Carbohydrate Research, 31 (1973) 255–263

EVALUATION OF THE BINDING CONSTANTS, BOUND CHEMICAL SHIFTS, AND EQUILIBRIUM STOICHIOMETRY FOR THE ASSOCIATION OF LANTHANIDE SHIFT REAGENTS WITH CARBOHYDRATE DERIVATIVES*

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(Received: May 21st, 1973; accepted for publication, July 3rd, 1973)

ABSTRACT

The molecular associations of tris(dipivalomethanato)europium(III) (**1**) and of tris(2,2-dimethyl-6,6,7,7,8,8,8,-heptafluoro-3,5-octanedionato)europium(III) (**2**) with six carbohydrate derivatives have been studied. The interactions of **2** in deuterochloroform solution appear to be "normal", *i.e.*, the plot of [sugar concentration] *versus* [induced chemical-shift change]$^{-1}$ is linear and can be interpreted in terms of the equilibrium binding constant (K_B) and the bound chemical shifts (Δ_B). Similar results are found for **1** in carbon tetrachloride solutions. However, in chloroform solutions, non-linear plots are obtained that cannot be simply interpreted. Possible reasons for this behaviour are given.

INTRODUCTION

It is now well known[3] that, in solution, certain chelate complexes of some of the lanthanides can induce very substantial changes in the chemical shifts of other organic molecules. In a previous paper[4], we discussed some of the factors that are relevant if the sole objective of an experiment is to obtain an optimally dispersed n.m.r. spectrum of the substrate substance. We now discuss a quantitative evaluation of the chemical equilibrium that necessarily occurs when a lanthanide shift** reagent (L) associates in solution with an organic substrate (S). This equilibrium can be

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*Part V of a series, "Nuclear Magnetic Resonance Studies of Paramagnetic Species". For a preliminary communication, see Ref. 1. For Part IV, see Ref. 2. An account of part of this work was given at a meeting of the Carbohydrate Discussion Group of the Chemical Society, held at the University of Sussex, Great Britain, April 1972.

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**Lanthanide ions in aqueous solutions can also[5,6] induce changes in chemical-shift. Although such systems follow many of the prerequisites discussed here, it is obvious that there are additional complications that merit separate discussion elsewhere.

defined[7]

$$L+nS \rightleftharpoons LS_n \qquad (1)$$

in terms of the equilibrium binding constant (K_B), the solution stoichiometry (n), and the bound chemical shifts (Δ_B) of each of the probe sites of the substrate. It should be realised at the outset that the sizes of the observed changes in chemical shift are dependent on the magnitudes of the values of both K_B and Δ_B.

In order that the lanthanide shift reagent induce any chemical shift change in the substrate, it is necessary that there be some degree of association between these two components and this leads to the following basic requirements.

(*a*) The lanthanide shift reagent must have at least one vacant "receptor" site, so that it can act as a Lewis acid and accept a donor bond from S. This condition is satisfied by the tris(dipivalomethanato)lanthanide(III) complexes [Ln(DPM)$_3$] and by the tris (2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato)lanthanide(III) complexes [Ln(FOD)$_3$]. Because of the electronegativity of the perfluoropropyl group, the latter chelates form the stronger complexes (higher K_B-values) and hence, all other factors being constant, induce larger observed shift changes than the DPM series of derivatives.

(*b*) A corollary of (*a*) is that the substrate (S) must have at least one site capable of forming a donor bond to the lanthanide. Since the lanthanides are "hard acids", substituent functionalities having oxygen or nitrogen atoms are suitable; those with a sulphur atom are less so. Fortunately, a variety of the functional groups commonly found in carbohydrate systems form reasonably strong donor bonds. The effectiveness of the donor properties of a molecule is reflected in the value of the binding constant (K_B).

(*c*) Measurements should be made in a solvent which is either unable to form a complex with the lanthanide or can interact only weakly. Thus, such solvents as acetone are unsuitable, and carbon tetrachloride is ideal. Such solvents as chloroform or benzene can also be used for qualitative experiments where the sole objective is to obtain an optimally dispersed n.m.r. spectrum; however, for quantitative studies, they can induce some complications (*vide infra*).

Besides inducing chemical shift changes, all lanthanide chelates necessarily induce changes in the nuclear relaxation times of the substrate molecule, and this in turn results in changes in the line widths of the substrate resonances[8]. Clearly, a shift reagent should have an optimal ratio of "induced shift" to "increase in line width"; for that reason, shift reagents based on europium, praseodymium, and, to a lesser extent, thulium have been widely studied. It is important to note that gadolinium(III) complexes cause no detectable changes in shifts, although they do induce substantial changes in line widths*.

The experiments described in this paper were intended to delineate the perspec-

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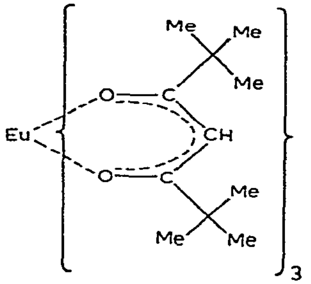
*This observation supports[9] the contention[10] that, for protons, shift changes are induced solely by the pseudo-contact mechanism[11].

tive of the above facts in the context of monosaccharide derivatives; particular attention was directed to the evaluation of the Δ_B-values. As will be seen, several additional points of interest have come from these studies. Two shift reagents were used, namely, tris (dipivalomethanato)europium(III) [**1**, Eu(DPM)$_3$] and tris (2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedianato)europium(III) [**2**, Eu(FOD)$_3$].



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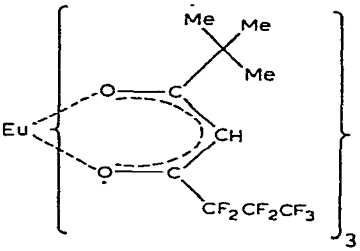

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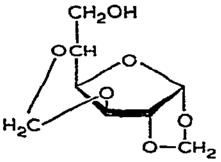
RESULTS AND DISCUSSION

The interaction between Eu(FOD)$_3$ and the compounds **3**–**7** were evaluated in the usual way. In each instance, a perfectly linear plot of $[S]_0$ *versus* $1/\delta$ was obtained, and the values of K_B and Δ_B derived from these plots (see Experimental) are given in Tables I and II. In some instances, the y-axis intercepts were too small for an accurate evaluation to be made of the K_B-value (see Fig. 1). However, in nearly every instance, the values of the bound chemical shifts (Δ_B-values) were accurately evaluated; the only exceptions were when the induced shift was very small. We shall discuss the Δ_B-values later, and, for the present, comment only that the interpretation of the K_B-values in chemical terms is not obvious. For example, on purely steric grounds, it might have been anticipated that **6** would bind more strongly than **7**, whereas the



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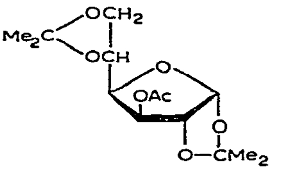

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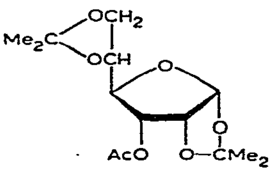

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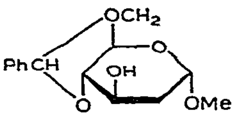

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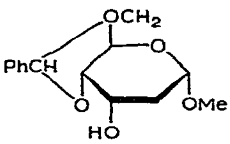

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TABLE I

CALCULATED VALUES[a] OF BOUND CHEMICAL SHIFTS (Δ_B-VALUES, p.p.m.) AND BINDING CONSTANTS (K_B-VALUES, l.mole^{-1}) FOR COMPLEXES OF FURANOSES WITH EITHER Eu(DPM)$_3$ OR Eu(FOD)$_3$

Substrate	*Shift reagent*	*Solvent*	*Parameter*	*Proton probe*				
				H-1	*H-2*	*H-3*	*H-4*	*H-5*
3	Eu(FOD)$_3$	CDCl$_3$	Δ_B	3.00	2.60	3.67	7.83	14.21
			K_B			≥ 100		
4	Eu(FOD)$_3$	CDCl$_3$	Δ_B	2.97	7.41	13.10	4.91	6.34
			K_B	69.8	65.2	79.0	[b]	[b]
5	Eu(FOD)$_3$	CDCl$_3$	Δ_B	[b]	4.62	12.80	8.57	14.37
			K_B			≥ 100		
8	Eu(DPM)$_3$	CCl$_4$	Δ_B	4.46	6.06	15.70	8.61	22.14
			K_B	32.7	31.0	29.0	[b]	30.0

[a]Based on experiments performed at 40.0°. [b]This value could not be determined with sufficient accuracy to justify inclusion here.

TABLE II

CALCULATED VALUES[a] OF BOUND CHEMICAL SHIFTS (Δ_B-VALUES, p.p.m.) AND BINDING CONSTANTS (K_B-VALUES, l.mole^{-1}) FOR COMPLEXES OF PYRANOSES WITH Eu(FOD)$_3$ IN DEUTEROCHLOROFORM SOLUTION

Substrate	*Parameter*	*Proton probe*									
		H-1	*H-2a*	*H-2e*	*H-3*	*H-4*	*H-5*	*H-6$_1$*	*H-6$_2$*	*PhCH*	*OMe*
6	Δ_B	2.29	9.93	8.17	13.66	ca. 6.3	2.58	ca. 9.4	5.41	3.98	1.33
	K_B				≥ 75						
7	Δ_B	9.64	9.41	15.95	6.67	2.33	[b]	3.39	6.61	2.47	5.59
	K_B				≥ 100						

[a]Based on experiments performed at 40°. [b]This value was not determined with sufficient accuracy to justify inclusion here.

data loosely imply the converse. It is interesting to note that, for **7**, intramolecular hydrogen-bonding is feasible.

Attention was next directed to experiments involving Eu(DPM)$_3$ (**1**). Two facts concerning the complexing properties of this reagent were already known. Firstly, it had previously been shown that, for any chosen substrate, **1** binds less strongly than Eu(FOD)$_3$. Secondly, it was known that the binding constants of **1** in carbon tetrachloride solution was approximately four times that measured for a deuterochloroform solution.

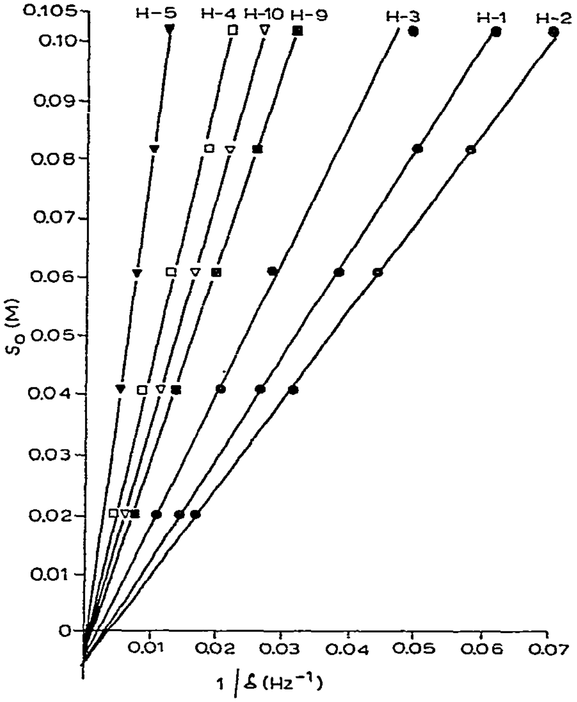
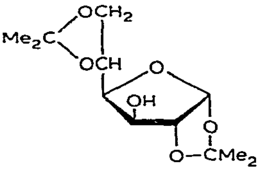


Fig. 1. Plot of $[S]_0$ versus $(1/\delta)$ for the interaction of 1,2:3,5-di-*O*-methylene-α-D-glucofuranose (3, 0.02 to 0.102M) with Eu(FOD)$_3$ (2, 5.9×10^{-3}M) in deuterochloroform solution. The precision of this plot is indicated by the close convergence of all lines to essentially the same *y*-axis intercept. The plots for protons H-1 to H-5, inclusive, follow the conventional numbering system; the plots labelled H-9 and H-10 are for two of the methylene protons.

The initial experiment involving 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (8) with Eu(DPM)$_3$ in carbon tetrachloride solution gave results identical with those from the Eu(FOD)$_3$ experiments. A linear plot of $[S]_0$ *versus* $1/\delta$ was obtained and



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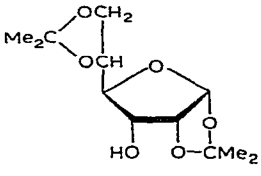

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now, as anticipated, the *y*-axis intercept was sufficiently large to permit an accurate evaluation of K_B (see Table I). Since this experiment seemed to represent "normal" responses, it was repeated with deuterochloroform as solvent. Although addition of a small molar ratio of **1** induced the anticipated shift changes, further additions caused only minor increases in these shifts. Instead, the major effect was that the line widths of the resonances of **8** increased substantially. Essentially the same behaviour was observed for 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**9**) and for **3** interacting with Eu(DPM)$_3$ in deuterochloroform solution, and also for **9** with Eu(DPM)$_3$ in carbon tetrachloride solution. In each experiment, the line broadening varied from resonance to resonance, suggesting that complexing was occurring but that it was not inducing the expected shift. Blank experiments on the solvent and all reagents eliminated the possibility that this phenomenon was due to the presence of such impurities as water. The simplest explanation for the leveling-off of the induced shifts on addition of further shift reagent would be that there is a two-step binding process, where the induced shift for the LS_2 complex is smaller than that for the LS complex:

$L+S \rightleftharpoons LS$ (large bound shift)

$LS+S \rightleftharpoons LS_2$ (smaller bound shift).

It may then be conjectured that binding of the second substrate molecule results in "crowding" of the first, so that both of them end up further away from the lanthanide atom, and hence have smaller Δ_B-values. This rationale has the additional merit that, if one of the two binding steps was relatively slow, the line-broadening effects that also characterise these experiments can be rationalised.

Further evidence concerning the difference between complexing of Eu(DPM)$_3$ in the carbon tetrachloride and deuterochloroform solutions came from the behaviour of the hydroxyl-proton resonances. In the absence of any lanthanide, the resonance of the hydroxyl proton always appears as a multiplet due to spin coupling with the adjacent ring hydrogen. Although, in all cases, addition of even the smallest quantities of Eu(DPM)$_3$ caused immediate removal of this splitting, in some instances, substantial quantities of Eu(FOD)$_3$ could be added before the coupling disappeared*. Although we shall not discuss these experiments here, it should be noted that Frankel[12] has made extensive studies of "chemical exchange spin-decoupling". It is also worthwhile commenting that the Eu(FOD)$_3$ complex has a greater affinity for water than does the Eu(DPM)$_3$ reagent, which, in view of the preceding observation, further substantiates the spurious effect of any water impurity.

One of the most important, potential applications of lanthanide shift reagents is to the solution of conformational and configurational problems, and that is why the accurate determination of Δ_B-values is so important. According to theory, the magnitude of the induced change in chemical shift is proportional to $(3\cos^2\theta-1)/r^3$, where r is the distance between the unpaired electron and the nucleus of interest, and θ is the angle between this distance vector and the principle magnetic axis of the complex. It follows from this relationship that there will in general be no simple

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*For example, in experiments with neopentyl alcohol, shifts of ~400 Hz could be induced into the hydroxyl resonance with the HO–CH$_2$ coupling still clearly resolved.

relationship between the distance of a particular proton from the principal binding-site and the Δ_B-value for that proton; instead, allowance must be made for the angular term. Clear demonstration of this point is given by the data for compounds **5**, **7**, and **8**, all of which show a "remote" proton undergoing the largest shift.

The situation is further complicated by the fact that the rotational lability between the substrate and the lanthanide can vary widely[2], ranging from essentially free rotation about the donor–lanthanide axis to essential rigidity. As a result, calculations of precise solution geometries can be somewhat tedious.

Fortunately, it may be possible to compare the configurations of related compounds simply by comparing shift ratios. However, this procedure will be reliable only if the compounds complex identically with the lanthanide, and that is difficult to predict. The Δ_B-values given in Tables I and II serve to show the magnitude of shift differential that can occur between pairs of compounds (**4**–**5** and **6**–**7**) that differ in configuration at a single centre. Further experiments to decide whether such simplistic comparisons are likely to be effective are planned.

CONCLUSIONS

The method by which a lanthanide shift reagent is used depends strictly on which final data are required. If optimally dispersed spectra, suitable for direct, first-order assignments are required, either a $Ln(DPM)_3$ or a $Ln(FOD)_3$ reagent can be used, preferably following a dropwise addition of a stock solution. As we have observed previously[4], the advantage of the $Ln(FOD)_3$ compounds is their higher solubility.

If reliable values for the bound chemical shifts (Δ_B-values) are required for the calculation of solution geometry, it is necessary to measure the induced shifts (δ) as a function of substrate concentration, with the lanthanide concentration held constant (at a suitably low value). Thereafter, plots of $[S]_0$ *versus* $1/\delta$ will lead to a straight line for each resonance, with a slope related to the magnitude of the Δ_B-value of the individual proton concerned. In principle, the same experiment also leads[7] to a reliable value of the equilibrium binding constant (K_B-value) for the association of the shift reagent with the substrate. In practice, the intercepts for experiments with $Ln(FOD)_3$ shift reagents only give a lower bound to the K_B-value.

Experiments with $Ln(DPM)_3$ reagents can give reliable Δ_B-values, but, with substrates having a hydroxyl donor group, complications can prevent the reliable determination of any parameters.

On this basis, it would appear that $Ln(FOD)_3$ reagents are to be preferred when the principal binding site is a hydroxyl function. The sole, possible disadvantage of these reagents is their tendency to self associate* in solution, and for that reason it is necessary to work with the lowest possible concentrations of lanthanide.

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*Self association of lanthanide shift reagents has been the subject of several conflicting reports. An early n.m.r. study of $Ln(DPM)_3$ reported[13] that, at higher concentrations, self association occurred. However, on the basis of a more recent n.m.r. study, using solutions that were rigorously anhydrous,

EXPERIMENTAL

All materials and equipment used in this study were rigorously dried before use.

The substrates were carefully purified by repeated recrystallisation and were dried in a vacuum desiccator over phosphorus pentaoxide for several weeks before use. Their melting points were in agreement with the literature values.

Carbon tetrachloride (A. R. Grade) was distilled and stored over sodium hydroxide pellets prior to use. Deuterochloroform (99.8%) from Merck, Sharp and Dohme (Montreal) was stored over Linde Molecular Sieve (4A), which had been previously dried at 100° for 24 h prior to use.

A sample of the lanthanide reagent was vacuum-sublimed immediately prior to an experiment. All apparatus used to make up the solutions, and all n.m.r. tubes were baked at 100° for at least 16 h prior to use. All manipulations and the preparation of solutions were conducted in a glove-bag that had previously been flushed with dry nitrogen gas.

All n.m.r. measurements were made with a Varian XL-100 (15) instrument operating in the conventional frequency-swept mode; the probe temperature was 40.0°. Tetramethylsilane was used as the internal reference signal for the field-frequency lock.

All plots of the experimental data were made with a Hewlett Packard Calculator (model 9100B) and a Hewlett Packard Plotter (model 9125A).

Although full details have previously been given[7,9] of the method used to make up the solutions required for these experiments, sufficient details are repeated here for this account to be self contained.

The lanthanide reagent [~0.042 g, 0.012M of $Eu(DPM)_3$; ~0.070 g, 0.012M $Eu(FOD)_3$] was dissolved in a mixture of 4.8 ml of solvent and 0.2 ml of tetramethylsilane, contained in a 5.0-ml volumetric flask. The substrate was dissolved separately in 5.0 ml of the same solvent. Aliquots (0.50 ml) of the lanthanide solution were added to each of a series (5 to 10) of graduated (1.0 ml) flasks. To these flasks were added various amounts of the stock solution of the substrate; *e.g.*, vial 1, 0.50 ml; vial 2, 0.40 ml; etc. The content of each flask was then made up to 1.0 ml with further solvent. Each solution was placed in a separate n.m.r. tube, that was then sealed and stored in a thermostatted water-bath until required.

THEORY

For full details of the theory for the analysis of the equilibrium depicted in equation (*1*), see refs. 7 and 9.

Provided that the lanthanide concentration is held constant at a low value

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Hart and co-workers concluded[14] that $Ln(DPM)_3$ reagents are invariably monomeric in solution. However, if the solutions were exposed to the atmosphere, other species were detected; most probably these were the species observed in the earlier study, and it was concluded that these were not the dimeric species. A definitive study, based on simple measurements of osmotic pressure to determine the number of particles in solution, showed unequivocally[15] that $Eu(DPM)_3$ did not dimerise in any solvent, but that dimerisation of $Eu(FOD)_3$ became progressively more serious as the solvent was varied from chloroform to carbon tetrachloride to hexane.

(<0.006M) and the concentration of the substrate (S) is much greater than the lanthanide (L) concentration, it follows that a plot of $[S]_0$ *versus* $1/\delta$ is linear with slope $[L]_0 \cdot \Delta_B$ and y-axis intercept $-\{1/K_B+[L]_0\}$.

The observed linearity of the above plot also provides evidence that the stoichiometry (n) of the association is unity. For 2:1 stoichiometry, the above plot is generally curved, but a linear plot is obtained for a plot of $[S]_0{}^2$ *versus* $[S]_0\,(1/\delta)$.

It should be noted that the above procedure only gives linear plots at low $[L]_0/[S]_0$ ratios. There are many factors that contribute to this; for Ln(FOD)$_3$ derivative, but not for the Ln(DPM)$_3$ series, these include self association of the shift reagent[12].

We have not repeated here any discussion of the uncertainties which attend attempts[16] to derive accurate values for K_B and Δ_B from experiments in which the substrate concentration is held constant and the lanthanide concentration is varied; for a detailed appraisal and rejection of those experiments, the reader is referred elsewhere[7]. Neither have we given details of the Scatchard Plot which can be used[9] in place of the method described above.

ACKNOWLEDGMENTS

This work was supported by operating grants from the National Research Council of Canada (A 1905 to L. D. H. and A 6178 to A. G. M.), and by funds from the Alfred P. Sloan Foundation.

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