac resonances, and minor changes in the chemical shifts of C-4Ac and C-6Ac resonances (columns

TABLE V

CHEMICAL-SHIFT DIFFERENCES OF CARBONYL CARBON RESONANCES ARISING FROM STRUCTURAL DIFFERENCES BETWEEN PERACETYLATED D-GLUCOSE AND D-MANNOSE DERIVATIVES[a]

Assignment	*Calculated ^{13}C chemical-shift differences*				
	δ_c(2a)–δ_c(2b)	δ_c(4a)–δ_c(4b)	δ_c(4a)–δ_c(2a)	δ_c(4b)–δ_c(2b)	δ_c(3a)–δ_c(1a)
C-1Ac	−0.13	−0.29	−0.68	−0.52	
C-2Ac	0.46	−0.47	0.08	1.01	−0.17
C-3Ac	0.17	0.20	−0.19	−0.24	−0.19
C-4Ac	−0.03	−0.06	0.16	0.25	0.21
C-6Ac	0.05	−0.02	0.04	0.11	0.00

[a]Chemical-shift differences were calculated from chemical shifts of resonances listed in Table I.

2 and 3 of Table V). Changes from an axially to an equatorially disposed substituent at C-1 results in a downfield shift in the C-1Ac and C-4Ac resonances, and an upfield shift in the C-3Ac resonance for both peracetylated D-glucose and D-mannose derivatives. Similar structural changes result in a downfield shift in the C-2Ac resonance of peracetylated D-mannose derivatives, and an upfield shift in the corresponding carbon resonance of peracetylated D-glucose derivatives. Apparently, the sign and the magnitude of these shifts depend on the configuration of C-2 (bearing the acetyl substituent) as well as C-1.

When the acetyl substituent at C-2 is moved from an axial, as in **4a**, to an equatorial position, as in **2a**, resonances arising from C-1Ac and C-3Ac undergo a downfield shift, while the C-4Ac resonance is shifted upfield (see columns 4–6 of Table V). The signs of these shift changes are similar to those calculated from the shift data of compounds **2b** and **4b**, where the substituent at C-1 is equatorial, not axial. Previously, Lemieux and Stevens had noted that pyranosyl ring-proton resonances are shifted upfield when substituents on the next-nearest-neighbor carbon atom (two bonds removed) were changed from an equatorial to an axial disposition[30,32]. This rule also seems to hold for the nonprotonated acetyl C-1Ac and C-3Ac carbon resonances when C-2 (bearing an acetyl substituent) undergoes a change in configuration. The configuration of C-1 (bearing substituent) appears to influence the magnitude of the changes in shift observed for the C-2Ac resonance. When the substituent on C-1 is equatorial, as in **2b** and **4b**, changing the configuration of C-2 results in a 1.01-p.p.m. shift in the C-2Ac resonance. However, when the substituent on C-1 is axial, as in **1a**, **2a**, **3a**, and **4a**, the absolute magnitude of this same shift-difference is <0.2 p.p.m.

It is interesting that the C-1Ac and C-6Ac resonances of **9a** are downfield of the corresponding resonances of **9b**, while, in the spectra of **8a** and **8b**, the respective order is reversed. It is quite possible that this reversal may be the result of intramolecular, head-to-tail interactions not present in the corresponding, peracetylated disaccharide.

In order to gain further insight as to the sensitivity of carbonyl carbon-resonance chemical-shifts to differences in the glycosidic linkages between adjoining, peracetylated D-glucose monomers, the assigned shifts of compounds **1–4** were subtracted from the assigned shifts of corresponding carbon atoms of peracetylated D-glucose di- and tri-saccharides. For example, chemical shifts of the carbonyl carbons of the α or β anomers of peracetylated D-glucose were subtracted from shifts of the corresponding carbon substituents on ring A of the α or β form of compounds **5–9**. Likewise, chemical shifts assigned to the carbonyl carbons of peracetylated methyl α-D-glucopyranoside were subtracted from shifts of corresponding carbon atoms in rings B and C of peracetylated forms of maltose, isomaltose, and isomaltotriose, while shifts assigned to the carbonyl carbon atoms of peracetylated methyl β-D-glucopyranoside were subtracted from shifts of corresponding carbon atoms in ring B of peracetylated β-cellobiose and β-gentiobiose. These shift differences are listed in Table VI. It is evident that shift differences

TABLE VI

CHEMICAL-SHIFT DIFFERENCES BETWEEN CARBONYL CARBON RESONANCES AND CORRESPONDING CARBON RESONANCES OF PERACETYLATED D-GLUCOPYRANOSES AND METHYL D-GLUCOPYRANOSIDES[a]

Assignment	*Compound*		
	5 *(β-Cellobiose)*	**6** *(β-Maltose)*	**7** *(β-Gentiobiose)*
C-1Ac	−0.08 (−0.20)	−0.12 (−0.45)	−0.07 (−0.10)
C-2Ac	0.25 (1.85)	0.39 (0.60)	0.04 (−0.10)
C-3Ac	−0.37 (−2.30)	0.04 (2.60)	0.06 (0.10)
C-4Ac			0.05 (0.50)
C-6Ac	−0.28 (0.05)	−0.10 (1.00)	
C-2'Ac	−0.41 (0.15)	0.39 (−0.75)	0.11 (−0.35)
C-3'Ac	−0.19 (−0.15)	−0.24 (−0.85)	−0.06 (−0.20)
C-4'Ac	−0.13 (0.25)	−0.21 (−0.55)	−0.02 (−0.05)
C-6'Ac	−0.29 (−0.35)	−0.22 (−0.40)	−0.06 (−0.05)

	8a *(α-Isomaltose)*	**8b** *(β-Isomaltose)*	**9a** *(α-Isomaltotriose)*	**9b** *(β-Isomaltotriose)*
C-1Ac	0.06	−0.04 (−0.20)	0.23	−0.08
C-2Ac	0.04[b]	0.07 (−0.25)	−0.06	0.01
C-3Ac	−0.07	0.10 (0.10)	0.02	0.04
C-4Ac	0.02	0.00 (0.55)	−0.02	0.05
C-6Ac				
C-2'Ac	0.13	0.18 (−0.95)	−0.02[b]	0.04[b]
C-3'Ac	−0.08	−0.14 (−0.15)	−0.25	−0.25
C-4'Ac	0.07[b]	0.04[b] (−0.20)	0.05	0.11
C-6'Ac	−0.07	−0.04 (−0.20)		
C-2"Ac			0.10	0.12
C-3"Ac			−0.14	−0.14
C-4"Ac			−0.01	−0.03
C-6"Ac			−0.12	−0.15

[a]Chemical-shift differences listed for substituents of ring A were calculated by subtracting assigned shifts of compounds **2a** or **2b** from shifts of corresponding carbonyl carbon atoms. Those calculated for substituent positions in rings B or C (α- or β-linked) were calculated by subtracting assigned shifts of compound **1a** or **1b** from shifts of corresponding carbonyl carbon atoms. Numbers in parentheses were calculated in the same manner for pyranose-ring carbon atoms, using the assigned shifts given in ref. 13. [b]Chemical-shift differences for compounds **8a** and **8b** or compounds **9a** and **9b** have not been assigned to carbonyl carbon atoms on a one-to-one basis.

calculated for the carbonyl carbon atoms in ring A of compounds **5–9** depend upon the substitution site and configuration of ring B, with those carbon atoms nearest the glycosidic linkage having the most sensitive shifts. For example, when ring A is substituted *via* a β-(1→4)glycosidic linkage to ring B (as in **5**), a shift difference of −0.37 p.p.m. is calculated for C-3Ac, while corresponding values of 0.04 and 0.10 p.p.m. are calculated when ring A is substituted *via* an α-(1→4) (as in **6**) or α-(1→6) linkage (as in **8b**). In ring B of **5–8** and ring C of **9**, C-2'Ac and C-3'Ac (or C-2"Ac and C-3"Ac) have shifts most sensitive to the anomeric configuration and the aglycon carbon atom involved in the glycosidic linkage. Hence, the calculated shift-

differences for C-2'Ac range from −0.41 p.p.m. in **5**, where ring B is β-(1→4)glycosidically linked to C-4 of ring A, to 0.39 p.p.m. in **6**, where ring B is α-(1→4)-linked to C-4. Furthermore, from our limited data-set, it appears that the remaining carbonyl substituents on ring B have chemical shifts sensitive to substituents at other ring-positions. Shift differences calculated for carbonyl substituents on ring B of peracetylated isomaltotriose are different from corresponding shift-differences calculated for ring B of any of the other peracetylated disaccharide residues. The sum of the combined effects of glycosidic linkages at both C-1' and C-6' do not account for these calculated shift-differences.

Although they are usually of much larger magnitude, the chemical-shift differences calculated for the pyranose-ring carbon atoms of the peracetylated di- and tri-saccharides (appearing in parentheses in Table VI) appear, in some instances, to be related to calculated shift-differences of their corresponding carbonyl carbon substituents. For example, pyranosyl-ring atoms C-1, C-3, C-4, C-3', and C-6' of compounds **5**, **6**, **7**, and **8b** all have negative chemical-shift differences, as do carbonyl carbon substituents at these positions. On the other hand, pyranosyl-ring C-2' atoms of the same compounds all have shift-differences of opposite sign, compared to the shift differences of their carbonyl carbon substituents.

CONCLUSIONS

To summarize, we have found that the ^{13}C-n.m.r. spectra of peracetylated mono- and oligo-saccharides of D-glucose contain resonances arising from the carbonyl carbon atoms in the region from ~168.7 to 170.6 p.p.m., and have chemical shifts sensitive to structure, with those carbon atoms nearest to the aglycon substitution site having shifts most sensitive to pyranosyl-ring configuration and the ring carbon atoms involved in the glycosidic linkage. Effects of larger magnitude on the chemical shifts of pyranosyl-ring carbon atoms had been noted previously for yeast D-mannans[4] and for permethylated or peracetylated disaccharides composed of D-glucose and D-galactose[11–19]. We conclude that both ring-carbon resonances of oligosaccharides and carbonyl carbon resonances of their corresponding peracetylated derivatives may act as indicators of carbohydrate structure. In addition, both methods of structural identification have certain advantages over the similar use of proton resonances arising from pyranose-ring protons, the primary advantage being the lack of overlapping resonances, each showing complex coupling-patterns. Clearly, one of the primary disadvantages of using either of these two ^{13}C-n.m.r. methods is sensitivity. However, in the case of the peracetylation method, ^{13}C enrichment is possible. A possible disadvantage of the method is the relatively long relaxation times of nonprotonated carbon nuclei, ultimately limiting the signal-to-noise ratio in spectra acquired over a fixed time period[33]. In addition, broadening of carbonyl resonances at high magnetic fields may arise as a result of relaxation due to chemical-shift anisotropy[34] (although no noticeable broadening was observed in our spectra acquired at 9.4 T).

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REFERENCES

1 P. A. J. Gorin, *Can. J. Chem.*, 51 (1973) 2375–2383.
2 D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, 93 (1971) 4463–4472.
3 T. Ogawa and H. Yamamoto, *Carbohydr. Res.*, 104 (1981) 271–283.
4 A. Allerhand and E. Berman, *J. Am. Chem. Soc.*, 106 (1984) 2400–2412.
5 A. Allerhand and E. Berman, *J. Am. Chem. Soc.*, 106 (1984) 2412–2420.
6 E. Berman and A. Allerhand, *J. Biol. Chem.*, 256 (1981) 6657–6662.
7 K. Dill, E. Berman, and A. A. Pravia, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 1–49.
8 K. Dill and A. Allerhand, *J. Biol. Chem.*, 254 (1979) 4524–4531.
9 E. Berman, A. Allerhand, and A. L. DeVries, *J. Biol. Chem.*, 255 (1980) 4407–4410.
10 J. F. G. Vliegenthart, H. van Halbert, and L. Dorland, *Pure Appl. Chem.*, 53 (1977) 45–77.
11 M. Haverkamp, J. A. de Bie, and J. F. G. Vliegenthart, *Carbohydr. Res.*, 37 (1974) 111–125.
12 J. Haverkamp, J. P. C. M. van Dngen, and J. F. G. Vliegenthart, *Tetrahedron*, 29 (1973) 3431–3439.
13 D. Y. Gagnaire, F. R. Taravel, and M. R. Vignon, *Carbohydr. Res.*, 51 (1976) 157–168.
14 D. Bassieux, D. Y. Gagnaire, and M. D. Vignon, *Carbohydr. Res.*, 56 (1977) 19–33.
15 B. Capon, D. S. Rycroft, and J. W. Thompson, *Carbohydr. Res.*, 70 (1979) 145–149.
16 E. Lee, J. O'Callaghan, and J. P. O'Reilly, *Carbohydr. Res.*, 105 (1982) 266–268.
17 K. Izumi, *J. Biochem. (Tokyo)*, 76 (1974) 535–544.
18 D. Y. Gagnaire, D. Mancier, and M. Vincendon, *Org. Magn. Reson.*, 11 (1978) 344–349.
19 G. Excoffier, D. Y. Gagnaire, and F. R. Taravel, *Carbohydr. Res.*, 56 (1977) 229–238.
20 A. S. Serianni, H. A. Nunez, and R. Barker, *Carbohydr. Res.*, 72 (1979) 71–78.
21 M. L. Hayes, N. J. Pennings, A. S. Serianni, and R. Barker, *J. Am. Chem. Soc.*, 104 (1982) 6764–6769.
22 M. L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1963) 211–215.
23 F. H. Newth, S. D. Nicholas, F. Smith, and L. F. Wigging, *J. Chem. Soc.*, (1949) 2550.
24 A. J. Shaka, J. Keeler, T. Frenkiel, and R. Freeman, *J. Magn. Reson.* 52 (1983) 335–338.
25 A. Bax, *Two-Dimensional Nuclear Magnetic Resonance In Liquids*, Delft Union Press, Delft, The Netherlands, 1982, Chapt. 2.
26 A. Bax and R. Freeman, *J. Magn. Reson.*, 44 (1981) 542–561.
27 G. Bodenhausen and R. Freeman, *J. Magn. Reson.*, 36 (1979) 221.
28 G. Bodenhausen, R. Freeman, and D. L. Truner, *J. Magn. Reson.*, 27 (1977) 511–514.
29 M. A. Bernstein, L. D. Hall, and S. Subramanian, *Carbohydr. Res.*, 103 (1982) c2–c6.
30 R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 43 (1965) 2059–2070.
31 N. Frank, H. Friebolin, G. Kewrlich, J. P. Merle, and E. Siefert, *Org. Magn. Reson.*, 4 (1972) 725–732.
32 R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 44 (1966) 249–262.
33 A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, 55 (1971) 189–198.
34 G. C. Levy and U. Edmund, *J. Am. Chem. Soc.*, 97 (1975) 5031–5032.
35 D. Horton and J. H. Lauterbach, *Carbohydr. Res.*, 43 (1975) 9–33.
36 D. Horton and J. H. Lauterbach, *J. Org. Chem.*, 34 (1969) 86–92.