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Note

Proton n.m.r. spectra of some 3,6-anhydro-D-galactose derivatives in the presence of a europium shift-reagent

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3,6-Anhydro-D-galactose and its enantiomorph are known to be widely distributed in polysaccharides from various species of the Rhodophyta[1-3]. The n.m.r. spectra of methyl 3,6-anhydro-α-D-galactopyranoside and its peracetate have recently been fully analyzed[4] and found to be useful in the structural determination of agar-type polysaccharides[5].

In recent years, certain chelated complexes of some lanthanide elements have been established as reagents able to induce very substantial changes in the chemical shifts of various compounds and give valuable information on their conformations[6,7]. The n.m.r. spectra of some peracetylated D-hexopyranosides and their 2-acetamido-2-deoxy derivatives have earlier been measured in the presence of tris(2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato)europium [$Eu(fod)_3$] and found to display appreciably different patterns of the induced downfield-shifts between the sugars of the gluco- and the galacto-pyranose series[8] as well as the neutral and the amino sugars[9]. In the present note, data on the bound chemical-shifts of methyl 2,4-di-*O*-acetyl-3,6-anhydro-α-D-galactopyranoside (**1**) and 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal (**2**) in the presence of $Eu(fod)_3$ are also described, and compared with those of the ordinary sugars not possessing the anhydro ring.

According to Armitage *et al.*[10], the bound chemical-shift (Δ_B), which means the difference in chemical shift between a monofunctional substrate and its state fully complexed with a lanthanide shift-reagent, may be determined from the slope of a straight line obtained by plotting the concentration of the substrate against the reciprocal of the difference in chemical shift at a constant concentration of the shift reagent. In the case of compounds **1** and **2** which are bi- and tri-functional substrates, respectively, the complexing with a shift reagent may be assumed to occur competitively between two or three functional groups (the acetoxyl groups); a Δ_B value obtained by the foregoing method is thought to indicate the weighted average of these complexing effects, of which the one induced by the complex having the highest stability makes the greatest contribution.

Straight lines were obtained for all of the protons in compound **1** by plotting the concentration (0.40–0.68M) of **1** against the reciprocal of the difference in chemical shift ($1/\Delta\delta$) of the respective signals between the spectra in the presence and in the absence of 0.1M Eu(fod)$_3$. Under these conditions, by the method of consecutive dilutions of the sample solutions[8], a 1:1 adduct has been confirmed to be mainly formed between related sugars and the shift reagent. The straight lines obtained for several protons in compound **1** are shown in Fig. 1, and bound chemical-shifts of all of the proton signals in the same compound, determined from the slopes of such lines, are listed in Table I. The Δ_B values for H-2, H-3, and H-4 are higher than those for other protons, as expected from the probable complexing of the europium atom with the 2- and the 4-acetoxyl groups. Comparison of the Δ_B values for compound **1** with those for methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside[9] (**3**) and 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose[8] (**4**), also described in Table I, shows that the values for H-2 and the protons in its vicinity (H-1, H-3, and *O*-methyl protons) become greater on formation of the 3,6-anhydro ring, suggesting that the complexing of europium with the 2-acetoxyl group is more extensive than that with the 4-acetoxyl group in compound **1**. The bound chemical-shifts of all of the proton signals for compound **2** were next obtained by the same method, and are also listed in Table I. The highest Δ_B value is also observed here for H-2. However, it may be noted that the values for H-1, H-3, and *O*-methyl protons are considerably lower, compared with those in compound **1**. This result means that the conformation of the furanoid ring may be extensively altered by opening of the pyranoid ring, as suggested before[4].

As the patterns of lanthanide-induced shifts have now been shown to differ

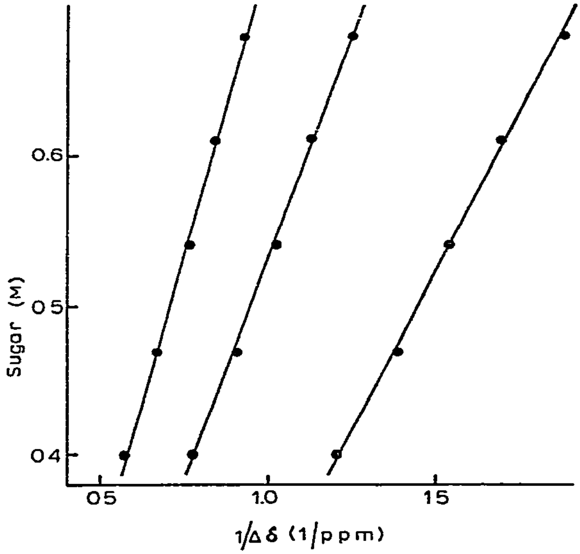


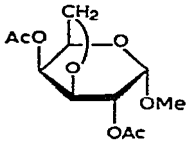
Fig. 1. A plot of the concentration of compound **1** against the reciprocal of downfield shifts of its proton signals (H-2, H-4, and H-6, respectively, from left to right) induced by the addition of 0.1M Eu(fod)$_3$.

TABLE I

BOUND CHEMICAL-SHIFTS (Δ_B, P.P.M.) TO LOWER FIELD FOR THE PROTON SIGNALS OF SEVERAL PER-ACETYLATED 3,6-ANHYDRO AND NORMAL D-GALACTOSE DERIVATIVES IN THE PRESENCE OF $Eu(fod)_3$ IN CHLOROFORM-*d*

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*O-Methyl*	*O-Acetyl*
1	5.22	7.92	7.84	6.02	3.83	4.29	4.78	1.98	3.06, 4.77
2	4.17	8.24	4.29	6.31	4.24	2.64	2.61	0.97, 1.18	1.05, 2.25, 2.46
3[a]	4.32	6.48	5.76	6.18	3.72	5.28	5.28	1.20	1.86, 3.60, 3.96, 4.74
4[b]	4.37	4.77	5.37	7.30	4.59	6.30	6.16		1.80, 1.84, 2.40, 3.08, 4.12

[a] See Ref. 9. [b] See Ref. 8.



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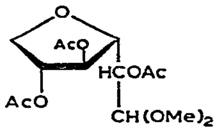

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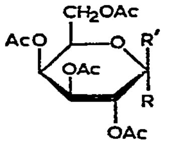

3 R = OMe, R' = H
4 R = H, R' = OAc

greatly between the ordinary peracetylated sugars of the galactopyranose series and their 3,6-anhydro derivatives, it was interesting to see what patterns are observed in experiments on mixtures of these sugars. Thus, the lanthanide-induced shifts of several protons in equimolar mixtures of the two types of sugars (0.47M each) were

TABLE II

THE LANTHANIDE [0.1M $Eu(fod)_3$]-INDUCED DOWNFIELD SHIFTS (%)[a] OF SOME PROTON SIGNALS IN MIXTURES OF SEVERAL PERACETYLATED 3,6-ANHYDRO- AND NORMAL D-GALACTOSE DERIVATIVES IN CHLOROFORM-*d*

Mixture	*Concentration* (M)	*H-1*	*H-2*	*H-3*	*H-4*	*O-Methyl*	*O-Acetyl*
1	0.47	95	93	91	93	97	89, 94
3	0.47	63	62	63	64	73	62, 62, 63, 64
1	0.47	78	79	78	77	76	81, 81
4	0.47	43	37	42	36		37, 39, 41, 44, 46
2	0.47	94	91	93	91	94, 95	92, 97, 98
4	0.47	28	22	27	22		27, 28, 29, 33, 34
2	0.47	64	65	66	66	63, 66	70, 71, 81
4	0.94	28	20	27	19		24, 24, 25, 26, 28

[a] Expressed as a percentage of the shift measured in the experiment with a single sugar [0.47M sugar and 0.1M $Eu(fod)_3$].

measured in the presence of $Eu(fod)_3$ (0.1M). The results are shown in Table II as a percentage of the shifts measured in experiments on the single sugar (0.47M) at the same concentration (0.1M) of the reagent. It is seen from Table II that the shifts of these protons of compounds **1** and **2** are markedly greater than those of compounds **3** and **4** [the differences were: 30–40% (**1**) and ~70% (**2**)]. This result means that preferential coordination to the europium by the acetoxyl groups in the 3,6-anhydro sugars occurs essentially without any pronounced disturbance by the ordinary sugars also present. The result obtained in a similar experiment on a mixture of compound **2** (0.47M) and twice the molar quantity of compound **4** (0.94M) is also given in Table II, which shows that the shifts of these protons in compound **2** are still appreciably greater than those in compound **4**. Such a strong affinity of the shift reagent toward 3,6-anhydrogalactose derivatives may be utilizable for analysis of these residues in the agar- and the carrageenan-type polysaccharides, and further development of the present research along these lines is currently under way.

EXPERIMENTAL

N.m.r. spectra were measured at 100 MHz, in the constant field-frequency-sweep mode, with a JNM-4H-100 spectrometer, using > 0.4M sugar solutions containing 0.1M $Eu(fod)_3$ in chloroform-*d*. Hexamethyldisiloxane was used as the lock signal and the internal standard. The temperature of the probe was 20°. Chemical shifts are expressed on the δ (p.p.m.) scale. Spectral assignments were generally made by first-order analysis, considering both the areas and the splitting patterns of each signal. The spin–spin couplings between some protons were also analyzed by assuming AB or ABX systems.

Compounds **1** and **2** were prepared from a synthetic sample of methyl 3,6-anhydro-α-D-galactopyranoside kindly provided by Dr. S. Hirase, Kyoto Technical University, by acetylating it with acetic anhydride–pyridine directly and also after opening of the pyranoid ring, as reported before[4]. Contamination of the final products with pyridine was avoided by washing their solutions in chloroform with dilute hydrochloric acid. $Eu(fod)_3$ was purchased from Nakarai Chemicals, Ltd., Kyoto and used after drying thoroughly in a desiccator over phosphorus pentaoxide. The effect of absorption of moisture by the shift reagent was negligible under the conditions used, and reproducible chemical-shift data were obtained with an accuracy of ±0.01 p.p.m.

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