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P.M.R. STUDIES ON FULLY METHYLATED DISACCHARIDES USING LANTHANIDE SHIFT REAGENTS: ASSIGNMENTS OF THE METHOXYL SIGNALS*

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ABSTRACT

The complexes of $Eu(fod)_3$ with per-*O*-methylated aldohexosylaldohexoses, consisting of D-glucopyranose and D-galactopyranose residues and having (1→2), (1→4), and (1→6) linkages, have been studied by using p.m.r. spectroscopy. It was found that $Eu(fod)_3$ binds preferentially to two neighbouring MeO-oxygens having an axial–equatorial relationship. Steric hindrance is a major factor in disfavouring certain sites. On the basis of $Eu(fod)_3$-effects on the methoxyl groups, and the comparison of the chemical shifts of corresponding groups in the permethylated mono- and di-saccharides, the signals for most of the MeO groups of the latter compounds were assigned. The shift increments of the signals for these MeO groups, with respect to those for the corresponding groups in the permethylated monomers, were related to the type and the configuration of the inter-sugar linkage. The potential of the shift increments for assignment purposes in other permethylated di- or higher-saccharides is discussed.

INTRODUCTION

P.m.r. spectroscopy is, in principle, a useful means of identifying per-*O*-methylated (PM) sugars. The MeO signals are particularly suitable, because each PM-sugar has a unique pattern of MeO signals. However, this method of identification requires assignment of the MeO signals, which may cause problems in the case of the higher PM-saccharides. This situation is reflected by the relative scarcity of data on MeO signals for such compounds, compared with the information available for PM-monosaccharides. For example, in the disaccharide series, only for PM-α- and -β-maltoside and for hepta-*O*-methyl-α- and -β-cellobiose have a few MeO signals been assigned[1]. As far as the higher saccharides are concerned, assignments have been made for some PM-derivatives of oligo- and poly-saccharides consisting

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of only one type of monomer, for example, cycloamyloses[1], amylose[1], and other (1→4)-linked glucans[1,2,3]. In all of these cases, assignments were made on the basis of comparison with the chemical shifts for the corresponding MeO groups in PM-monosaccharides.

It is clear that the method of preparing partially deuteriomethylated analogues to elucidate MeO assignments, as in commonly used for the PM-monosaccharides[4], is not practicable for the higher oligomers, because of the complex synthetic work involved. We now report that, for PM-disaccharides (and probably also for higher PM-saccharides), lanthanide shift reagents (LSR) are a valuable aid in the assignment of methoxyl-proton signals.

We have recently studied the complexes of LSR with PM-aldohexopyranosides[5,6] and with their 6-deoxy analogues[6]. By evaluating the lanthanide-induced shifts (LIS) and lanthanide-induced broadening by Eu(III), Pr(III), and Gd(III) tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione) [$Eu(fod)_3$, $Pr(fod)_3$, and $Gd(fod)_3$, respectively], it was found that these reagents bind to the PM-monosaccharides in a highly specific manner, in spite of the large number of possible binding-sites. It was possible to express[6] the preference for the various binding-sites in a sequence rule.

In the present study, these results have been applied to a number of PM-disaccharides, made up of D-glucose and D-galactose residues and having (1→2), (1→4), or (1→6) linkages. The effects of $Eu(fod)_3$ on the methoxyl-proton signals were interpreted by using the sequence rule. Together with additional information derived from the $Eu(fod)_3$-effects on the anomeric protons, this approach has now led to the assignment of the signals for nearly all of the MeO groups of the PM-disaccharides. Once the assignments were made, the influence of the inter-sugar linkages on the chemical shifts of the MeO groups could be traced.

EXPERIMENTAL

Materials. — Commercially available disaccharides were used. The lanthanide shift reagent $Eu(fod)_3$ (Merck) was stored over P_2O_5 before use, and chloroform-*d* (Merck) was dried over Linde Molecular Sieve type 3A.

After per-*O*-methylation of the disaccharides by Kuhn's method[7], individual anomers were isolated after t.l.c. separation on silica gel (Merck) with hexane–acetone (3:2). The sugars were revealed under u.v. light after spraying of the plates with 1% methanolic quercitin, and extracted with chloroform.

P.m.r. spectroscopy. — Drying of the equipment used in p.m.r. spectroscopic measurements, preparation of samples, and the mode of operation were as described earlier[6]. Chemical shifts for spectra recorded at 100 MHz are given relative to Me_4Si on the δ-scale, within 0.01 p.p.m.

Eu(fod)$_3$ experiments. — Known amounts of $Eu(fod)_3$ were added to 0.1M solutions of PM-disaccharides in $CDCl_3$; after each addition, the chemical shifts of MeO and anomeric protons were recorded Up to a molar ratio $X = 0.1$, $Eu(fod)_3$

was added by syringe from a stock solution in $CDCl_3$, and for $0.1 < X \leqslant 1$, as a solid powder {where $X = [Eu(fod)_3]/[sugar]$}. For each compound, the chemical shifts were plotted against X. The graph obtained for PM-α-maltoside (3) is given (Fig. 1) as a typical example; with few exceptions, the MeO signals are shifted to lower field with increasing X. Shift gradients G (in p.p.m., for $X = 1$) were determined from the initial slopes of curves relating the value of δ to X.

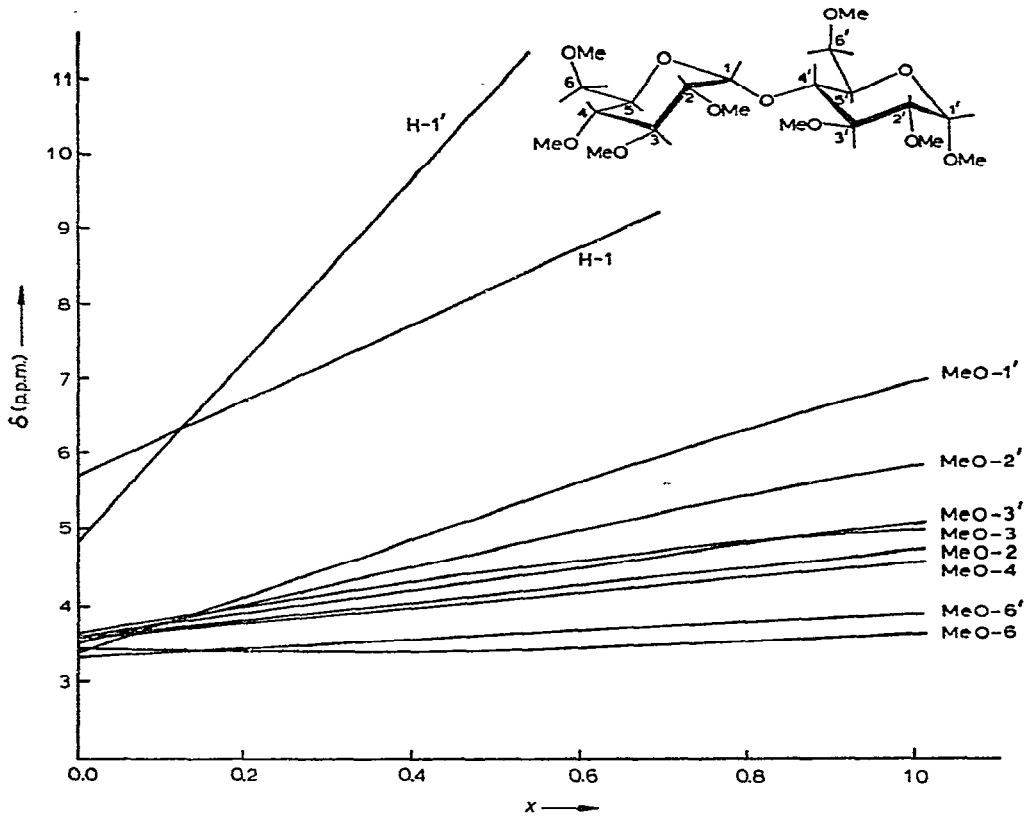


Fig. 1. Shifts of the methoxyl groups and the anomeric protons, H-1 and H-1′, of PM-α-maltoside 3, induced by Eu(fod)$_3$ (solvent $CDCl_3$, $X = [LSR]/[sugar]$). The assignments for MeO-2 and MeO-4 may be reversed.

RESULTS AND DISCUSSION

P.m.r. spectra of PM-disaccharides

The p.m.r. spectra of the following PM-disaccharides were recorded: PM-α- and β-cellobioside (1 and 2), PM-α- and β-maltoside (3 and 4), PM-β-lactoside (5), PM-α- and β-gentiobioside (6 and 7), PM-β-melibioside (8), PM-β-sophoroside (9), and PM-β-kojibioside (10). Each spectrum consists of a set of eight MeO signals (some of which overlap), two doublets for the anomeric protons H-1 and H-1′, and

a complex pattern of peaks (not discussed further) for the non-anomeric protons other than MeO. Spectral assignments for the anomeric protons of PM-disaccharides dissolved in $CDCl_3$ have been made by Minnikin[8].

Initial assignments of the MeO signals were made by comparison with those of the constituent monosaccharides, PM-α- and -β-D-glucopyranoside (**11** and **12**) and PM-α- and -β-D-galactopyranoside (**13** and **14**), collected in Table I from data recorded in Ref. 6. The following assumptions were made. (*a*) MeO groups located in positions remote from the inter-sugar linkage have essentially the same chemical shifts as corresponding groups in **11** to **14**. (*b*) Chemical shifts of the MeO groups of the non-reducing units are practically uninfluenced by anomeric change in the reducing units; if any, the influence would be most apparent in the (1→2)-linked disaccharides. Although the signals for certain MeO groups may be confidently assigned in this way, for most, only approximate regions of resonance can be given. For example, in PM-α-cellobioside (**1**, Table II; see also subsequent discussion), the only signal assigned unambiguously is at δ 3.63 (MeO-3 of the non-reducing unit). The remainder are divided into three groups: δ 3.40–3.42 (MeO-1′,6,6′), 3.51–3.53 (MeO-2′,4), and 3.57–3.58 (MeO-2,3′), where primed numbers refer to carbons of the reducing unit. The results of the LSR experiment were then applied for specific assignments within such groups of signals, wherever possible.

TABLE I

CHEMICAL SHIFTS (δ, IN p.p.m.) OF THE ANOMERIC PROTONS AND THE METHOXYL GROUPS, AND LSR EFFECTS [SHIFT GRADIENTS, *G* (IN p.p.m., FOR $X = 1$), AND RELATIVE BROADENING, *B*[a]] INDUCED BY $Eu(fod)_3$ ON THESE GROUPS, FOR THE PER-*O*-METHYLATED MONOSACCHARIDES **11**–**14**[b]

Per-O-methyl derivative of		*H-1*[c]	*MeO-1*	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*
	δ	4.82 (3.4)	3.43	3.52	3.64	3.55	3.43
α-D-Glc*p* (**11**)	*G*	>15[d]	4.2	2.4	1.1	2.9	0.3
	B	++++	+++	++		+	
	δ	4.15 (7.0)	3.54	3.58	3.64	3.54	3.42
β-D-Glc*p* (**12**)	*G*	11.2	7.1	3.9	2.4	2.6	5.9
	B	+	+				+
	δ	4.86 (3.3)	3.41	3.51	3.51	3.57	3.40
α-D-Gal*p* (**13**)	*G*	>15[d]	8.6	7.7	0.5	3.3	1.8
	B	++++	+++	+++			
	δ	4.15 (7.0)	3.52	3.59	3.53	3.57	3.41
β-D-Gal*p* (**14**)	*G*	5.0	2.7	5.0	3.8	1.3	2.8
	B		+	+	+		+

[a]Broadenings are given qualitatively: ++++, very strong; +++, strong; ++, moderate; and +, weak; the remaining signals show no broadening at all. [b]Data for methoxyl groups taken from Ref. 6. [c]Coupling constants $J_{1,2}$ (in Hz) are given in brackets. [d]*G*-value cannot be determined accurately due to extreme broadening of signal.

TABLE II

CHEMICAL SHIFTS (δ, IN p.p.m.) OF THE ANOMERIC PROTONS AND METHOXYL GROUPS[a], AND LSR EFFECTS [SHIFT GRADIENTS, G (IN p.p.m., FOR $X = 1$), AND RELATIVE BROADENING, B[b]] INDUCED BY Eu(fod)$_3$, FOR THE PM-DISACCHARIDES 1–10

Per-O-methyl derivative of		*Anomeric protons*[c]		*MeO groups, non-reducing unit*				*MeO groups, reducing unit*				
		H-1	*H-1'*	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*	*MeO-1'*	*MeO-2'*	*MeO-3'*	*MeO-4'*	*MeO-6'*
β-D-Glc*p*-(1→4)-	δ	4.29 (7.2)	4.82 (3.6)	3.57[d]	3.63	3.53	3.41	3.42	3.51	3.58[d]	—	3.40
α-D-Glc*p* (1)	*G*	2.5	>15[f]	0.7	0.7	0.7	0.6	6.7	3.8	1.2	—	0.9
	B	+	++++					+++	++		—	+
β-D-Glc*p*-(1→4)-	δ	4.32 (7.2)	4.16 (7.4)	3.56	3.63	3.54	3.41	3.53	3.58	3.59	—	3.40
β-D-Glc*p* (2)	*G*	2.7	6.6	0.9	0.8	0.9	1.4	3.7	2.5	2.1	—	3.2
	B							+			—	+
α-D-Glc*p*-(1→4)-	δ	5.66 (3.6)	4.83 (3.6)	3.54[d]	3.64	3.55[d]	3.41	3.40	3.49	3.57	—	3.34
α-D-Glc*p* (3)	*G*	5.6	11.7	1.2	1.8	1.2	−0.2	3.8	2.7	1.6	—	0.6
	B	+	++					+++	++		—	++
α-D-Glc*p*-(1→4)-	δ	5.62 (3.6)	4.16 (7.4)	3.55[d]	3.64	3.55[d]	3.41	3.52	3.55	3.57	—	3.34
β-D-Glc*p* (4)	*G*	7.9	6.3	1.3	2.8	1.6	0.5	4.3	2.5	1.6	—	4.3
	B	+	+					+			—	+
β-D-Gal*p*-(1→4)-	δ	4.33 (7.0)	4.15 (7.4)	3.56	3.52	3.57	3.39	3.52	3.58	3.57	—	3.39
β-D-Glc*p* (5)	*G*	4.9	10.9	2.0	2.7	4.0	1.8	6.0	3.4	3.2	—	5.7
	B		+					+			—	+
β-D-Glc*p*-(1→6)-	δ	4.28 (7.0)	4.81 (3.2)	3.61	3.63	3.54	3.41	3.42	3.52	3.63	3.57	—
α-D-Glc*p* (6)	*G*	2.4	>15[f]	2.8	0.2	1.3	1.5	9.3	9.3	1.1	2.8	—
	B		++++					+++	+++			—
β-D-Glc*p*-(1→6)-	δ	4.32 (7.0)	4.14 (7.1)	3.59[d]	3.63[e]	3.54	3.42	3.54	3.58[d]	3.62[e]	3.52	—
β-D-Glc*p* (7)	*G*	4.5	4.9	1.9	1.6	1.4	2.4	2.4	1.9	1.6	2.9	—
	B											—
α-D-Gal*p*-(1→6)-	δ	5.08 (3.3)	4.15 (7.4)	3.52	3.49	3.57	3.40	3.54	3.58	3.63	3.50	—
β-D-Glc*p* (8)	*G*	8.5	1.6	13.6	1.4	1.9	1.1	−0.3	0.8	0.3	10.4	—
	B	+		+++							+++	—
α-D-Glc*p*-(1→2)-	δ	4.58 (7.0)	4.30 (7.1)	3.62[d]	3.62[d]	3.54	3.39	3.49	—	3.60	3.53	3.41
β-D-Glc*p* (9)	*G*	4.6	7.9	1.6	1.4	1.6	3.2	12.7	—	1.9	2.2	12.1
	B		+					+	—			+
α-D-Glc*p*-(1→2)-	δ	5.49 (3.6)	4.32 (7.2)	3.50[d]	3.64	3.54	3.41[e]	3.50[d]	—	3.57	3.54	3.41[e]
β-D-Glc*p* (10)	*G*	11.8	3.8	1.4	6.0	6.3	0.1	2.4	—	5.2	0.1	1.0
	B	+							—			

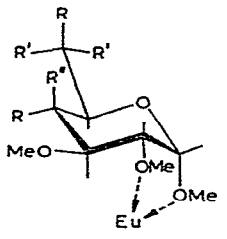
[a]Confidence in the different MeO assignments varies as set out in the text. [b]Broadenings are given qualitatively; see Table I, footnote *a*. [c]Coupling constants $J_{1,2}$ (in Hz) are given in brackets. [d,e]Assignments might be reversed; probable assignments given. [f]*G*-value cannot be determined accurately, due to extreme broadening.

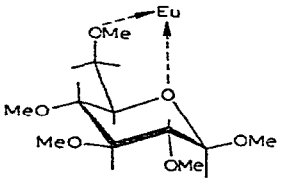
Structures of PM-disaccharide–LSR complexes

In order to interpret LSR data for the protons of a compound complexed with LSR, in terms of the structure of the complex, two relationships can be applied[9], *viz.*, the McConnell–Robertson equation[10] [LIS = $K(3\cos^2\theta-1)/r^3$, where the lanthanide-induced shift depends upon r, the distance between the lanthanide atom and the observed nucleus, and upon θ, the angle between the magnetic axis of the complex and the vector along which r is measured]; and the Sternlicht equation[11], which relates LSR-broadening to the term r^{-6}. This must be done with particular care in the case of PM-sugars, which possess several sites of comparable, intrinsic coordinating capacity (the MeO- and ring-oxygens), so that the observed effects are the time-averaged sum of contributions from complex formation at various sites; nor is the position of the magnetic axis always known for certain. In this study, therefore, qualitative use only has been made of these relationships.

The conclusions reached[6] in investigating $CDCl_3$ solutions of PM-aldohexopyranosides and their 6-deoxy analogues can be summarized by stating the preferences for different binding-sites towards $Eu(fod)_3$, which are as follows: O-1(eq)–O-2(ax) ≈ O-2(ax)–O-3(eq) ≈ O-1(ax)–O-2(eq) > O-6(ax)–O-5 > O-1(eq) ≈ O-2(eq) ≈ O-3(eq) ≈ O-6(eq) > O-4(ax or eq). Using $Pr(fod)_3$ or $Gd(fod)_3$, the sequence is similar, except that for these LSR: O-2(ax)–O-3(eq) > O-1(ax)–O-2(eq) ≈ O-6(ax)–O-5. In the study of PM-disaccharides, it seems advantageous to use $Eu(fod)_3$ because of its ability to differentiate between O-1(ax)–O-2(eq) and O-6(ax)–O-5, whereas the relative occupancy of the two sites by $Pr(fod)_3$ and $Gd(fod)_3$ could easily be influenced by steric factors in disaccharides containing an α-D-Glc*p* residue. The preferred binding-sites for $Eu(fod)_3$ in **11–13** are shown in Fig. 2; the equatorial MeO groups in **14** are favoured equally.

The information in Table II enables the following conclusions to be drawn regarding the preferred binding-sites for $Eu(fod)_3$ in the compounds **1–10**, and the MeO signal assignments. A number of the $Eu(fod)_3$–PM-disaccharide complexes are illustrated in Fig. 3.





12

11 R = OMe; R' = R" = H

13 R = H; R' = OMe, H; R" = OMe

Fig. 2. The preferred binding-sites of $Eu(fod)_3$ in the PM-monosaccharides **11–13**. In **14** (PM-β-D-Gal*p*), there is no preference for any particular site. For **13**, *both* favoured rotamers of the C-5–CH_2OMe group are given (C-6–O-6 can be antiparallel to C-5–C-4 or to C-5–O-5). See Ref. 6 for terminology (ax or eq) used for the C-6–O-6 bond.

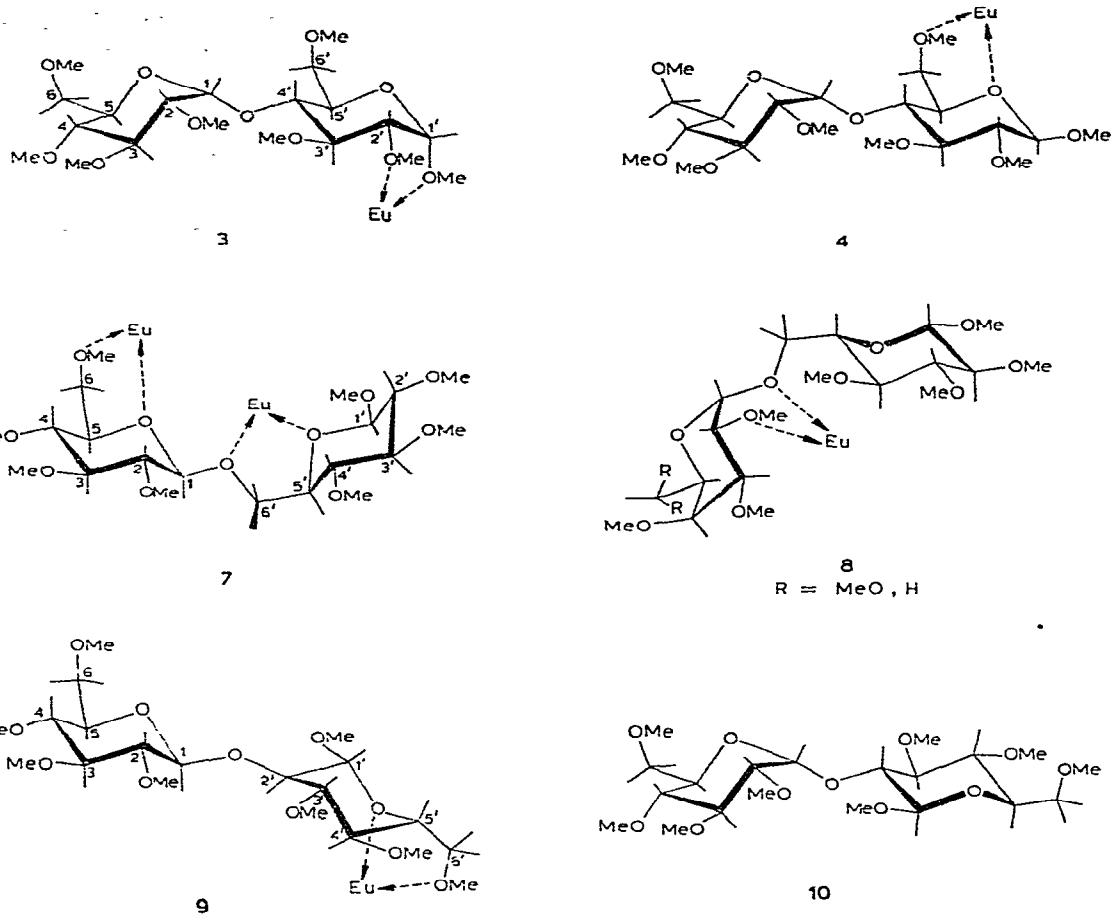


Fig. 3. The preferred binding-sites of $Eu(fod)_3$ in various PM-disaccharides. For **10**, no preferred binding-site(s) can be given (see text). Compounds **1**, **2**, **5**, and **6** are not shown, because the favoured binding-site in **1** and **6** is the same as in **3** (O-1′–O-2′), and in **2** and **5** it is the same as in **4** (O-6′–O-5′). For the D-Gal*p* unit of **8**, *both* favoured[6] rotamers of the C-5–CH_2OMe group are shown.

LSR complexes with (1→4)-linked PM-disaccharides

PM-α-cellobioside (**1**). — The large *G*-value and considerable line-broadening associated with each of the MeO signals at δ 3.42 and 3.51, with supporting evidence from the large LIS and broadening of the H-1′ signal compared with H-1, leads to their assignment to MeO-1′ and MeO-2′, respectively; this conforms to the sequence rule which requires preferential binding of $Eu(fod)_3$ at the O-1′(ax)–O-2(eq) site of **1**. Distinction between the other signals in the δ 3.40–3.42 group may be made on the basis of the broadening and larger *G*-value of MeO-6′ compared with the more distant MeO-6.

PM-β-cellobioside (**2**). — Here, both sugar units have the *β*-D-*gluco* configuration, and of the two preferred sites for binding (both O-6–O-5), the O-6′(ax)–

O-5′ is clearly favoured; this follows from the larger *G*-value and moderate broadening of one of the MeO-6 signals at δ 3.40 and 3.41, taken in conjunction with the enhanced LIS of H-1′ compared with H-1. Molecular models confirm this to be the more-exposed site. For PM-β-D-Glc*p* (**12**), it was found (see Table I) that with Eu(fod)$_3$ bound to MeO-6, the MeO-1 signal shows an even larger LIS and a similar broadening; the signal at δ 3.53 ($G = 3.7$) is consequently assigned to MeO-1′, and that at δ 3.54 to MeO-4. The cluster of three peaks δ 3.56, 3.58, and 3.59 may be assigned to MeO-2, MeO-2′, and MeO-3′, respectively, on the basis of *G*-values for MeO-2 and MeO-3 in **12**. The remaining signal at δ 3.63 is due to MeO-3.

PM-α-maltoside (**3**). — Of the O-1(ax)–O-2(eq) sites in **3**, that in the original non-reducing unit is sterically less suitable for binding (see Fig. 1), because O-1 is involved in the inter-sugar linkage. The signals at δ 3.40 ($G = 3.8$) and at δ 3.49 ($G = 2.7$) were accordingly assigned to MeO-1′ and MeO-2′, respectively. The signal at highest field $\delta = 3.34$ is attributed to MeO-6′, because it shows line-broadening similar to MeO-6′ in **1**, and because the larger shift-increment (*cf.* **11**) is expected of MeO-6′, as it is closer to the inter-sugar linkage than is MeO-6 (δ 3.41). The tentative assignments of MeO-2, MeO-4, and MeO-3′ can only rest on a comparison with **11**.

PM-β-maltoside (**4**). — The site expected from the sequence rule to have the highest LSR-affinity is inaccessible to Eu(fod)$_3$ (*cf.* **3**, above). Results of the LSR-experiments show that Eu(fod)$_3$ binds instead to O-6′(ax)–O-5′, rather than O-6(ax)–O-5; the MeO-6′ signal at δ 3.34 corresponds to its counterpart in **3** (compare also with **12**), and relative to MeO-6 has the greater *G*-value and line-broadening. The fact that the H-1′ signal shows a comparable *G*-value and line-broadening to that of H-1 confirms this view. With Eu(fod)$_3$ bound thus, the signal at δ 3.52 ($G = 4.3$ and moderate broadening) must be due to MeO-1′ (*cf.*, **2** and **12**). Signals for MeO-6, MeO-3, and MeO-3′ are assigned after comparison with **3**, and for MeO-2′ on the ground that the *G*-value (2.5) lies, as for corresponding methoxyl groups in **12**, between the values (4.3 and 1.6) for MeO-1′ and MeO-3′.

PM-β-lactoside (**5**). — As in **2** and **4**, Eu(fod)$_3$ binds preferentially to the O-6′(ax)–O-5′ site of **5**, a conclusion supported by the LSR effects on the anomeric protons. Of the two MeO-6 groups resonating at δ 3.39, that with the larger *G*-value (5.7) is due to MeO-6′, and of the groups resonating at δ 3.52 (MeO-1′ and MeO-3), the *G*-value of 6.0 distinguishes MeO-1′. MeO-2′ was assigned to the peak at δ 3.58 by comparison with data for **2**; configurational change at C-4 would not be expected to influence the situation at MeO-2′. Probable assignments of the group of three signals δ 3.56–3.57 to MeO-2, MeO-4, and MeO-3′ rest on the structural identity of **5** to **2** in the vicinity of the inter-sugar linkage, which suggests MeO-2 for the signal with the lowest *G*-value, and the trend $G_{MeO-2} > G_{MeO-3}$ for β-D-Glc*p* units in **2**, **4**, and **12**, which means that the signal with $G = 3.2$ is best assigned to MeO-3′.

LSR complexes with (1→6)-linked PM-disaccharides

PM-α-gentiobioside (**6**). — According to the sequence rule, Eu(fod)$_3$ will bind to MeO-1′ and MeO-2′; the observation of large *G*-values for MeO groups at δ 3.42

(axial MeO-1) and 3.52 (where MeO-2 in **11** is found), and a large LIS and excessive broadening of H-1′, substantiates this conclusion. Because of the relatively large distance between the sugar units, no great changes in chemical shift are expected for the MeO groups in comparison with those of the PM-monomers. Having established the assignment of MeO-1′, the peak at δ 3.41 must be due to MeO-6. The two MeO-3 groups evidently resonate at δ 3.63, that with the larger LIS being MeO-3′ (comparing with **11** and the reducing units in **1** and **3**). By inspection of **1** and **2**, the position of MeO-4 is found. This leaves MeO-4′ and MeO-2, for which a probable assignment is given; the *G*-values, being identical, yield no further information.

PM-β-gentiobioside (**7**). — The observation that both MeO-2 and both MeO-3 groups have identical LIS, and that MeO-6 shows a relatively large LIS, suggests that, in accordance with the sequence rule, $Eu(fod)_3$ favours both O-6(ax)–O-5 sites. The generally low *G*-values, and absence of line-broadening, indicate that there is steric hindrance to the approach of the lanthanide. Of the two signals at δ 3.54, that having the larger LIS is assigned to MeO-1′, because it is located closer to $Eu(fod)_3$ than is MeO-4. The large LIS of MeO-4′ must be due to the combined effects of the two preferred binding-sites.

PM-β-melibioside (**8**). — The largest LIS and broadenings are observed in the cluster of signals between δ 3.49 and 3.52, assigned to MeO-2, MeO-3, and MeO-4′. Of these, only the first (together with the axial oxygen of the inter-sugar linkage) can be expected, from the sequence rule, to be involved in firm binding with $Eu(fod)_3$; the large LIS of H-1 supports this view. The MeO signal with the largest *G*-value (13.6) is therefore attributed to MeO-2, and that with $G = 10.4$ to MeO-4′, rather than to MeO-3, on the basis of a comparison with **13**. Assignments of the δ 3.40, 3.54, and 3.63 signals are straightforward; of the remaining pair, δ 3.57 is more likely (*cf.* **13**) to be due to MeO-4 than to MeO-2′.

LSR complexes with (1→2)-linked PM-disaccharides

PM-β-sophoroside (**9**). — Of the two similar sites with the highest priority, O-6′(ax)–O-5′ is preferred on steric grounds, and on account of the large LIS of H-1′, which is nearly twice that of H-1; hence, the assignment of MeO-6′ and MeO-1′ on the basis of their large *G*-values, and of MeO-6. The two signals at δ 3.53 and 3.54 are assigned to MeO-4′ and MeO-4, upon comparison of their *G*-values with those of **12**. Of the group at δ 3.60–3.62, MeO-3′ has the *G*-value closest to that of MeO-4′ (*cf.* **12**), MeO-3 is expected to show the least deviation in δ from the value for **12**, and MeO-2 may be assigned on the basis that its *G*-value exceeds that of MeO-3.

PM-β-kojibioside (**10**). — The site with the highest priority, O-1(ax)–O-2(eq), is inaccessible to $Eu(fod)_3$ as in **2** and **4**, and the small LSR effects on the MeO-6 and MeO-6′ signals at δ 3.41 show that the O-6–O-5 sites are not favoured either. Weak binding to the various equatorial MeO groups is indicated by lack of broadening of the signals, though a preference for the non-reducing unit is indicated by the large LIS of H-1. By comparison with **11** and **12**, the signal δ 3.64 is assigned to MeO-3, and of

TABLE III

THE SHIFT INCREMENTS ($\Delta\delta$, IN p.p.m.) OF THE MeO GROUPS IN **1–10**, RELATIVE TO THE RESONANCE POSITION OF THE CORRESPONDING MeO GROUPS IN **11–14**

Per-O-methyl derivative of	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*	*MeO-1'*	*MeO-2'*	*MeO-3'*	*MeO-4'*	*MeO-6'*
β-D-Glc*p*-(1→4)-α-D-Glc*p* (**1**)	−0.01[a] (0.00)	−0.01	−0.01	−0.01	−0.01	−0.01	−0.06[a] (−0.07)	—	−0.03
β-D-Glc*p*-(1→4)-β-D-Glc*p* (**2**)	−0.02	−0.01	0.00	−0.01	−0.01	0.00	−0.05	—	−0.02
α-D-Glc*p*-(1→4)-α-D-Glc*p* (**3**)	+0.02[a] (+0.03)	0.00	0.00[a] (−0.01)	−0.02	−0.03	−0.03	−0.07	—	−0.09
α-D-Glc*p*-(1→4)-β-D-Glc*p* (**4**)	+0.03	0.00	0.00	−0.02	−0.02	−0.03	−0.07	—	−0.09
β-D-Gal*p*-(1→4)-β-D-Glc*p* (**5**)	−0.03	−0.01	0.00	−0.02	−0.02	0.00	−0.07	—	−0.03
β-D-Glc*p*-(1→6)-α-D-Glc*p* (**6**)	+0.03	−0.01	0.00	−0.01	−0.01	0.00	−0.01	+0.02	—
β-D-Glc*p*-(1→6)-β-D-Glc*p* (**7**)	+0.01[a] (0.00)	−0.01[b] (−0.02)	0.00	0.00	0.00	0.00[a] (+0.01)	−0.02[b] (−0.01)	−0.02	—
α-D-Gal*p*-(1→6)-β-D-Glc*p* (**8**)	+0.01	−0.02	0.00	0.00	0.00	0.00	−0.01	−0.04	—
β-D-Glc*p*-(1→2)-β-D-Glc*p* (**9**)	+0.04	−0.02	0.00	−0.03	−0.05	—	−0.04	−0.01	−0.01
α-D-Glc*p*-(1→2)-β-D-Glc*p* (**10**)	−0.02	0.00	−0.01	−0.02	−0.04	—	−0.07	0.00	−0.01

[a,b]Assignments of MeO groups might be reversed; least-probable $\Delta\delta$-values given between brackets.

the two at 3.54, the signal with $G = 6.3$ is more likely to be due to MeO-4 than to MeO-4'.

Influence of the inter-sugar linkage on chemical shifts of MeO groups

By comparison of the chemical shifts of the MeO groups in the PM-disaccharides **1–10** with those of the corresponding MeO groups in the PM-monosaccharides **11–14**, shift increments due to the presence of the inter-sugar linkage may be calculated (Table III). The following conclusions can be drawn.

(*a*) *The (1→4)-linked disaccharides* (**1–5**). — MeO-3' shows a considerable upfield-shift (0.05–0.07 p.p.m.), and so does MeO-6', although this effect is more pronounced for an α- (0.09 p.p.m.) than a β-linkage (0.02–0.03 p.p.m.). On the other hand, MeO-2 shows an upfield shift when the linkage is β, and a downfield shift when α. MeO-6 shows a small upfield-shift of 0.01–0.02 p.p.m. In general, larger incremental shifts are found for all MeO groups of the reducing unit in the case of an α-linkage, compared with the β-linkage. This finding is in agreement with the fact that the former type of linkage is more constricted[12], leading to stronger, steric perturbations for the MeO groups.

(*b*) *The (1→6)-linked disaccharides* (**6–8**). — The situation is less clear-cut than for the previous group of sugars: MeO-4' shows a downfield shift in **6** (β-linked), and an upfield shift in **7** and **8** (β- and α-linked, respectively). The incremental shifts of MeO-2 appear to be independent of the configuration at O-1, being downfield in all three cases. Similarly, for MeO-3', upfield shifts are found. The incremental shifts of the remaining MeO groups are small or negligible, in agreement with the fact that the relatively extended (1→6)-linkage will give rise only to small steric perturbations for the MeO groups located close to the linkage.

(*c*) *The (1→2)-linked disaccharides* (**9** *and* **10**). — Although only two compounds have been investigated, certain trends can be seen. Substantial incremental-shifts are observed for the MeO groups close to the linkage, MeO-1', MeO-3', MeO-6, and MeO-2, indicating considerable steric-crowding for these groups. Except for MeO-2, all increments are upfield. MeO-2 in **9** shows a downfield shift (β-linkage), and an upfield shift in **10** (α-linkage).

In conclusion, the shift increments can be related to the type and configuration of the inter-sugar linkage, and, in principle, they can give information about the conformation of the linkage. Furthermore, the observed trends in shifts can be utilized for assignment purposes of other PM-disaccharides.

From the incremental shifts recorded in Table III, the p.m.r. resonances for MeO groups of higher PM-saccharides can be estimated. Good results from such a procedure can be expected for PM-oligomers having α- or β-(1→6) or β-(1→4) linkages, as the observed incremental-shifts for their dimers are small or negligible (see **1**, **2**, and **6–8**). However, the estimations must be made with care for the more-constrained oligomers having α-(1→4) or α- or β-(1→2) linkages. Here, the influence of steric factors may change drastically in going from dimer to oligomer. For instance, in α-linkages, the conformation of the C-1–O-1 bond is dominated[13] by the exo-

anomeric effect, which results in a favoured conformation of this bond antiparallel to C-1–O-2. From molecular models, it can be inferred that in α-(1→4)-linked D-glucose disaccharides, this leads to a considerable steric crowding for the predominantly "axial" MeO-6 group of the reducing unit. Relief from this crowding can be obtained by a shift in rotamer population of this C-6–OMe group to the rotamer with C-6–O-6 antiparallel to C-4–C-5 (hence, the large shift-increment for MeO-6′ in **3** and **4**). However, molecular models of the α-(1→4) oligomer show that this relief from crowding is largely annihilated in the oligomer. Therefore, deviations from the incremental shift reported in Table III for this group can be expected for the oligomer. Similarly, in the case of (1→2)-linkages, extra steric constraints are introduced for the MeO groups close to the glycosidic linkage, in going from the dimer to the oligomer. It is clear that the determination of the MeO chemical-shifts for the middle units of PM-trimers will improve the estimations made for PM-oligomers containing the latter types of linkage.

CONCLUSIONS

This study demonstrates that the binding of $Eu(fod)_3$ to PM-disaccharides is specific, in spite of the large number of possible binding-sites. The sequence rule derived for the PM-monosaccharides is applicable to the PM-disaccharides, when the extra steric-hindrance factors are taken into account. When only one binding-site is preferred by $Eu(fod)_3$, the observed LSR effects can readily be interpreted, and be used for the assignment of the MeO groups. When more than one binding site is preferred (as in **7** and **10**), the LSR effects are more complicated and, as yet, no conclusions regarding MeO assignments can be drawn. The results give rise to the expectation that LSR experiments will also be useful for MeO assignments in small PM-oligosaccharides (such as trisaccharides) when the LSR binds preferentially to one unit.

Comparing the data in Tables I and II, it can be observed that, in some cases, the LIS of the MeO groups, to which $Eu(fod)_3$ is preferentially bound, are larger in the PM-disaccharide than in the corresponding monosaccharide (*cf.*, for instance, MeO-1′ and MeO-2′ in **1** with MeO-1 and MeO-2 in **11**). This difference is probably due to the stronger preference for this site in the disaccharide; the other sites are more sterically hindered. An alternative explanation, that $Eu(fod)_3$ approaches closer to the site in the case of the disaccharide (which would make r in the McConnell–Robertson equation smaller, and, consequently, the LIS larger), is unlikely, considering the larger steric hindrance involved.

When more PM-disaccharides have been investigated by using LSR, it is expected that it will be possible to draw conclusions regarding the conformation of these compounds in solution (*viz.*, the torsion angles of the inter-sugar linkages). An indication that such information can, in principle, be deduced from LSR studies is obtained when the LIS observed for the MeO groups of the non-reducing unit of the

cellobiosides **1** and **2** are compared with those for the maltosides **3** and **4**. In **1** and **3**, $Eu(fod)_3$ is preferentially bound to the same site, O-1′–O-2′; nevertheless, the LIS of the MeO groups of the non-reducing units show a characteristic difference. In **1**, they are practically the same (0.6–0.7), but in **3**, they vary from −0.2 to 1.8. A similar difference is observed for **2** and **4**. These data indicate a difference in the relative positions of the two pyranose rings in **1** and **2** with respect to **3** and **4**. However, for further elaboration on this point, more data are required regarding, for instance, the position of the magnetic axis of the LSR-sugar complex. Further, the degree of exclusiveness of the preferred binding-site is important: whether or not the contributions from the binding of LSR to other sites are negligible.

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