Tetrahedron. Vol. 29, pp. 3431 to 3439. Pergamon Press 1973. Printed in Great Britain

PMR AND CMR SPECTROSCOPY OF METHYL 2,3,4,6-TETRA-O-METHYL-α- AND -β-D-GLUCOPYRANOSIDE

AN APPLICATION TO THE IDENTIFICATION OF PARTIALLY METHYLATED GLUCOSES

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(*Received in the UK* 19 *March* 1973; *Accepted for publication* 10 *July* 1973)

Abstract—The proton and ^{13}C magnetic resonance spectra of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside are completely assigned. For this purpose specific deuterium and ^{13}C labelling, spin decoupling and spectrum simulation are employed. The NMR data are discussed and compared with those of the free monomers and the methylglucosides. A partially methylated glucose obtained as a degradation product in the permethylation analysis of a carbohydrate can be identified after permethylation of the compound with labelled Me groups and comparison of the PMR or CMR spectra with those of reference permethylglucoses. From these spectra information is obtained about type and ring form of the monomers and position(s) of the glycosidic bond(s) in the original oligo- or polysaccharide. The number of reference compounds necessary for identification is strongly reduced. The method can be extended to other monomers because there are significant differences between the NMR data of the various permethylmonosaccharides.

INTRODUCTION

Structural analysis of carbohydrates by permethylation, followed by cleavage of the glycosidic bonds and identification of the formed products is still a method of great importance in carbohydrate chemistry. However, the identification of the formed partially methylated monosaccharides frequently presents problems. Only little attention has been paid to the applicability of NMR spectroscopy in the identification of these compounds.[1-3] During the last 10 years a lot of PMR spectral data about carbohydrates have been published,[4,5] but recently also some CMR spectral data of several carbohydrates, carbohydrate derivatives and related compounds have been reported.[6-16] For the assignment of both PMR and CMR data the specific introduction of substituents or the transition of substituents from equatorial to axial positions are used besides the generally applied NMR techniques.

In this paper the complete assignment of the PMR and CMR spectra of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside is described, which is performed by specific deuterium or ^{13}C labelling and also by spin decoupling and spectrum simulation. It will be shown that these spectral data can be used for the determination of the positions of OMe groups in partially methylated glucoses and consequently for the determination of the positions of glycosidic bonds in oligosaccharides.

RESULTS

PMR spectra of methyl 2,3,4,6-*tetra*-O-*methyl*-α-*and* -β-D-*glucopyranosides.* By comparison of the chemical shifts of the OMe signals in the 100 MHz spectra of the series of methyl tetra-O-methyl-glucopyranosides, selectively labelled with trideuteromethoxyl groups as given in Table 1, these resonances could unequivocally be assigned (Fig 1). The δ-values in acetonitrile-d_3 differ slightly from those in chloroform–benzene (6:1) as reported by Gagnaire and Odier,[1] however, the sequence of the chemical shifts of the various OMe groups is identical.

The large OMe proton singlets coincide with the resonances of the glucose skeleton protons H_2 up to and including H_6H_6. For this reason the last resonances were assigned in the 220 MHz spectra of methyl-d_3 tetra-O-methyl-d_3-α- and -β-D-glucopyranosides (Figs 2, 3). Due to small $\Delta\delta/J$ values these spectra are complex. Approximate δ- and J values were obtained as follows. Irradiation of proton H_1 gave a good estimation for the chemical shift of H_2 and the coupling constant $J_{2,3}$. The chemical shift of H_5 and the coupling constant $J_{4,5}$ were determined by comparison of the 100 MHz spectra of methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-α- and -β-D-glucopyranoside with the 100 MHz spectra of the 6,6′-d_2 analogues. The values for the other protons were obtained by calculation from the 220 MHz spectra.

By spectrum simulation the experimental PMR data were refined. The theoretical spectra were calculated from the initial experimental parameters in an iterative interactive procedure with the spin

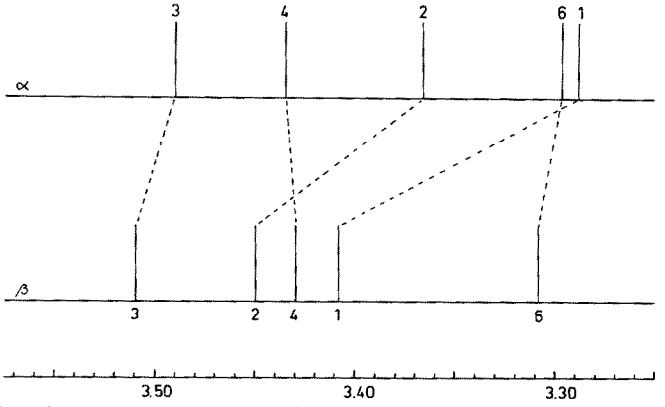

Fig 1. Correlation of the methoxyl resonances in the 100 MHz PMR spectra of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside.

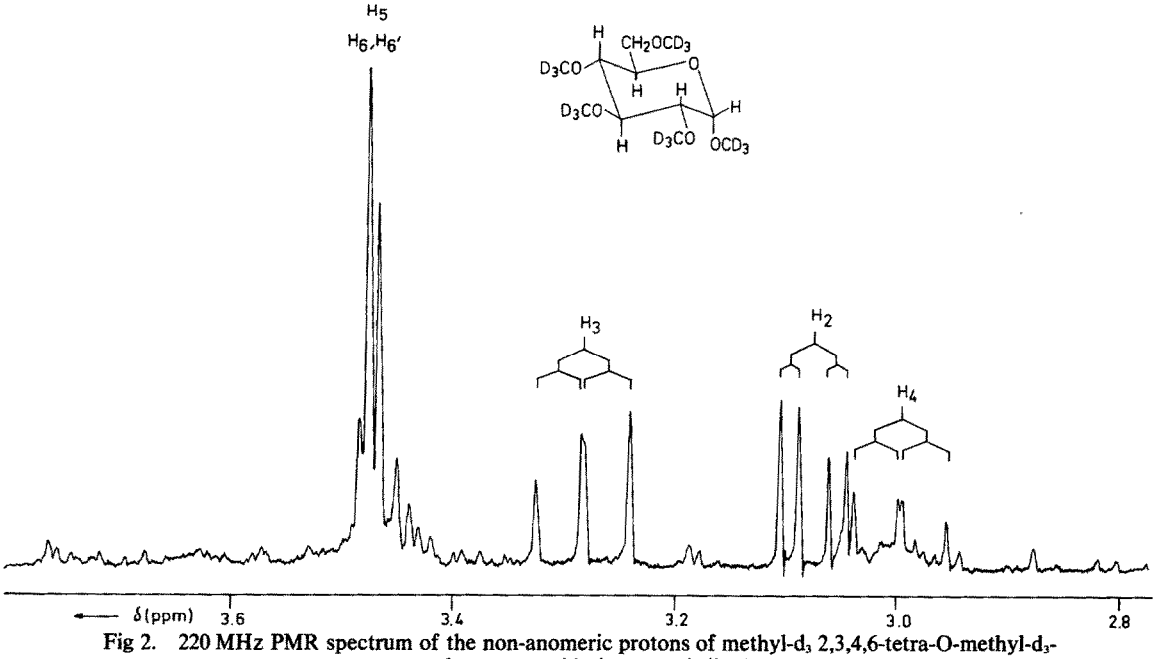

Fig 2. 220 MHz PMR spectrum of the non-anomeric protons of methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-α-D-glucopyranoside in acetonitrile-d_3.

Table 1. PMR chemical shifts[a,b] of OMe groups for methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranosides

Starting compound —permethylation→[c]	Permethyl ether with OCH_3 substituent in position(s)	1-OMe α	1-OMe β	2-OMe α	2-OMe β	3-OMe α	3-OMe β	4-OMe α	4-OMe β	6-OMe α	6-OMe β
D-Gp	1 2 3 4 6	3·29	3·41	3·36	3·45	3·49	3·51	3·43	3·43	3·30	3·31
Me D-Gp	1	3·29	3·41	—	—	—	—	—	—	—	—
3-O-Me-D-Gp	3	—	—	—	—	3·48	3·51	—	—	—	—
2,3-di-O-Me-D-Gp	2 3	—	—	3·37	3·45	3·49	3·51	—	—	—	—
Me 2,3,6-tri-O-Me-D-Gp	1 2 3 6	3·29	3·41	3·37	3·45	3·49	3·51	—	—	3·30	3·31
Me 2,3,4-tri-O-Me-D-Gp	1 2 3 4	3·29	3·41	3·37	3·45	3·49	3·51	3·44	3·43	—	—

[a]Determined at 100 MHz for solutions in acetonitrile-d_3; δ-values are given in ppm relative to TMS.
[b]— = signal is missing as a result of the introduction of OCD_3 at this position.
[c]α-D-Gp and β-D-Gp were permethylated, the other compounds were pertrideuteromethylated.

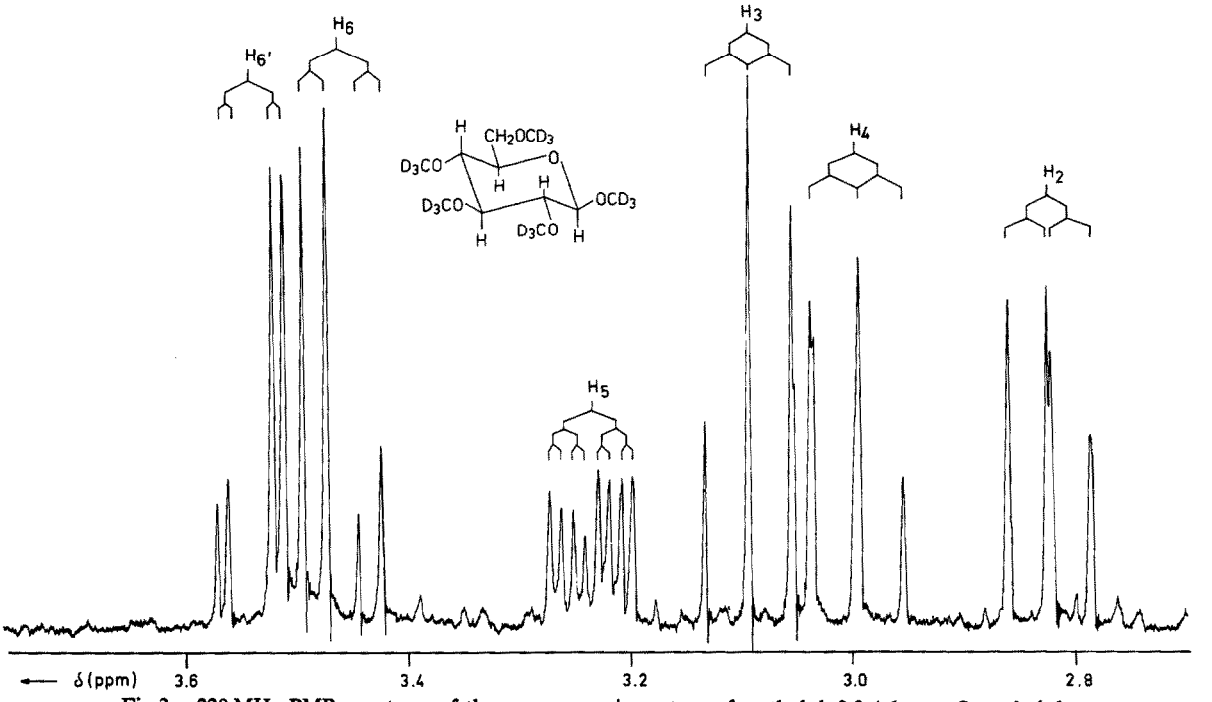

Fig 3. 220 MHz PMR spectrum of the non-anomeric protons of methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-β-D-glucopyranoside in acetonitrile-d_3.

simulation program SIMEQ. The proton systems in both methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-α- and -β-D-glucopyranoside were treated as seven-spin systems ABCDEFG (H_1–$H_{6'}$). All vicinal coupling constants were taken positive but the geminal coupling constant $J_{6,6'}$ was taken negative.[22] The theoretical spectra agree satisfactorily with the experimental ones. The values of coupling constants and chemical shifts (Fig 4) obtained for these systems are given in Table 2. By means of the modified Karplus equation:[23]

$$J_{H,H'} = (6{\cdot}6 - 1{\cdot}0\cos\phi + 5{\cdot}6\cos 2\phi)\left(1 - \sum_i^n f_i \Delta X_i\right)$$

which represents the dependence of the vicinal coupling constant $J_{H,H'}$ on the dihedral angle ϕ between H and H', the ring conformation of both anomers is determined. The experimental coupling constants were compared with those for ideal conformations obtained by calculation from the dihedral angles in Dreiding molecular models. The electronegativity factor f_i was taken 0·15 for $\theta > 90°$ and 0·05 for $\theta < 90°$ (θ is the dihedral angle between the substituent R and the proton H in the system R—C—C—H). The electronegativity X of the substituents is: $X_{O_r} = 3{\cdot}3$; $X_{—C—O—} = 2{\cdot}5$; $X_{OMe} = 3{\cdot}3$ and $X_H = 2{\cdot}1$. The experimental coupling constants agree well with those calculated for Cl (D) conformations (Table 3).

CMR spectra of methyl 2,3,4,6-*tetra*-O-*methyl-α-and -β-*D-*glucopyranosides.* For the assignment of the OMe signals in the CMR spectra the same series of partially methylated glucoses was used as for PMR spectroscopy, but the free OH groups were protected with 5 or 10% ^{13}MeI. Quite analogous to the PMR spectra, the CMR spectra reveal striking differences in the chemical shifts of the OMe groups at positions 1 and 2 for the α and β anomers, whereas the resonances of the OMe groups at the positions 3, 4 and 6 are almost uneffected by anomeric change (Table 4, Fig 5). The axial 1-OMe group in the α anomer resonates at higher field (1·7 ppm) than the corresponding equatorial OMe group

Table 2. PMR parameters[a] of the skeleton protons of methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-α- and -β-D-glucopyranoside

Anomer	H_1	H_2	H_3	H_4	H_5	H_6	$H_{6'}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
α	4·76	3·09	3·29	3·01	3·47	3·48	3·48	3·5	9·6	8·8	9·8	3·4	3·4	—[b]
β	4·12	2·83	3·09	3·01	3·24	3·47	3·54	7·8	8·9	9·0	9·6	4·9	2·1	−10·8

[a]Determined at 220 MHz for solutions in acetonitrile-d_3; δ-values are given in ppm relative to TMS, coupling constants are given in Hz.

[b]No $J_{6,6'}$ is observed.

Table 3. Coupling constants $J_{H,H'}$ observed and calculated for ideal Cl (D) conformations, and the calculated dihedral angles $\phi_{H,H'}$

Permethyl ether of	Observed values				Calculated values							
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$\phi_{1,2}$	$\phi_{2,3}$	$\phi_{3,4}$	$\phi_{4,5}$
α-D-Gp	3·5	9·6	8·8	9·8	2·1	11·1	11·1	11·1	47	157	151	159
β-D-Gp	7·8	8·9	9·0	9·6	10·6	11·1	11·1	11·1	147	152	152	157

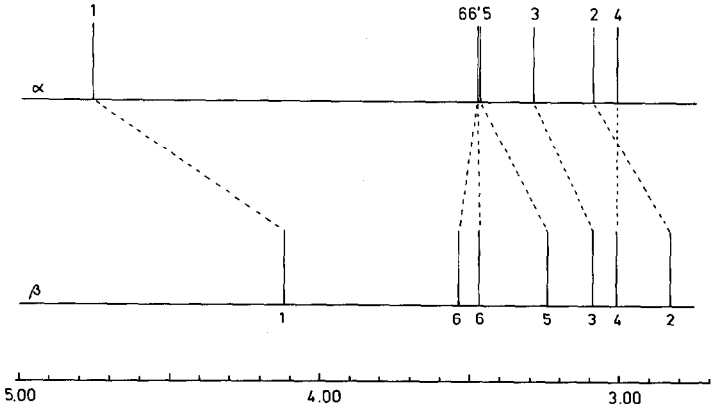

Fig 4. Correlation of the skeleton proton resonances in the 220 MHz PMR spectra of methyl-d_3 2,3,4,6-tetra-O-methyl-d_3-α- and -β-D-glucopyranoside.

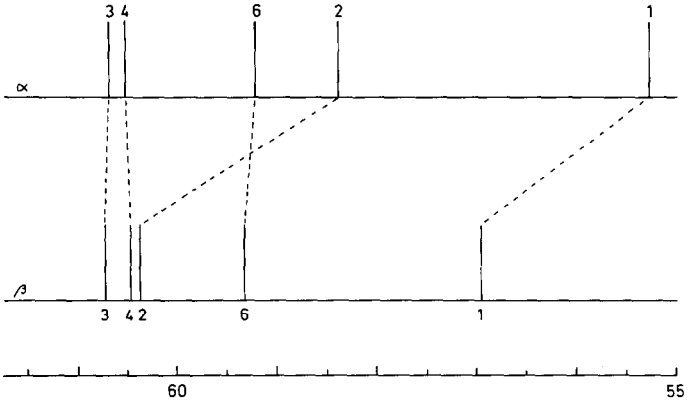

Fig 5. Correlation of the methoxyl resonances in the CMR spectra of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside.

in the β anomer; a similar sequence of signals has been reported for the CMR spectra of methylglycosides[14] and methylglycoside peracetates.[10] The 2-OMe group is shifted upfield over rather 2·0 ppm by change from β to α anomer. The skeleton carbons C_1–C_6 resonate downfield from the OMe carbons (Table 5, Fig 6). For the assignment of these resonances use was made of heteronuclear off-resonance selective spin decoupling.[21] Various single frequencies at intervals of 20 Hz in the range of the proton resonances were irradiated. The plot of the resonance frequencies of the partially decoupled FT-CMR spectra of the α anomer against the proton irradiation frequencies is given in Fig 7. Assignment of the carbon resonances is possible because the assignment of the proton spectrum is known.

The chemical shifts of the carbons in the positions 4 and 6 alter hardly by anomeric change because these carbons are relatively remote from the anomeric centre (Fig 6). This is in accordance with the changes in the positions of the corresponding protons (Table 2). As indicated for the glycosides,[6,8,14] the anomeric C_1 resonates at lower field than the carbons C_2–C_6 as a result of its attachment to two O atoms. The anomeric carbon in the

Table 4. CMR chemical shifts[a,b] of methoxyl groups for methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranosides

Starting compound —permethylation→[c]	Permethyl ether with 5 or 10% $O^{13}CH_3$ substituent in position(s)	1-OMe		2-OMe		3-OMe		4-OMe		6-OMe	
		α	β	α	β	α	β	α	β	α	β
Me D-Gp	2 3 4 6	55·27	56·97	58·36	60·47	60·69	60·78	60·52	60·57	59·24	59·30
3-O-Me-D-Gp	1 2 4 6	55·28	57·02	58·43	60·42	60·75	60·78	60·60	60·51	59·22	59·34
2,3-di-O-Me-D-Gp	1 4 6	55·27	56·91	58·41	60·34	60·71	60·69	60·55	60·41	59·24	59·30
Me 2,3,6-tri-O-Me-D-Gp	4	55·31	56·96	58·41	60·36	60·72	60·72	60·53	60·45	59·25	59·34
Me 2,3,4-tri-O-Me-D-Gp	6	55·24	56·96	58·38	60·37	60·68	60·72	60·49	60·44	59·22	59·35

[a] Determined at 25·2 MHz for solutions in acetonitrile-d_3; δ-values are given in ppm relative to TMS.
[b] The resonances of the methoxyl groups enriched in ^{13}C are underlined.
[c] The compounds were permethylated with 5 or 10% ^{13}MeI.

Table 5. CMR chemical shifts[a] of the skeleton carbons of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside

Anomer	C_1	C_2	C_3	C_4	C_5	C_6
α	98·16	82·58	84·28	80·61	70·98	72·41
β	105·00	84·58	87·21	80·48	75·38	72·36

[a]δ-values are given in ppm relative to TMS.

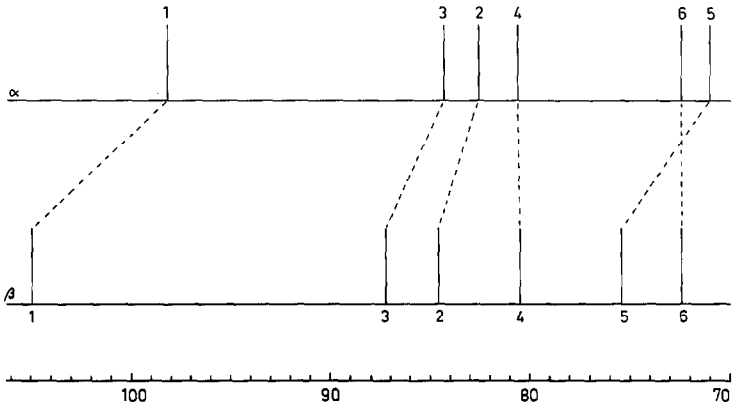

Fig 6. Correlation of the skeleton carbon resonances in the CMR spectra of methyl 2,3,4,6-tetra-O-methyl-α- and -β-D-glucopyranoside.

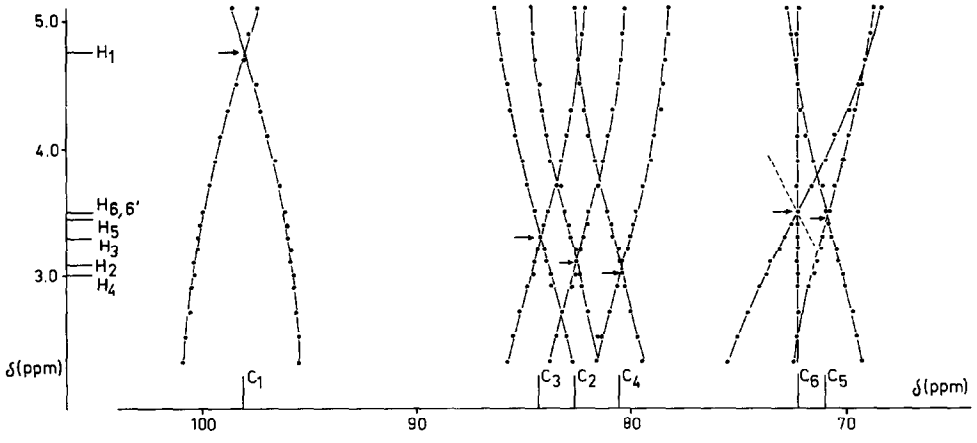

Fig 7. Plot of peak frequencies in the proton off-resonance selectively decoupled CMR spectra of methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside as a function of the position of irradiation in the proton spectrum. Chemical shifts are given in ppm relative to TMS. On the ordinate the peak positions in the proton off-resonance decoupled CMR spectra are plotted, and on the abscissa the proton decoupling frequencies. The lines on the ordinate show the carbon resonance positions in the proton noise decoupled CMR spectrum. The projection on the abscissa of the points of collapse (arrows) of the respective carbon multiplets shows the relative positions of the connected protons in the PMR spectrum (Fig 4).

α-anomer (axial OMe) resonates at higher field than that in the β anomer (equatorial OMe). A downfield shift is also observed for C_2, C_3 and C_5 by change of configuration from α to β.[8] Only the chemical shifts of the carbons 1, 2, 3 and 5 are influenced by anomeric change; the chemical shifts of C_3 and C_5 are more effected than the shift of C_2. A possible explanation of this observation is the occurrence of a 1,3-diaxial interaction in the α anomer between the 1-OMe group and the protons H_3 and H_5, by which a polarization of charge along the H_3–C_3 and H_5–C_5 bonds are induced.[7,8] It has to be noted that the carbon and proton chemical shifts in the positions 1, 2, 3 and 5 are effected inversely by

anomeric change; a decreased shielding of a carbon is accompanied by an increased shielding of the directly attached proton.[8] In contrast to this observation the carbons of the 1- and 2-OMe groups and also their appended protons resonate in the α anomer at higher field than in the β anomer, reflecting differences in anisotropic and solvatation effects rather than van der Waals interactions.

The resonances of the carbons at positions 1, 2, 3, 4 and 6 are shifted downfield by 6–13 ppm relative to the corresponding resonances in the spectra of the unsubstituted hexopyranoses (in D_2O).[7] This agrees with the observation of Dorman and Roberts,[7] that methylation of an OH group has a deshielding effect on the directly attached carbon. C_5 occupies a unique position, because it bears no OMe substituent; the resonance of this carbon is shifted slightly upfield (0·3–0·5 ppm) relative to the resonance in the free sugar. As a result of the strong downfield shifts of the OMe-bearing carbons, the resonance of C_6 in the α anomer is not situated at the highest field relative to the resonances of the other ring carbons. This is in contrast with the spectra of unsubstituted α-D-glucose and methyl α-D-glucopyranoside.[7,14]

DISCUSSION

The suitability of permethylation as a method in the structure elucidation of oligo- and polysaccharides depends on the availability of adequate techniques for the separation and identification of the partially methylated monosaccharides, which are obtained after the breakdown of the permethylated compound. In several approaches to this problem the degradation products are chemically modified and the free OH groups are blocked. Frequently the alditol acetate method[24] is used, which is based on the reduction of partially methylated glycoses to alditols, followed by acetylation of the OH groups. The identification of the alditol acetate is accomplished by comparison with reference compounds e.g. carried out by a combination of TLC,[25] GLC and mass spectrometry. It is a disadvantage of this and related techniques that so many reference compounds are needed (14 for one aldohexopyranose already), which renders problems in the unequivocal identification of components in a mixture derived from an oligo- or polysaccharide.

The complexity of the mixture to be separated and the number of reference compounds are reduced if the free OH functions are blocked with labelled Me groups, provided that the positions of labelled groups can be determined unambiguously. The results of this investigation show that by means of PMR or CMR spectroscopy the location of trideuteromethoxyl groups or ^{13}C-enriched OMe groups can be established on the basis of the chemical shifts and the intensities of the OMe signals in the spectra of permethylated D-glucose.

An application of this technique for the structure determination of oligosaccharides is given in the assignments of the 4-OMe and 6-OMe resonances of the permethyl-D-glucoses by methanolysis of permethyl maltose and -gentiobiose respectively (Fig 8). Little variations in the chemical shift of a definite OMe group due to the presence of free OH groups in the monosaccharide derivative are circumvented. A further advantage is that the configuration of the glycosidic bond(s) in the permethyl oligosaccharide can be directly inferred from the PMR spectrum of the intact compound.[26] For identification purposes the assignment of the OMe signals in the pyranose and furanose forms of each permethyl monosaccharide must be known. This

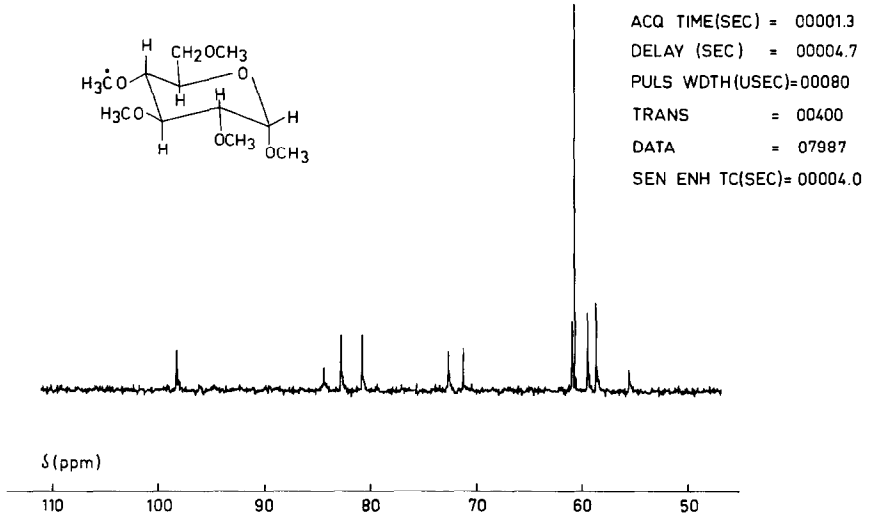

Fig 8. CMR spectrum of the permethylated α-D-glucopyranose obtained after methylation with 5% ^{13}MeI of the partially methylated components in the methanolysate of permethyl maltose.

study is still in progress, but we have already observed that the OMe signals in PMR and CMR spectra of the various permethylated monosaccharides show appreciable differences. It is essential that the degree of ^{13}C-labelling is sufficient to overcome the variations in resonance intensities, arising from differences in relaxation times and NOE contributions; *5% labelling is normally sufficient.* Under the used conditions we found that the resonances of the OMe groups and of the skeleton carbons at different positions in the molecule, but at the same isotopic abundance have almost identical intensities. Besides the NMR parameters of the methoxyl groups, also those of the ring protons and ring carbons, give valuable information about the monosaccharides. On the basis of the coupling constants in the PMR spectrum of the per-deuteromethylated monosaccharide the ring conformation can be determined. The CMR spectrum, rather than the PMR spectrum, can be used for identification of the monomer in question.

The choice of deuterium or ^{13}C label depends on the type of problem to be solved. Labelling with ^{13}C increases the intensity of the OMe signal, whereas deuterium labelling decreases the intensity of the carbon resonance due to ^{13}C-D spin-spin couplings. Therefore the preference for one of these labels will often be determined by the amount of material available for analysis. The recent improvements in the NMR apparatus and the extension with Fourier Transform have lowered the amounts of material necessary for analysis; for PMR less than one mg and for CMR about 10 mg of a monosaccharide derivative is sufficient.

EXPERIMENTAL

Materials. D-glucose, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside and maltose were obtained from Baker Chemicals N.V.; 3-O-methyl-D-glucose from Calbiochem; 2,3-di-O-methyl-D-glucose from Koch-Light Laboratories Ltd; gentiobiose from Pierce Chemicals Comp and D-glucose-6,6′-d_2 from Merck Sharp Dohme. Methyl 2,3,4-tri-O-methyl-α,β-D-glucoside and methyl 2,3,6-tri-O-methyl-α,β-D-glucoside were prepared from gentiobiose and maltose respectively. MeI (5 and 10% ^{13}MeI) was prepared by dilution of MeI (61% ^{13}MeI) (Merck Sharp Dohme). Trideuteromethyliodide (99% CD_3I) (Merck) was distilled before use.

Methods. Sugars were permethylated according to the procedure of Kuhn[17] by treatment with MeI and Ag_2O in dry DMF. Methylation of glucose derivatives was performed with CD_3I for PMR spectroscopy and with MeI (5 or 10% ^{13}MeI) for CMR spectroscopy. The methyl 2,3,4,6-tetra-O-methyl-glucopyranosides were separated into α- and β-anomers by TLC[1] on 0·5 mm plates of silica-G (Merck) in the solvent system benzene:methanol (96:4). After spraying with 1% morin in MeOH three zones were detected under UV light; a broad one (A) near the starting point containing DMF, impurities and incompletely methylated material; (B) containing methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside (R_f~0·40) and (C) containing methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside (R_f~0·55). The zones (B) and (C) gave clear syrups after extraction with chloroform and evaporation of the solvent. Disaccharides were permethylated with MeI until the OH absorptions in the IR spectrum had disappeared. Purification of the permethyl disaccharides was performed by column chromatography (column 35×1·5 cm, Sephadex LH 20; eluant EtOH–$CHCl_3$ 2:1). Sugars were detected in 0·1 ml aliquots with the phenol-sulfuric acid reagent.[18]

Methanolysis (1 M MeOH–HCl, 85°, 24 hr)[19] of permethyl gentiobiose and permethyl maltose resulted in the formation of methyl 2,3,4-tri-O-methyl-α,β-D-glucopyranoside and methyl 2,3,6-tri-O-methyl-α,β-D-glucopyranoside respectively, besides the methyl 2,3,4,6-tetra-O-methyl glucopyranosides. The last named compounds were removed by TLC as described before.

The purity of the permethylated glucopyranosides was tested by GLC using a F & M Model 700 gaschromatograph at a nitrogen flow rate of 28 ml/min. The column (S.S., 2·70 m×3·2 mm O.D.) contained 3% w/w of ECNSS-M on chromosorb-W-AW-DMCS (80–100 mesh) and was operated at a temperature of 125°. The retention time of the β-anomer was 0·65 relative to that of the α-anomer.

NMR spectroscopy. The PMR spectra were recorded at 100 MHz with a Varian HA-100 spectrometer (Organic Chemical Institute T.N.O., Utrecht) and at 220 MHz with a Varian HR-220 spectrometer (T.N.O. Central Laboratories, Delft). 5–20% Solns of the glucose derivatives in acetonitrile-d_3 were used. Chemical shifts are given relative to TMS in the δ-scale with an accuracy of about 0·01 ppm. Acetonitrile-d_3 was used as an internal standard: δ = 1·94 ppm. The accuracy of the coupling constants is about 0·2 Hz. Spectrum simulations were run on a 16 k Varian 620 i computer coupled with a Varian XL 100 spectrometer, using a modified SIMEQ spin simulation program.[20]

Proton-noise decoupled CMR spectra of 5–20% solutions of ^{13}C-labelled glucose derivatives in acetonitrile-d_3 were recorded at 25·2 MHz on a Varian XL 100–15 FT spectrometer, operating in the deutero-lock mode. Chemical shifts are given relative to TMS internal (δ-scale) with an accuracy of 0·04 ppm. Assignment of the resonances of the ring carbons is based on a special proton off-resonance decoupling technique.[21]

Acknowledgements—We thank Miss T. Volp (Organic Chemical Institute T.N.O., Utrecht), Dr. E. Talman and Mr. J. L. Hoogendoorn (T.N.O. Central Laboratories, Delft) and Mr. D. Seykens for recording the spectra. This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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