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Note

Proton-n.m.r spectra of peracetylated derivatives of methyl L-arabinofuranosides in the presence of a europium shift-reagent

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L-Arabinofuranoses are widely distributed in many cell-wall and intercellular-matrix polysaccharides from higher plants. Few proton-n.m.r. data of these sugars have thus far been reported, except for those of some benzoylated derivatives of their D enantiomorphs[1,2], because of serious overlap of several signals in their spectra.

Some chelated complexes of europium and praseodymium having β-diketone-type ligands induce very substantial changes in the chemical shifts of other compounds and thus facilitate first-order analyses of spectra[3,4]. One of these lanthanide shift-reagents, tris(2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato)europium [Eu(fod)$_3$], has been used with certain peracetylated derivatives of pyranoid sugars to clarify the different patterns of downfield proton shifts between neutral and amino sugars[5], between gluco- and galacto-pyranose derivatives[6], between normal and 3,6-anhydro sugars[7], and between pento- and hexo-pyranosides[8]. Similar spectral and conformational analyses by use of this shift reagent have been made with some isopropylidene[9] and permethylated derivatives[10,11] of hexopyranoses, and with related disaccharides.

This investigation concerns the Eu(fod)$_3$-induced shifts for peracetylated methyl L-arabinofuranosides; and the chemical shifts, coupling constants, and bound chemical-shifts for all of the well-resolved proton-signals are reported here, with particular reference to comparisons between the α and β anomers as well as between the furanoid and pyranoid sugars.

Chemical shifts of all proton signals in the n.m.r. spectra of methyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranoside (**1**), methyl 2,3,5-tri-*O*-acetyl-β-L-arabinofuranoside (**2**), methyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside (**3**), and methyl 2,3,4-tri-*O*-acetyl-β-L-arabinopyranoside (**4**) in chloroform-*d* are listed in Table I. The data for the pyranosides (**3** and **4**) are in gross agreement with those reported by Durette and Horton[12] for their D enantiomorphs in chloroform-*d* or acetone-d_6. Comparison of the data between the furanosides (**1** and **2**) and the pyranosides (**3** and **4**) shows that the δ values for H-1 and *O*-methyl protons in compounds **1** and **2** differ greatly from those in compound **3** and are close to those in compound **4**, suggesting that H-1

TABLE I

CHEMICAL SHIFTS (δ) OF THE PROTON SIGNALS OF PERACETYLATED METHYL L-ARABINOFURANOSIDES (1 AND 2) AND L-ARABINOPYRANOSIDES (3 AND 4) IN CHLOROFORM-*d*

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	O-*Methyl*	O-*Acetyl*
1	4.89	5.02	4.95	4.18	4.13	4.36	3.34	2.04, 2.04, 2.04
2	5.06	5.04	5.30	4.05	4.16	4.32	3.32	2.04, 2.04, 2.05
3	4.30	5.10	5.02	5.21	3.60	3.98	3.44	1.96, 2.01, 2.07
4	4.88	5.11	5.26	5.24	3.85	3.61	3.34	1.94, 2.02, 2.08

TABLE II

COUPLING CONSTANTS BETWEEN METHINE AND METHYLENE PROTONS, AND APPROXIMATE DIHEDRAL ANGLES (ϕ) CALCULATED FROM THEM, FOR PERACETYLATED METHYL L-ARABINOFURANOSIDES IN CHLOROFORM-*d*

Compound	*Coupling constants (Hz)*						*Angles (degrees)*		
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$	$\phi_{1,2}$	$\phi_{2,3}$	$\phi_{3,4}$
1	<0.5	1.2	4.8	6.0	3.5	12.0	73–80	112	134
2	4.4	6.7	5.0	7.3	3.5	11.4	45	145	136

and the methoxyl group are oriented quasi-equatorially and quasi-axially, respectively, in both the α and β anomers of the L-arabinofuranosides. Similar data have also been reported[13] for the anomeric protons in per(trimethylsilyl)ated derivatives of L-arabinofuranoses in chloroform-*d*.

Table II records first-order coupling-constants (J) between methine and methylene protons in compounds **1** and **2**, measured in the presence of Eu(fod)$_3$. The J values in the absence of Eu(fod)$_3$ may also be taken to be nearly the same as these values, as the coupling constants are generally not greatly influenced by addition of a shift reagent[14], and the J values in Table II were found invariable in several experiments employing various concentration ratios of shift reagent to substrate.

Significant differences in the $J_{1,2}$ as well as $J_{2,3}$ values may be observed between the α and β anomers, suggesting a marked conformational distinction between them. Thus, the dihedral angles (ϕ) between all of the vicinal ring-protons (H-1 to H-4) for compounds **1** and **2** were calculated from the $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ values by applying the Karplus equation as modified by Abraham *et al.*[15], and are also shown in Table II. The most favored conformations of the furanoid rings in compounds **1** and **2** are deduced to be twist ($^1T_0$) and envelope (E_1) forms, respectively, from these dihedral-angle data. These conformations may also be deduced by applying a version of the Karplus equation as modified by Karplus[16], and it is probable that they are stabilized by a relatively favorable interaction between the C-1–O-1 and

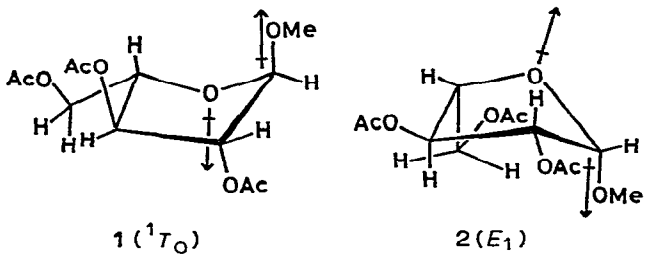

Fig. 1. The most favored conformations of the furanoid rings in peracetylated methyl α- (**1**) and β-L-arabinofuranosides (**2**) deduced from the dihedral angles between various protons. (↔ dipole; Me, methyl; Ac, acetyl)

C-4–O-4 dipoles, as depicted in Fig. 1. It may be noted that the orientations of H-1 and the methoxyl group in these conformations are consistent with those previously suggested from the chemical shifts of H-1 and the *O*-methyl protons. Stevens and Fletcher[1] have proposed from proton-n.m.r. data the $^0T_1$ and $^1T_2$ conformations for methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside in acetonitrile and 1,3,5-tri-*O*-benzoyl-2-*O*-mesyl-β-D-arabinofuranose in chloroform-*d*, respectively. It is worth noting that these conformations are the same or the closest to the D-enantiomorphic forms ($^0T_1$ and ^1E) of those deduced here for the L-arabinofuranosides.

On the other hand, Table II indicates that there are appreciable differences between the $J_{4,5}$ and $J_{4,5'}$ values in both anomers, suggesting that rotation about the C-4–C-5 bond is somewhat restricted. As the three staggered rotamers (**a**, **b**, and **c**, as shown in Fig. 2) may be considered the most important, the mole fractions n_a, n_b, and n_c of these rotamers were calculated from the *J* values according to the method described by Streefkerk *et al.*[17] as follows: $n_a = 0.44$, $n_b = 0.17$, $n_c = 0.39$ for compound **1**, and $n_a = 0.59$, $n_b = 0.16$, $n_c = 0.25$ for compound **2**. The n_a value is much higher than the n_c value in compound **2**, whereas these values are approximately the same in compound **1**. This difference probably arises because rotamer **c** in compound **2** (containing a quasi-axially oriented C-5) is rather unstable on account of steric hindrance between the methoxyl group and the acetoxyl group at C-5.

The magnitude of a lanthanide-induced shift in an n.m.r. spectrum may be expressed quantitatively in terms of bound chemical-shift, Δ_B, the difference in chemical shift between a free substrate and its state fully complexed with a lanthanide

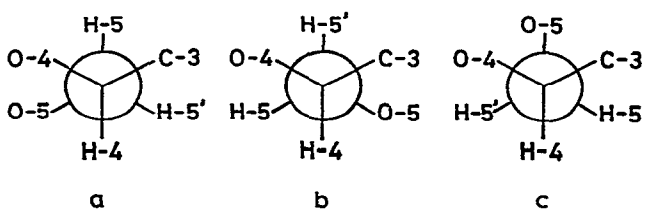

Fig. 2. The three staggered rotamers (**a**, **b**, and **c**) of the C-4–CH_2O-acetyl group in peracetylated L-arabinofuranosides.

TABLE III

BOUND CHEMICAL-SHIFTS (Δ_B, P.P.M.) TO LOWER FIELD FOR THE PROTON SIGNALS OF PERACETYLATED METHYL L-ARABINOFURANOSIDES IN CHLOROFORM-*d* IN THE PRESENCE OF Eu(FOD)$_3$

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	*O-Methyl*	*O-Acetyl*
1	3.78	7.26	10.02	7.26	9.36	10.44	1.50	2.28, 3.60, 5.10
2	4.62	6.12	9.06	7.74	8.82	11.94	2.28	3.48, 3.84, 4.80

shift-reagent[4]. The Δ_B value may be determined from the slope of a straight line obtained by plotting the difference in chemical shift against the mole ratio of the shift reagent to the substrate, which is at rather high concentration[18]. For peracetylated sugars, substrate concentrations not <0.3M have been found suitable for determination of bound chemical-shifts by this method[6], and hence the Δ_B values for the L-arabinofuranosides were measured at 0.40, 0.45, 0.52, and 0.60M in the presence of 0.1M Eu(fod)$_3$-d_{27}.

The bound chemical-shifts (Δ_B) are to lower field for all protons in compounds **1** and **2**, and are given in Table III. It is noteworthy that the Δ_B values for H-3, H-5, and H-5' are generally higher than those for other protons, suggesting the formation of relatively stable complexes between the shift reagent and the acetoxyl groups at C-3 and C-5. The relatively low Δ_B values for acetyl protons suggests that the europium may, as pointed out before[6], bind preferentially to the carbonyl oxygen atom in an extended form of the acetoxyl groups, having a nearly *cis*-disposition toward the methyl group. Favored complexation of the acetoxyl group at C-5 is understandable from steric considerations, resembling those at C-6 in the hexopyranosides[5]. However, there seems to be a marked difference in the affinity toward europium between the 2- and 3-acetoxyl groups, which are both linked directly to the furanoid ring. Any special type of stabilization should therefore be considered only with regard to complexation involving the 3-acetoxyl group. Thus, it may be postulated that a more-stable, bidentate complex is formed preferentially between europium and the 3- and 5-acetoxyl groups in these arabinofuranosides, as in the case of the sugars of the galactopyranose series, which have been considered[6] capable of binding bidentately to europium through their 4- and 6-acetoxyl groups. The formation of such a complex is feasible in the aforementioned, preponderant rotamers (**a** and **c** for **1**, and **a** for **2**) by slightly displacing the carbonyl oxygen atoms at C-3 and C-5 closer to each other, as established by inspection of molecular models. The coordinated europium is markedly closer to H-5' than to H-5 in such a complex formed from rotamer **a** only; this is consistent with the fact that the Δ_B values for H-5' are much greater than those for H-5, particularly in compound **2** (Table III).

As the patterns of lanthanide-induced shifts differ appreciably between peracetylated furanoid and pyranoid sugars, it is of interest to examine mixtures of these sugars. The lanthanide-induced shifts of the methine and methylene protons

TABLE IV

THE LANTHANIDE [0.1M Eu(FOD)$_3$]-INDUCED DOWNFIELD-SHIFTS (%)[a] OF THE METHINE AND METHYLENE PROTON-SIGNALS IN EQUIMOLAR MIXTURES (0.3M EACH) OF PERACETYLATED DERIVATIVES OF L-ARABINO-FURANOSIDES AND SEVERAL PENTO- AND HEXO-PYRANOSIDES IN CHLOROFORM-*d*

Mixture	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5'*	*H-6*	*H-6'*
1	52	52	52	51	52	52		
2	49	48	48	49	48	48		
1	62	62	63	62	62	61		
3	31	35	34	36	30	31		
2	65	66	66	66	66	65		
4	46	47	45	45	42	45		
1	59	57	58	58	58	57		
5	40	43	43	42	39	40		
2	55	55	57	57	56	56		
5	41	44	45	45	40	41		
1	63	62	63	63	63	63		
6	36	37	36	38	34		37	37
2	63	63	62	63	62	61		
6	38	39	38	40	37		40	40

[a]Expressed as a percentage of the shift measured in an experiment with a single sugar [0.3M sugar and 0.1M Eu(fod)$_3$].

in equimolar mixtures of two types of sugar (0.3M each) were measured in the presence of Eu(fod)$_3$-d_{27} (0.1M). The results are shown in Table IV as a percentage of the shifts measured in experiments on the single sugar (0.3M) at the same concentration (0.1M) of reagent. This percentage may be regarded as manifesting a relative binding-strength of each sugar toward Eu(fod)$_3$. Table IV shows that the shifts of these protons in the L-arabinofuranosides (**1** and **2**) are significantly greater than those in the corresponding L-arabinopyranosides (**3** and **4**, respectively), whereas the shifts do not differ appreciably between compounds **1** and **2**. The relatively higher affinities of the arabinofuranosides for Eu(fod)$_3$ are also clear from experiments on mixtures of these and other pyranoid sugars, namely methyl 2,3,4-tri-*O*-acetyl-β-D-xylo-pyranoside (**5**) (which is known among methyl pentopyranosides to have the strongest affinity for the reagent[8]) and methyl 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-pyranoside (**6**), as shown in Table IV. It is noteworthy that a similar difference in the affinity for the shift reagent between the sugars containing furanoid and pyranoid rings has been observed with a mixture of 2,4,5-tri-*O*-acetyl-3,6-anhydro-D-galactose dimethyl acetal and 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose[7].

EXPERIMENTAL

Spectra. — N.m.r. spectra were recorded at 100 MHz, in the constant field–frequency-sweep mode, with a JNM-4H-100 spectrometer, for 0.3–0.6M sugar solutions containing up to 0.1M Eu(fod)$_3$-d_{27} in chloroform-*d*. Hexamethyldisiloxane (HMDS) was used as the lock signal and internal standard. The temperature of the probe was 20°. Chemical shifts are expressed on the δ (p.p.m.) scale (HMDS : 0.05)[19], and coupling constants (J) are given in Hz. Spectral assignments were generally made by first-order analysis, and spin–spin couplings between geminal and vicinal protons analyzed by assuming AB or ABX systems. Overlapping signals were gradually shifted and separated from one another by incremental addition of Eu(fod)$_3$-d_{27}, and original chemical-shift data were assigned by extrapolation.

Compounds. — Methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, and Eu(fod)$_3$-d_{27} were purchased from Nakarai Chemicals, Ltd., Kyoto. Eu(fod)$_3$-d_{27} was dried thoroughly 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.

Methyl L-arabinofuranosides and methyl L-arabinopyranosides were prepared by glycosidation of L-arabinose in hot methanol containing Dowex-50 X8 (H^+ form) resin, with subsequent column-chromatography on Dowex-1 X2 (OH^- form) resin according to the general method of Austin *et al.*[20]. Methyl L-arabinosides were eluted from the column in the order β-pyranoside, α-pyranoside, β-furanoside, and α-furanoside, with about ten bed-volumes of water, the yields of these products being 35, 20, 8, and 18%, respectively. The L-arabinopyranosides were crystallized as described in the literature[21], but the L-arabinofuranosides, which are known to be extremely hygroscopic[22], were handled as syrups.

Methyl glycosides were peracetylated with acetic anhydride–pyridine. Contamination of the final products with pyridine was avoided by washing the solutions in chloroform with dilute hydrochloric acid. The final crystalline or syrupy products were identified by comparing the signal areas of the *O*-methyl protons (δ 3.3–3.5) and the methine and methylene protons (δ 3.5–5.5) with those of the acetyl protons (δ 1.9–2.1) in the n.m.r. spectra.

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