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# N.M.R. SPECTRA ($^1$H- AND $^{13}$C-) OF METHYL 2-ACETAMIDO-2-DEOXY-D-HEXOPYRANOSIDES IN THE PRESENCE OF LANTHANIDE IONS

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ABSTRACT

The $^1$H- and $^{13}$C-n.m.r. spectra of 2-acetamido-2-deoxy derivatives of methyl D-gluco- and D-galacto-pyranoside were measured in the presence of lanthanide ions in deuterium oxide. The signals of the *N*-acetyl protons, H-2, the carbonyl and methyl carbon atoms in the *N*-acetyl groups, and C-2 were more markedly shifted than others by the addition of praseodymium ion. The $^{13}$C-spin–lattice relaxation-times were also measured in the presence of gadolinium ion, and the distances between it and several carbon atoms were estimated. Based on all of the data, it is suggested that lanthanide ions may preferentially form a complex with the acetamido group, probably binding to the nitrogen atom in the amino sugars.

## INTRODUCTION

Amino sugars, especially 2-acetamido-2-deoxy derivatives of D-gluco- and D-galacto-pyranoses, are widely distributed in cell-wall and intercellular-matrix polysaccharides as well as in glycoproteins of cellular origin. N.m.r.-spectral analysis of these amino sugars is, in principle, a useful tool for the structural investigation of such polysaccharides and glycoproteins.

In 1974, it had been established that the lanthanide shift reagents, complexes of lanthanide metals with some $\beta$-diketone-type ligands[1], can induce a remarkably different pattern of the changes in $^1$H-chemical shift between several peracetylated derivatives of amino sugars and of the corresponding neutral sugars in chloroform[2]. The signals of *N*-acetyl and amido protons, H-2, and H-3 in peracetylated 2-acetamido-2-deoxy derivatives of methyl D-gluco- and D-galacto-pyranoside have been confirmed to be shifted more markedly than others by addition of the reagent.

On the other hand, certain characteristic shifts induced by lanthanide cations in aqueous medium have also been reported for the $^1$H-n.m.r. spectra of methyl $\alpha$-D-gulopyranoside[3] and $\alpha$-D-allopyranoside[4], and the $^{13}$C-n.m.r. spectra of sodium D-gluco- and D-galacto-pyranuronate[5]. It had been suggested that bidentate and tridentate complexes are formed between these sugars and lanthanide ions. Consequently, the $^1$H- and $^{13}$C-n.m.r. spectra of 2-acetamido-2-deoxy derivatives of methyl D-gluco- and D-galacto-pyranoside were measured in the presence of

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lanthanide ions, and some features of the shift and relaxation data are given here with special regard to the nature of the binding between the sugars and the metal ions.

## EXPERIMENTAL

*Materials.* — The sugar samples and lanthanide nitrates (hexahydrates) were of reagent grade from Nakarai Chemicals, Ltd. Deuterium oxide (99.75% D) was purchased from Merck & Co., Inc.

*Measurement of n.m.r. spectra.* — The $^1$H-n.m.r. spectra were recorded at 200 MHz with a Varian XL-200 spectrometer. The spectra were measured for 1.5M sugar solutions in $D_2O$ in a 5-mm tube at 22°. About 60 transients were collected with a pulse width of 3.5 $\mu$s and an acquisition time of 3.077 s.

The $^{13}$C-n.m.r. spectra were recorded at 20 MHz with a Varian FT-80 spectrometer, under conditions of complete proton-decoupling. The spectra were measured for 1.5M sugar solutions in $D_2O$ in a 5-mm tube at 35°. Usually, 1500 transients were collected, with a pulse width of 10 $\mu$s, an acquisition time of 1.024 s, and a pulse-delay time of 6 s.

The chemical shifts in both types of spectra are expressed on the $\delta$ (by definition, in p.p.m.) scale relative to that of tetramethylsilane, using the $^1$H-n.m.r. signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate ($\delta$ 0.02) and the $^{13}$C-n.m.r. signal of 1,4-dioxane ($\delta$ 67.28) as the internal standard.

*Measurement of spin–lattice relaxation-time.* — $^{13}$C-Spin–lattice relaxation-times ($T_1$) were measured by the 180°–$t$–90° pulse sequence (inversion-recovery) method[6]. At least six $t$ values were used in each determination of the $T_1$ value, which was calculated by a least-squares fit to the semilogarithmic plot. The partial

TABLE I

$^1$H-CHEMICAL SHIFTS ($\delta$, IN P.P.M.) AND THEIR CHANGES ($\Delta\delta$, IN P.P.M.) INDUCED BY 0.4M $Pr^{3+}$ FOR 1.5M METHYL 2-ACETAMIDO-2-DEOXY-D-HEXOPYRANOSIDES (1–4)

| *Proton* | *Methyl glycosides of* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | α-D-*GlcNAc*p (1) | | β-D-*GlcNAc*p (2) | | α-D-*GalNAc*p (3) | | β-D-*GalNAc*p (4) | |
| | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ |
| H-1 | 4.76 | 0.13 | 4.45 | 0.12 | 4.80 | 0.13 | 4.39 | 0.11 |
| H-2 | 3.92 | 0.49 | 3.69 | 0.35 | 4.17 | 0.41 | 3.90 | 0.31 |
| H-3 | 3.71 | 0.20 | 3.54 | 0.16 | 3.89 | 0.13 | 3.72 | 0.11 |
| H-4 | 3.49 | 0.01 | 3.45 | 0 | 3.99 | −0.08 | 3.94 | −0.08 |
| H-5 | 3.67 | 0.06 | 3.46 | 0.05 | 3.91 | 0.03 | 3.69 | 0.02 |
| H-6a | 3.88 | 0.02 | 3.94 | 0.01 | 3.78 | −0.02 | 3.80 | −0.03 |
| H-6b | 3.78 | 0.03 | 3.76 | 0.01 | 3.78 | −0.02 | 3.79 | −0.03 |
| OMe | 3.39 | 0.07 | 3.51 | 0.02 | 3.40 | 0.05 | 3.52 | 0 |
| NAc | 2.05 | 0.63 | 2.04 | 0.65 | 2.06 | 0.68 | 2.05 | 0.72 |

spectra for the carbonyl and the other carbon atoms were recorded separately, collecting 200–2000 transients with a pulse-delay time of ~5 $T_1$ value. Reported $T_1$ values are represented as the mean of three determinations under the same conditions, with standard deviations of less than 10% (for carbonyl carbon atoms) or 5% (for other atoms).

## RESULTS AND DISCUSSION

*$^1$H-N.m.r. spectra in the presence of $Pr^{3+}$ ion.* — Chemical shifts ($\delta$) of several proton signals and their changes ($\Delta\delta$) induced by 0.4M praseodymium nitrate for 1.5M aqueous solutions of methyl 2-acetamido-2-deoxy-$\alpha$-D-glucopyranoside (**1**), 2-acetamido-2-deoxy-$\beta$-D-glucopyranoside (**2**), 2-acetamido-2-deoxy-$\alpha$-D-galactopyranoside (**3**), and 2-acetamido-2-deoxy-$\beta$-D-galactopyranoside (**4**) are listed in Table I. Assignments of the signals for compounds **1** to **3** were made by reference to the reported $\delta$ values[7,8]. The signals for compound **4** were assigned by comparison with the foregoing $\delta$ values and those for methyl D-galactopyranosides[9], and the results were confirmed by spin-decoupling experiments.

It is noteworthy that the chemical shifts of the signals for H-6a and H-6b in compounds **3** and **4** are virtually indistinguishable, whereas those in compounds **1** and **2** differ clearly from each other. A similar spectral pattern regarding the methylene protons had also been found for D-gluco- and D-galacto-pyranoses and their methyl glycosides[9], and explained on the basis of a marked difference in rotamer distribution of the hydroxymethyl group between the sugars of the D-gluco- and D-galacto-pyranose series[10].

When increasing amounts of lanthanide salts were added to the sugar solutions at a constant concentration (1.5M), a linear relationship was always established between the magnitude of the induced shifts and the concentration of the lanthanide ions in a range of not more than 0.4M. Thus, the $\Delta\delta$ values in Table I may be taken as those representing the shift-gradients[4] intrinsic to the sugars and the lanthanide salts.

It should be noted that the $\Delta\delta$ values for *N*-acetyl protons are the highest, and those for H-2 and H-3 (or H-1) are next highest, in that order, for all of the compounds. Because the shift-gradients in a $^1$H-n.m.r. spectrum are known to consist mainly of the pseudocontact component and hence satisfy the so-called McConnell–Robertson equation[1], the general feature of the induced shifts given here suggests that the praseodymium ion is bound to a site in the vicinity of the acetamido group in these amino sugars.

It is also to be noted that the $\Delta\delta$ values for H-2 and *O*-methyl protons in the $\alpha$ anomers are higher than those in the $\beta$ anomers and those for H-3 and H-5 in compounds **1** and **2** are slightly higher than those in compounds **3** and **4**.

*$^{13}$C-N.m.r. spectra in the presence of $Pr^{3+}$ ion.* — Chemical shifts ($\delta$) of all of the carbon signals and their changes ($\Delta\delta$) induced by 0.2M praseodymium nitrate for 1.5M aqueous solutions of compounds **1** to **4** are listed in Table II. Assignments

TABLE II

$^{13}$C-CHEMICAL SHIFTS ($\delta$) AND THEIR CHANGES ($\Delta\delta$) INDUCED BY 0.2M $Pr^{3+}$ FOR 1.5M METHYL 2-ACETAMIDO-2-DEOXY-D-HEXOPYRANOSIDES (1–4)

| *Carbon atom* | *Methyl glycosides of* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | α-D-*GlcNAcp* (1) | | β-D-*GlcNAcp* (2) | | α-D-*GalNAcp* (3) | | β-D-*GalNAcp* (4) | |
| | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ | $\delta$ | $\Delta\delta$ |
| C-1 | 98.78 | 0.04 | 102.59 | 0.03 | 98.87 | 0.03 | 103.12 | −0.04 |
| C-2 | 54.39 | 0.16 | 56.21 | 0.19 | 50.62 | 0.16 | 53.02 | 0.16 |
| C-3 | 71.92 | 0.04 | 74.72 | 0.01 | 68.54 | −0.01 | 71.90 | −0.12 |
| C-4 | 70.86 | −0.06 | 70.79 | −0.03 | 69.26 | −0.04 | 68.62 | −0.12 |
| C-5 | 72.45 | −0.04 | 76.61 | −0.02 | 71.44 | 0.01 | 75.82 | −0.05 |
| C-6 | 61.45 | −0.07 | 61.59 | −0.05 | 61.99 | 0.01 | 61.74 | −0.02 |
| OMe | 55.86 | 0.03 | 57.63 | 0.15 | 55.85 | 0.02 | 57.69 | 0.08 |
| NAc C=O | 174.94 | 2.99 | 175.13 | 3.70 | 175.18 | 2.84 | 175.42 | 4.25 |
| NAc Me | 22.70 | 0.26 | 22.97 | 0.27 | 22.75 | 0.25 | 23.02 | 0.29 |

for most of the signals for compounds **1** to **3** were made by reference to the reported $\delta$ values[8,11]. Other signals were assigned by comparison with the $\delta$ values for the 2-acetamido-2-deoxy derivatives of D-gluco- and D-galacto-pyranose[12]. The $\delta$ values for the *O*-methyl carbon atom in the $\alpha$ anomers are shown to be somewhat lower than those for that in the $\beta$ anomers, as reported generally for other types of methyl glycosides[13].

The $\Delta\delta$ values may also be taken here as the shift-gradients, as a relationship similar to that found for the proton signals has again been established between the magnitude of the induced shifts and the concentration of the lanthanide ions. The carbon signals arising from the carbonyl group have remarkably higher $\Delta\delta$ values than the others, and the values for the signals of the methyl carbon atoms in the acetyl groups and for that of C-2 are also moderately higher, in that order, for all of the compounds.

Lanthanide-induced shifts in a $^{13}$C-n.m.r. spectrum are generally known to involve the components of the contact and complex-formation shifts in addition to the pseudocontact shift[14,15]. Thus, the $\Delta\delta$ values for compounds **1** and **2** in Table II were divided into these three components, employing $La^{3+}$-, $Eu^{3+}$-, and $Nd^{3+}$-induced shifts, by a combined method[5] according to Ajisaka *et al.*[16] and Reilley *et al.*[17]. The so-called geometric factors ($G_i$), which represent the magnitude of the pseudocontact component, were then calculated, and are shown in Table III.

The carbonyl carbon atoms have the highest $G_i$ values, and the values for the methyl carbon atoms in the acetyl groups and C-2 are next highest, in that order, suggesting again that the lanthanide ion is bound to a site in the vicinity of the acetamido group in compounds **1** and **2**. A similar type of binding may be presumed to occur in compounds **3** and **4**, because differences in the pattern of the $\Delta\delta$ values in Table II are hardly discernible among all the compounds, and this pattern of the

TABLE III

$^{13}$C-GEOMETRIC FACTORS ($G_i$) CALCULATED FROM $La^{3+}$-, $Eu^{3+}$-, $Nd^{3+}$-, AND $Pr^{3+}$-INDUCED SHIFTS FOR METHYL 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSIDES (**1**, **2**)

| *Carbon atom* | $G_i$ *(ratio)* | |
|---|---|---|
| | *Me α-D-GlcNAcp* (**1**) | *Me β-D-GlcNAcp* (**2**) |
| C-1 | 0.06 | 0.02 |
| C-2 | 0.12 | 0.10 |
| C-3 | 0.04 | 0.01 |
| C-4 | 0.05 | 0.03 |
| C-5 | 0.05 | 0.02 |
| C-6 | 0.04 | 0.02 |
| OMe | 0.05 | −0.01 |
| NAc C=O | 1.00 | 1.00 |
| NAc Me | 0.23 | 0.18 |

$Pr^{3+}$-induced shifts is similar to that of the $G_i$ values for compounds **1** and **2**, except for the carbonyl carbon atoms which show an excessively higher $\Delta\delta$ value owing to a greater contribution of the contact shift.

*$^{13}$C-Spin–lattice relaxation-times in the presence of $Gd^{3+}$ ion.* — Spin–lattice relaxation-times ($T_1$) of all the carbon signals in the presence and absence of 0.3mM gadolinium nitrate for 1.5M aqueous solutions of compounds **1** and **2** are listed in Table IV. From these $T_1$ values, it may be seen that the relaxation times of the carbonyl carbon atoms are most significantly lessened by the addition of the

TABLE IV

$^{13}$C-SPIN-LATTICE RELAXATION-TIMES ($T_1$) IN THE PRESENCE AND ABSENCE OF 0.3mM $Gd^{3+}$ FOR 1.5M METHYL 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSIDES (**1**, **2**) AND THE DISTANCES BETWEEN $Gd^{3+}$ AND THE CARBON ATOMS IN THESE SUGARS AS CALCULATED FROM THEIR $T_1$ VALUES

| *Carbon atom* | *Me α-D-GlcNAcp* (**1**) | | | *Me β-D-GlcNAcp* (**2**) | | |
|---|---|---|---|---|---|---|
| | $T_1$ *(s)* | | *Distance (ratio)* | $T_1$ *(s)* | | *Distance (ratio)* |
| | *Without addition* | *With $Gd^{3+}$* | | *Without addition* | *With $Gd^{3+}$* | |
| C-1 | 0.56 | 0.55 | [a] | 0.61 | 0.58 | [a] |
| C-2 | 0.60 | 0.50 | 1.24 | 0.59 | 0.50 | 1.24 |
| C-3 | 0.51 | 0.47 | [a] | 0.56 | 0.56 | [a] |
| C-4 | 0.56 | 0.51 | [a] | 0.56 | 0.51 | [a] |
| C-5 | 0.54 | 0.51 | [a] | 0.59 | 0.59 | [a] |
| C-6 | 0.33 | 0.33 | [a] | 0.33 | 0.33 | [a] |
| OMe | 1.87 | 1.44 | 1.44 | 1.93 | 1.64 | 1.65 |
| NAc C=O | 10.80 | 0.85 | 1.00 | 9.77 | 0.92 | 1.00 |
| NAc Me | 1.92 | 0.93 | 1.12 | 1.81 | 0.99 | 1.15 |

[a]Estimated to be >1.4.

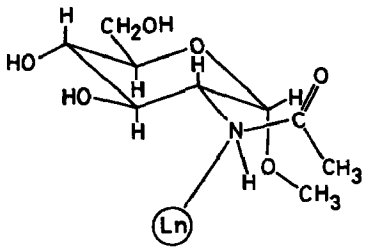


Fig. 1. The most probable binding site of a lanthanide ion (Ln) for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**).

gadolinium salt for both compounds. The more or less outstanding alterations are also found for C-2 and the two kinds of methyl carbon atoms.

Thus, the distances (ratio) between the gadolinium ion and these carbon atoms were estimated by calculating the reciprocals of the sixth root of the spin–lattice relaxation-rates (ratio) in the $Gd^{3+}$-bound molecules obtained from the foregoing $T_1$ values according to the method of Barry *et al.*[18]. The results, given in Table IV, show the gadolinium ion to be nearest to the carbonyl carbon atoms and next nearest to the methyl carbon atoms in the acetyl groups and C-2, in that order, for both compounds. It is also to be noted that the distance between the ion and the *O*-methyl carbon atoms in the α anomer (**1**) is appreciably shorter than that in the β anomer (**2**).

These results are almost compatible with the data for the changes in the $^1H$- and $^{13}C$-chemical shifts induced by the praseodymium ion, suggesting that the acetamido group in these sugars is responsible for binding of the lanthanide ions. The acetamido group in 2-acetamido-2-deoxy-α-D-glucopyranose is generally known to be in a fully extended form with an almost *trans* configuration between the amido proton and H-2 as well as the carbonyl oxygen atom[19]. Thus, assuming a similar configuration of the acetamido group and considering the data in Table IV, the most probable binding site of a lanthanide ion in compound **1** was postulated to be at a distance of approximately 2.8, 3.0, 3.4, and 3.7 Å from the nitrogen atom, carbonyl carbon atom, methyl carbon atom in the acetamido group, and C-2, respectively, as shown in Fig. 1. The lanthanide ion is here presumed to form a complex with the nitrogen atom (which has a rather higher basicity), as reported similarly for several peracetylated derivatives of amino sugars[2].

Such a favored binding site was found to be roughly in harmony with the data for pseudocontact shifts of the $^1H$- and $^{13}C$-n.m.r. signals for compound **1** (see Tables I and III) by setting the principal magnetic axis of the lanthanide ion on a suitable direction close to the one toward the nitrogen atom and calculating the geometric factors according to the McConnell–Robertson equation. All of the data in Tables I to IV further suggest that a fundamentally analogous type of complex can be formed between lanthanide ions and compounds **2**, **3**, or **4**. In the case of compound **2**, a longer distance between the lanthanide ion and the *O*-methyl

carbon atom (see Table IV) may probably show the latter to be slightly nearer to the ring oxygen atom because of steric hindrance between the methyl protons in the methoxyl and acetyl groups.

In biological systems, calcium ions (which are similar in size to lanthanide ions[20]) are known to be widely distributed and characteristically associated with several polysaccharides. Therefore, the interactions reported here between the amino sugars and lanthanide ions, which are regarded as probes used instead of calcium ions, are of interest in connection with the biological significance of these sugars.

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