Carbohydrate Research, 69 (1979) 39–46
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

APPLICATION OF LANTHANIDE SHIFT REAGENTS TO ^{1}H- AND ^{13}C-N.M.R. SPECTRA OF SOME CARBOHYDRATE DERIVATIVES

PATRICK MCARDLE, JOHN O. WOOD, ELIZABETH E. LEE,
Department of Chemistry, University College, Galway (Ireland)

AND MICHAEL J. CONNEELY
Department of Mathematical Physics, University College, Galway (Ireland)

(Received December 29th, 1977; accepted for publication, March 2nd, 1978)

ABSTRACT

N.m.r. line-shifts (^{1}H and ^{13}C) induced by Eu(fod)$_3$ in the spectra of methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside (**1**), methyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2**), and methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**3**) have been measured. The shift data allow a complete assignment of the ^{1}H- and ^{13}C-n.m.r. spectra of **1**. However, it was necessary to consider the spectra of the component monosaccharides, together with the shift data, in assigning the ^{13}C-spectra of **2** and **3**. Atom positions were measured on Drieding models, and shifts calculated by using the axial symmetry approximation were in excellent agreement with the observed values.

INTRODUCTION

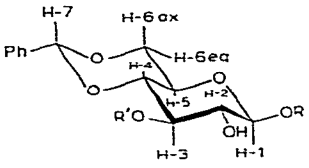
Previous n.m.r. studies involving lanthanide shift reagents on carbohydrate systems have been largely of a qualitative nature[1]. Exceptions were the studies of Armitage and Hall, where lanthanide–substrate equilibrium constants were determined[2] and the effect of rotation about the lanthanide–donor axis was examined[3]. The qualitative use of shift reagents in the simplification of n.m.r. spectra will continue to be of great value in aiding both the direct assignment of ^{1}H-spectra and the assignment of ^{13}C-spectra by selective ^{1}H-decoupling. A quantitative approach is necessary, however, if any of the structural information inherent in the shift data is to be extracted.

In general, a ligand co-ordinated to a paramagnetic ion can experience (*i*) contact effects, proportional to unpaired electron density at the nuclei examined; and (*ii*) dipolar or pseudo-contact effects, described by equation *1* for lanthanide–substrate systems with one set of axially symmetric, magnetic axes,

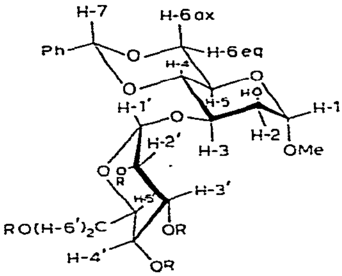
$$\Delta H = \frac{K\,.\,(3\cos^2\theta - I)}{R^3} \tag{1}$$

so that relative shifts depend only on the lanthanide–nucleus distance, R, and the angle θ made by this vector and the principal magnetic axis. The dipolar effect has been exploited in some studies of lanthanide–substrate configuration in solution[3-5]. Both dipolar and relaxation data have been used to obtain solution conformations of the structurally similar nucleotides[6]. In view of the lack of data on carbohydrate systems, this shift-reagent study was undertaken.

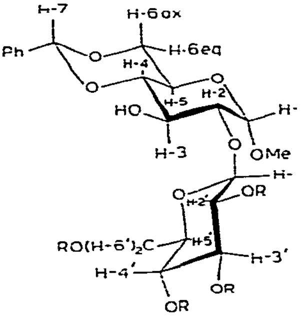
Systems were chosen which possessed two characteristics: a single, relatively dominant, donor group, to ensure a range of shifts and define a magnetic axis, and a set of nuclei of known fixed geometry whose shifts could be used to calculate a europium position. The compounds chosen were methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-glucopyranoside[7] (**1**), methyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside[8] (**2**), and methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside[9] (**3**). The ^{1}H- and ^{13}C-n.m.r. shifts induced by Eu(fod)$_3$ (fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethylocta-4,6-dione) were obtained and compared with values calculated by using a computer search procedure. At present, the program is being modified to allow the position of substituent groups, with variable conformation relative to the set of fixed nuclei, to be determined. It is hoped that this work will provide information on the solution structures of some di- and oligo-saccharides.



1 R = R' = Me
4 R = R' = PhCH$_2$
5 R = PhCH$_2$, R' = Me



2 R = Ac



3 R = Ac

RESULTS AND DISCUSSION

Compound **1**. The ^{1}H-n.m.r. spectrum of **1** (100 MHz, CDCl$_3$) could be only partially assigned directly, as follows: δ 7.15 (m, 5 H, Ph), 5.3 (s, 1 H, PhC*H*), 3.54 (s, 3 H, OMe), 3.44 (s, 3 H, OMe), and 2.85 (broad s, 1 H, OH; confirmed by deuterium exchange). A 2-proton resonance at δ 4.2 was probably due to H-1 and H-6eq, by analogy with the spectra of related compounds previously reported[10]. Resonances due to the remaining pyranoside-ring protons and H-6ax were overlapping and obscured by the methoxyl signals. After the first addition of Eu(fod)$_3$, the signal at δ 4.2 shifted and was resolved into a doublet ($J_{1,2}$ 7 Hz) for H-1, and a doublet of doublets ($J_{6,6'}$ 10, $J_{6,5}$ 4 Hz) for H-6eq. Further additions of europium resolved the reso-

TABLE I

^{1}H-N.M.R. CHEMICAL-SHIFT DATA[a]

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6ax*	*H-6eq*	*H-7*	*OH*	*H-1'*[b]	*H-2'*	*H-3'*	*H-4'*	*H-5'*	*H-6'*	*OMe*	*Ph*	*OAc*
1[c]	*4.21*	3.39	3.35	3.62	3.40	3.40	4.19	*5.3*	*2.85*	—	—	—	—	—	—	*3.44,* *3.54*	*7.15*	—
2[c]	4.78	4.12	4.17	4.30	3.83	3.85	4.35	*5.6*	*2.80*	5.28	5.38	5.28	5.15			*3.40*	*7.35*	*1.98–2.10*
3[d]	4.80	*3.58*[e]	*(3.4*———	———	———	——— *3.9)*	*4.30*	*5.5*	*2.20*	4.80	5.00	5.10	4.95	*3.7*[e]	4.10	*3.40*	*7.35*	*1.96–2.04*

[a] δ units. Italicised values were observed directly. All others were obtained from shift-reagent plots (see text). [b] Superscripts refer to the acetoxyl-substituted ring in disaccharide derivatives. [c] Measured in $CDCl_3$ solution. [d] Measured in CD_2Cl_2 solution. [e] Assignments made by spin-decoupling experiments on normal spectrum.

TABLE II

^{13}C-N.M.R. CHEMICAL-SHIFT DATA (P.P.M. DOWNFIELD FROM Me_4Si)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-1'*[a]	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*OMe-1*	*OMe-3*	*Ph*	*PhCH$_2$-1*	*PhCH$_2$-3*	*OAc*
1	104.3	74.1	82.4	81.5	66.3	68.7	101.3	—	—	—	—	—	—	57.4	60.9	126–137	—	—	—
4	102.3	74.5	80.4	81.4	66.5	68.8	101.4	—	—	—	—	—	—	—	—	125–139	71.4	74.6	—
5	102.3	74.3	82.3	81.6	66.5	68.8	101.4	—	—	—	—	—	—	—	60.9	126–137	71.5	—	—
2	101.5	71.0	73.8	78.5	63.5	68.8	101.5	98.5	69.0	69.3	66.6	69.0	62.2	55.0	—	126–137	—	—	20.67
6[13]	101.7	70.6	68.0	78.5	62.9	68.4	101.7	—	—	—	—	—	—	54.4	—	125–138	—	—	—
7	—	—	—	—	—	—	—	98.7	69.1	69.6	66.4	68.5	62.6	55.3	—	—	—	—	20.60
3[b]	100.5	82.2	69.8	81.8	62.5	69.4	102.2	102.2	71.9	73.0	69.0	72.4	62.4	55.8	—	126–137	—	—	20.80
8[13]	99.9	72.4	70.5	80.8	62.0	68.5	101.5	—	—	—	—	—	—	54.9	—	126–138	—	—	—
9	—	—	—	—	—	—	—	101.6	71.3	73.0	68.6	71.8	62.1	56.9	—	—	—	—	20.50

[a] Superscripts refer to carbon atoms of the acetoxyl-substituted ring. [b] Measured in CD_2Cl_2 solution; all other spectra were measured in $CDCl_3$ solution.

nances due to H-2, H-3, and H-4, which were all triplets with $J \sim 9$ Hz. This result is as expected for β-D-glucopyranoses in the $^4C_1$ conformation, where the ring protons are all trans-diaxial. These assignments were confirmed by spin-decoupling experiments.

Good straight-line plots of induced shift *vs.* molar ratio [Eu(fod)$_3$/substrate] were obtained. Extrapolation to zero concentration of europium gave the chemical-shift values listed in Table I. Although these chemical shifts were derived by ignoring possible AB interactions (and are therefore approximate), they correlate well with known values for similar systems[10-12].

The ^{13}C-n.m.r. spectrum of **1** is given in Table II. This assignment is in good agreement with assignments reported by Guthrie *et al.*[13] for a series of related compounds. However, ^{13}C europium-shift data and selective ^{1}H-spin-decoupling experiments allowed direct assignment of all resonances. The methoxyl resonances were individually assigned by comparing the spectrum of **1** with those[8] of **4** and **5**.

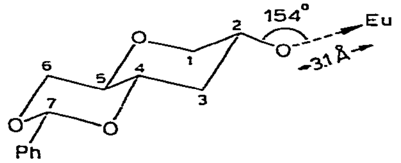
Compound **2**. The ^{1}H-n.m.r. spectrum of **2** (CDCl$_3$) was assigned in a manner similar to that described for **1**; the ^{1}H-chemical shifts are given in Table I. Extrapolated chemical-shift values were not obtained for poorly resolved resonances (H-5′, 6′).

The ^{13}C-n.m.r. spectrum was partially assigned by using Eu(fod)$_3$ shift data and selective ^{1}H-decoupling. The remaining resonances were assigned by comparison with spectra of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**6**) and methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**7**). The ^{13}C-spectrum of the former has been assigned previously[13], and that of **7** was completely assigned by selective ^{1}H-spin-decoupling. The shift data for **2**, **6**, and **7** are given in Table II.

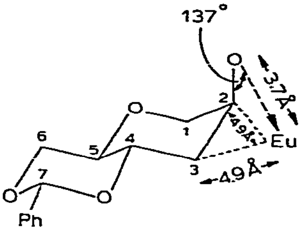
Compound **3**. On addition of Eu(fod)$_3$ to a CD$_2$Cl$_2$ solution of **3**, the ^{1}H-n.m.r. spectrum was not as well resolved as that of **2**. The extrapolated chemical shifts obtained for well-resolved resonances are given in Table I.

The ^{13}C spectrum was again partially assigned by using the shift reagent. The complete assignment given in Table II was obtained by comparison with the spectra of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[13] (**8**) and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**9**). The spectrum of the latter was assigned by selective ^{1}H-spin-decoupling.

Calculation of Eu(fod$_3$)-induced shifts. The relative shift gradients, *G* (p.p.m./mol of Eu(fod)$_3$/mol of substrate), derived from the experimental plots of induced shift *vs.* mole ratio, are given in Table III. Drieding models of **1**, **2**, and **3** were constructed and (*x,y,z*) co-ordinates for ring carbon and hydrogen atoms were measured with respect to an arbitrary origin. An 0.2-Å grid within a 12-Å cube was generated about the molecule. Geometric factors were calculated by using equation *1* for each point in the grid, and scaled to the experimental ^{1}H-shift gradients by the least-squares procedure. The best fit, that which had the lowest R-factor, was obtained.



10



11

TABLE III

^{1}H RELATIVE SHIFT GRADIENTS[a] (G)

Compound	*Proton*	*Experimental*	*Calculated*	*R-factor*
1	1	13.80	13.84	0.4%
	2	21.00	21.01	
	3	14.70	14.67	
	4	6.90	6.79	
	6eq	3.20	3.25	
2	1	3.80	3.88	2.5%
	2	6.70	6.68	
	3	3.40	3.28	
	4	5.20	5.25	
	5	2.30	2.16	
	6ax	1.20	1.23	
	6eq	0.94	1.08	

[a] P.p.m. per mol of Eu(fod)$_3$ per mol of substrate.

$$\mathrm{R} = \left(\frac{\sum(G_{\mathrm{obs}} - G_{\mathrm{calc}})^2}{\sum(G_{\mathrm{obs}})^2}\right)^{0.5}$$

This fit was refined by searching a smaller cube (3.0-Å side, 0.08-Å grid) about the calculated europium position.

The refined position of europium for **1** yielded an excellent R-factor of 0.4%, and placed Eu 3.1 Å from O-2, equidistant (5.3 Å) from C-1 and C-3; Eu was 26° off the C-2–O-2 line, directed towards H-2 (**10**). Calculated and observed shift-gradients are given in Table III.

Co-ordinates for **2** were determined for ring A only (that containing the fused benzylidene acetal), and the europium position was refined as before. An R-factor of 2.5% was obtained by using the ^{1}H-data in Table III. Eu was located 3.7 Å from O-2, equidistant (4.9 Å) from C-2 and C-3, and 6.3 Å from C-1, and the Eu–O-2–C-2 angle was 137° (**11**). The europium–oxygen distance (3.7 Å) is longer than expected. Steric hindrance about O-2 is unlikely, but models indicate that competition for Eu by the acetoxyl groups of the B ring is possible. The presence of some acetoxyl competition for the dominant OH co-ordination would tend to alter the effective magnetic axis away from the Eu–O-2 line to an unknown position. This would also explain the somewhat larger R-factor obtained in this case.

The Eu(fod)$_3$-induced ^{1}H-shifts for ring A in **3** were small and the resonances were not resolved. In contrast, the resonances of ring-B hydrogens *were* resolved (Table I), strongly suggesting that, in this case, acetoxyl competition for Eu coordination is dominant. Therefore, it is not possible to define a magnetic axis position, and calculations were not performed for this molecule. Examination of models indicates that there are several possible configurations in which acetoxyl groups could chelate Eu effectively. It has recently been demonstrated[14] that favourably disposed C=O groups complex strongly with Eu.

CONCLUSION

The use of lanthanide shift reagents in aiding the assignment of ^{1}H- and ^{13}C-n.m.r. spectra of complex molecules has been demonstrated. There is a correlation between the calculated position of Eu and substrate properties. Where there is one predominant, co-ordinating atom, europium–substrate geometry is well-defined; however, when competing acetoxyl groups are present, competition effects lead to uncertainty in the choice of magnetic axes, and poorer agreement between observed and calculated shifts.

EXPERIMENTAL

^{1}H-N.m.r. spectra (100 MHz) were recorded with a JEOL MH-100 spectrometer, and ^{1}H- and ^{13}C-n.m.r. spectra (59.75 MHz and 15.03 MHz, respectively) were recorded with a JEOL FX-60 spectrometer. Chemical shifts are in δ units (^{1}H) and p.p.m. downfield from Me_4Si (^{13}C). $CDCl_3$ (99.8 atom-% D) and CD_2Cl_2 (99+ atom-% D) were supplied by Aldrich Chemical Co., Ltd.

Appropriate quantities of solid Eu(fod)$_3$ (supplied by Fluorochem, Ltd.) were added to sample solutions, and spectra were recorded after each addition.

ACKNOWLEDGMENT

This work was supported by the National Science Council of Ireland.

REFERENCES

1 For example, I. ARMITAGE AND L. D. HALL, *Can. J. Chem.*, 49 (1971) 2770–2776; S. J. ANGYAL AND D. GREEVES, *Aust. J. Chem.*, 29 (1976) 1223–1230; S. J. ANGYAL, D. GREEVES, L. LITTLEMORE, AND V. PICKLES, *ibid.*, 29 (1976) 1231–1235.

2 I. ARMITAGE, L. D. HALL, A. ARDUINI, AND A. G. MARSHALL, *Carbohydr. Res.*, 31 (1973) 255–263.

3 I. ARMITAGE, L. D. HALL, A. G. MARSHALL, AND L. G. WERBELOW, *J. Am. Chem. Soc.*, 95 (1973) 1437–1443.

4 M. OCHIAI, E. MIZUTA, O. AKI, A. MORIMOTO, AND T. OKADA, *Tetrahedron Lett.*, (1972) 3245–3248.

5 M. R. Willcott, R. E. Lenkinski, and R. E. Davis, *J. Am. Chem. Soc.*, 94 (1972) 1742–1744; R. E. Davis and M. R. Willcott, *ibid.*, 94 (1972) 1744–1745.
6 C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Nature (London)*, 232 (1971) 236–245.
7 H. R. Bolliger and D. A. Prins, *Helv. Chim. Acta*, 29 (1946) 1116–1120.
8 E. E. Lee and J. O. Wood, unpublished work.
9 B. Coxon and H. G. Fletcher, Jr., *J. Org. Chem.*, 26 (1961) 2892–2894.
10 E. O. Bishop, C. B. Barlow, P. R. Carey, and R. D. Guthrie, *Carbohydr. Res.*, 9 (1969) 99–105.
11 R. D. Guthrie and L. F. Johnson, *J. Chem. Soc.*, (1961) 4166–4172.
12 B. Coxon, *Tetrahedron*, 21 (1965) 3481–3503.
13 R. D. Guthrie, E. Conway, S. D. Gero, G. Lukacs, and A.-M. Sepulchre, *J. Chem. Soc., Perkin Trans. 2*, (1974) 542–546.
14 L. F. Lindoy and W. E. Moody, *J. Am. Chem. Soc.*, 99 (1977) 5863–5870.