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Note

Partial n.m.r. assignment of methyl groups in *O*-methylsucroses*

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In a continuation of our investigation of the mechanisms of alkaline degradation of sucrose[1], we wished to examine the relative rates of degradation of mixtures of partially methylated sucroses. The assignment of the position of the methyl groups by n.m.r. spectroscopy appeared the most direct method for identification and analysis of such mixtures.

^{1}H-N.m.r. chemical shifts of methylated monosaccharide sugars have been extensively studied. Much of this work was motivated by the fact that unequivocal identification of partially methylated residues would provide a rapid means for assigning polysaccharide structure after methylation analysis[2]. Another line of research, which has led to an interest in the ready identification of partially substituted sugars, is the study of selective reactivity of hydroxyl groups in carbohydrates[3,4]. The approach in all instances has been to synthesize specifically substituted derivatives and thereby identify the methyl singlets.

A number of studies have been conducted on monomethyl hexoses with D_2O as solvent, and di-*O*-methyl, tri-*O*-methyl, and tetra-*O*-methyl derivatives of D-galactose have been studied in D_2O and in benzene, other solvents having been found unsatisfactory[5]. These studies confirmed that the chemical shift of a methoxyl group is strongly dependent upon its own orientation (either axial or equatorial) and upon the presence and orientation of adjacent substituents. It was also apparent that identification was not possible by this method without the use of a large number of reference compounds.

A much simpler means of identification, originally used on partially acetylated sugars[3], was employed successfully by Gagnaire and Odier[6]. In this method, methyl glucopyranosides that had been partially methylated were subsequently fully substituted at the remaining hydroxyl groups by $-OCD_3$ groups. Although the n.m.r. spectrum was thus effectively that of a permethylated sugar, the methyl singlets

*Dedicated to Professor Stephen J. Angyal on the occasion of his retirement.

were then absent for those positions that carried -OCD_3. The chemical shifts of the methyl groups were assigned previously by synthesis of specific mono-*O*-methyl sugars which had themselves been perdeuteriomethylated before the n.m.r. spectrum was taken. The same method has been used to characterise *O*-methyl derivatives of D-galactose[7] and to study substitution patterns in methylated pentopyranosides[4]. The spectra of permethylated methyl α- and β-D-glucopyranoside and α- and β-D-galactopyranoside have been fully assigned[1] at[8] 220 MHz. Khan[9] has reviewed the derivatives of sucrose, including the methyl ethers, and has made ^{1}H-n.m.r. studies of certain partially methylated and acetylated derivatives. However, thus far, no study of octa-*O*-methyl sucrose has been published and there has been no assignment of any of the methyl singlets in any methylated sucrose derivative containing more than one such group.

The ^{1}H-n.m.r. spectrum of octa-*O*-methylsucrose was taken in chloroform-*d*–benzene-d_6 mixtures of various proportions. These two solvents were chosen as they were used by Gagnaire and Odier[6] in their study of permethylated D-glucopyranosides. Pure benzene-d_6 was found to give the best resolution of the methyl singlets, and the spectrum showed seven distinct singlets in the region 3.1–3.6 p.p.m. A solution in pyridine-d_5 gave partial resolution of all eight peaks, but the separations were very small. In order to achieve high precision of shift-measurements it was essential to ensure that conditions of temperature, concentration, and solvent were maintained constant throughout the range of samples. The precision of the spectrometer was 0.002 p.p.m. and the errors quoted are calculated as standard deviations.

The seven methyl singlets overlay the signals for the ring protons, and the chemical shifts of the seven peaks and their assignments are given in Table I. The Roman numerals in column 1 indicate the order of the peaks numbered downfield from tetramethylsilane, and the identities of the reference compounds are listed in Table II.

TABLE I

^{1}H CHEMICAL-SHIFTS[a] OF METHYL GROUPS IN REFERENCE COMPOUNDS

Peak	*Compound*					
	1[b]	**2**	**3**[c]	**4**[c]	**5**[c]	*Assignment*
I	3.173 ±0.003		3.176 ±0.000		3.165 ±0.007	1′
II	3.202 ±0.002		3.205 ±0.0004			6′
III	3.235 ±0.003					6
IV	3.250 ±0.001			3.256 ±0.002	3.251 ±0.005	
V, VI	3.367 ±0.001			3.366 ±0.001	3.362 ±0.001	
VII	3.531 ±0.003		3.533 ±0.002	3.528 ±0.001	3.531 ±0.001	4
VIII	3.601 ±0.002			3.601 ±0.003	3.603 ±0.004	possibly 3

[a]Shifts in p.p.m. downfield from internal Me_4Si; solvent: benzene-d_6. [b]Mean of five spectra. [c]Mean of three spectra.

TABLE II

REFERENCE COMPOUNDS

Number	*Name*
1	Octa-*O*-methylsucrose
2	Octa-*O*-(methyl-d_3)sucrose
3	4,1′,6′-Tri-*O*-methyl-2,3,6,3′,4′-penta-*O*-(methyl-d_3)sucrose
4	2,3,4,3′,4′-Penta-*O*-methyl-6,1′,6′-tri-*O*-(methyl-d_3)sucrose
5	2,3,4,1′,3′,4′-Hexa-*O*-methyl-6,6′-di-*O*-(methyl-d_3)sucrose

TABLE III

^{1}H CHEMICAL-SHIFTS[a] OF METHYL GROUPS IN METEYL 2,3,4,6-TETRA-*O*-METHYL-α-D-GLUCOPYRANOSIDE (A) AND METHYL 1,3,4,6-TETRA-*O*-METHYL-β-D-FRUCTOFURANOSIDE (B)

δ p.p.m.[b]	*Assignment*[c]
A	
3.171 ±0.003	1
3.215 ±0.0004	2,6
3.482 ±0.001	4
3.574 ±0.0008	3
B	
3.140 ±0.001	
3.327 ±0.0004	
3.379 ±0.0009	2
3.402 ±0.0004	

[a]Shifts in p.p.m. downfield from internal Me_4Si; solvent: benzene-d_6. [b]Mean of three samples. [c]After ref. 6.

Examination of the spectra of the monosaccharide residues that constitute the sucrose molecule may afford some hints, at least, as to which moiety the unassigned peaks belong to. For this reason, the spectra of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside and methyl 1,3,4,6-tetra-*O*-methyl-β-D-fructofuranoside were determined, and the chemical shifts are recorded in Table III. Assignment of the methyl peaks in Table III is after the order given by Gagnaire and Odier[6]. This assignment appears justified, despite the difference in solvent, as a small and continuous variation was noted in the shift of methyl peaks of octa-*O*-methylsucrose when changing from 12:1 $CDCl_3$–C_6D_6 to pure C_6D_6. In addition, Haverkamp *et al.*[10], using acetonitrile-d_3, found the same sequence of methyl peaks as Gagnaire and Odier, thus confirming a relative insensitivity to solvent-effect. It should be noted that correspondence of assigned peaks between sucrose and monosaccharide derivatives is not exact, and care should be exercised in correlating these results with those in Table I. However,

the 4-OCH_3 group of the glucose residue might be expected to be least affected by the nature of the aglycon, and the two shift-values differ by only 0.05 p.p.m. Similarly, the difference for the 6-OCH_3 assignments is 0.20 p.p.m. For this reason, it is possible to make a tentative assignment of peak VII as 3-OCH_3, but no attempt has been made to assign 2-OCH_3 in this manner, as this position would be most strongly affected by the nature of the aglycon.

The foregoing conclusions are entirely qualitative. Initially, it was hoped that the method would yield quantitative estimates of the constituents of mixtures such as partially methylated sucroses, thus giving information on relative reactivities of hydroxyl groups. The ring C–H absorptions, however, underlie many of the methyl singlets (cf. ref. 6) and, especially for minor constituents, the only procedure that would give reasonably accurate measurement of the various methyl groups would be the integration of the "difference" spectrum obtained by subtracting the spectrum of octa-*O*-(methyl-d_3)sucrose from that of (say) a mixture of mono-*O*-methylsucroses. An initial attempt was made to conduct this procedure on the spectra of octa-*O*-methyl- and octa-*O*-(methyl-d_3)-sucroses at 270 MHz. The "difference" spectrum, however, contained anomalous negative peaks and could not be satisfactorily integrated. The difference spectra were obtained from spectra collected over 3600 Hz in 32K data points, and it is therefore unlikely that the negative peaks could be associated with an inadequate number of data points. It is concluded that the substitution of deuterium for hydrogen in the methyl groups affects J values and splitting patterns of actual and "virtual" long-range coupling between ring protons and methyl groups. These factors cause line shifts and line-width variation, so that the two spectra are non-equivalent. An alternative possibility of integrating the ^2H spectrum was considered, but this required deconvolution techniques to resolve the eight methyl peaks and as a result, accurate integration was not possible.

In ^{13}C-n.m.r. spectroscopy, the effect of solvent is relatively unimportant, and acetone-d_6 was chosen as giving a solvent signal conveniently removed from the region of interest. To give maximum resolution, the frequency-width observed was 1500 Hz instead of the usual 3000 Hz.

The spectrum of octa-*O*-methylsucrose showed eight peaks corresponding to *O*-methyl carbon atoms between 51 and 54 p.p.m., with the other carbon resonances occurring between 64 and 84 p.p.m. The spectrum of octa-*O*-(methyl-d_3)sucrose showed only slight noise in the 50–55 p.p.m. region, attributed to background created by deuterated methyl carbon atoms. The spectra of the other three model compounds were determined; the chemical shifts and peak assignments are listed in Table IV and show excellent correlation between the different compounds. The peaks are lettered in order from high field to low field.

Independent evidence[11], to be published in detail later, suggests that peak B may correspond to the C-2 methyl group, as it is present in relatively large excess after partial methylation of sucrose, and that peak C may represent the C-3′ methyl group, as the corresponding mono-*O*-methylsucrose is relatively stable to alkaline degradation[11].

TABLE IV

^{13}C CHEMICAL-SHIFTS[a] OF THE CARBON ATOMS OF THE METHYL GROUPS IN REFERENCE COMPOUNDS

Peak	*Compound*					
	1	**2**	**3**	**4**	**5**	*Assignment*
A	51.76			51.76	51.76	
B	51.83			51.83	51.83	
C	51.90			51.90	51.90	
D	52.27		52.27			6′
E	52.46					6
F	52.76		52.76		52.76	1′
G	53.73		53.73	53.73	53.73	4
H	53.97			53.97	53.97	

[a]Shifts in p.p.m. downfield from internal Me_4Si; solvent: acetone-d_6.

This study has allowed the unequivocal assignment of four plus a possible fifth of the eight methyl groups in the ^1H-n.m.r. spectra of *O*-methyl sucroses, and four of the methyl carbon atoms in the ^{13}C-n.m.r. spectra. Complete assignment of the spectra will require the synthesis of new, partially methylated sucroses, and the distinction between the 3′ and 4′ positions will be particularly difficult. We also conclude that the method shows little promise in accurate quantitative analysis of mixtures of partially methylated sucroses.

EXPERIMENTAL

^1H-N.m.r. spectroscopy. — Spectra at 100 MHz were measured with a Jeol JNM-MH-100 spectrometer at a probe temperature of 33 ±0.5°. Concentration of samples was constant at 50 mg per 420 μL of solvent. The internal standard was 1% tetramethylsilane, which was locked internally at 4000 Hz. Shift measurements were taken in p.p.m. downfield from Me_4Si by using a digital frequency-counter. Spectra at 270 MHz were taken on a Bruker HX-270 spectrometer at the National NMR Centre of the Australian National University by Dr. A. J. Jones.

^{13}C-N.m.r. spectroscopy. — Spectra were measured at 15 MHz with a Jeol JNM-FX60Q FT spectrometer at probe temperature, in acetone-d_6.

Synthesis of reference compounds. — Permethylations and perdeuteriomethylations were conducted by the Hakomori method as described by Lindberg *et al.*[12]. These procedures were applicable for methylation of either free or acetylated hydroxyl groups.

4,1′,6′-Tri-*O*-methylsucrose was prepared by methylation[13] of 2,3,6,3′,4′-penta-*O*-acetylsucrose[14] with diazomethane, followed by perdeuteriomethylation to yield reference compound 3. 2,3,4,3′,4′-Penta-*O*-methylsucrose was prepared *via* tri-*O*-tritylsucrose as described earlier[1], and was perdeuteriomethylated to yield

reference compound **4**. 6,6'-Di-*O*-tritylsucrose[15] was successively permethylated, detritylated, and perdeuteriomethylated to yield reference compound **5**.

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