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The Application of Paramagnetic Shift Reagent Eu(FOD)$_3$ in the Nuclear Magnetic Resonance Studies of Acetylated Aryl Glycopyranosides

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The nuclear magnetic resonance spectra of anomeric pairs of acetylated aryl D-glycopyranosides were measured in deuteriochloroform at 60 MHz. The shift reagent Eu(FOD)$_3$ proved to be useful to distinguish the H-1 proton of the β-anomer and permitted the assignment of all the ring protons in some cases. The virtual long-range coupling of the H-5 proton with the H-3 proton of aryl β-D-glucopyranosiduronates was confirmed with an aid of the shift reagent.

All of the acetoxy signals were well resolved by the addition of the shift reagent.

In the previous papers dealing with the nuclear magnetic resonance (NMR) spectra of anomeric pairs of several acetylated glycopyranosides[2] as well as di- and poly-D-glucopyranoses,[3] it was demonstrated that the spectral pattern difference between α- and β-anomers was useful to assign the anomeric configuration, especially when the anomeric proton was obscured by overlapping with other proton signals, as were observed in the cases of acetylated aryl β-D-glycopyranosides[2,4,5] and oligo- or polysaccharides.[3,6,7] In the α-anomer, the 1,3-diaxial deshielding effect of the axial oxygen at C-1 caused the axial H-3 or H-5 protons to appear in the lower field as compared with those of β-anomer, allowing the first-order assignment of the ring protons, measured in deuteriochloroform at 60 MHz. In the β-anomer, on the other hand, the H-2, H-3, and H-4 proton signals appeared in the narrow range and their assignments were usually difficult at 60 or 100 MHz. Moreover, the H-1 proton signals of acetylated aryl β-D-glucopyranosides and β-D-glucopyranosiduronates were overlapped with other proton signals, so that Kiss, *et al.*[5] were unable to establish their anomeric configurations at 60 or 100 MHz, but able to distinguish the H-1 proton signals finally at 220 MHz, confirming the β-glycosidic linkages. While Wick, *et al.*[8] established the β-configuration of ipalbidine tetra-O-acetyl-D-glucopyranoside (phenolic glucoside) by comparing its pattern of 100 MHz spectrum with those of the anomeric pair of *p*-cresyl tetra-O-acetyl-D-glucopyranosides.

Recently the NMR shift reagents, tris(dipivalomethanato)europium (Eu(DPM)$_3$)[9] and tris(dipivalomethanato)praseodymium (Pr(DPM)$_3$)[10] were introduced to simplify the NMR spectra of organic compounds. They act as Lewis acids and form complexes with a variety of organic Lewis bases, such as amines, alcohols, ketones, ethers, and esters. Usually, the europium chelate causes downfield shifts of proton signals while the praseodymium chelate

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[1] Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
[2] M. Matsui and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **18**, 2129 (1970).
[3] M. Matsui and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **19**, 395 (1971).
[4] Y. Tsuzuki and K. Tanaka, *Bull. Chem. Soc. Japan*, **40**, 1208 (1967).
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[6] D. Gagnaire, L. Odier, and M. Vincendon, *J. Polymer Sci.*, Part C, 27 (1969).
[7] B. Casu, M. Reggiani, G.G. Gallo, and A. Vigevani, *Carbohyd. Res.*, **12**, 157 (1970).
[8] A.E. Wick, P.A. Bartlett, and D. Dolphin, *Helv. Chim. Acta*, **54**, 513 (1971).
[9] C.C. Hinckley, *J. Am. Chem. Soc.*, **91**, 5160 (1969); J.K.M. Sanders and D.H. Williams, *Chem. Commun.*, 422 (1970); *idem, J. Am. Chem. Soc.*, **93**, 641 (1971).
[10] J. Briggs, G.H. Frost, F.A. Hart, G.P. Moss, and M.L. Staniforth, *Chem. Commun.*, **1970**, 749 J. Briggs, F.A. Hart, and G.P. Moss, *ibid.*, **1970**, 1506

upfield shifts. Rondeau, *et al.*[11] used the chelates, designated Eu(FOD)$_3$ and Pr(FOD)$_3$, which are complexes of 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octane-dione with europium and praseodymium, respectively. They increase the solubility and the acidity of the metal complexes, thus being superior to Eu(DPM)$_3$ and Pr(DPM)$_3$ as shift reagent. The applications of Eu(DPM)$_3$ or Pr(DPM)$_3$ to the NMR spectra of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose,[12] N- or O-acetylated carbohydrates and nucleosides[13] have proved the usefulness of these shift reagents for the spectral analysis of the carbohydrate. The present paper describes the utility of the shift reagent Eu(FOD)$_3$ for the configurational analysis of the acetylated aryl glycopyranosides.

The NMR spectrum[14] of methyl (phenyl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate is shown in Fig. 1, demonstrating that all the protons were unequivocally assigned from the first-order analysis (Table I). The spacing of the H-1 proton signal was 3.5 Hz, indicating the α-configuration. On the other hand, the NMR spectrum of methyl (phenyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate exhibited an entirely different pattern (Fig. 2a). Thus, the H-1, H-2, H-3, and H-4 protons appeared in the narrow range of 0.5 ppm and their assignment was not feasible. In contrast to the α-anomer, the H-5 proton signal of the β-anomer showed a complicated splitting which was explained by the virtual long-range coupling[15] of the H-5 proton with the H-3 proton due to the large $J_{3,4}$ as compared with $\Delta\nu_{3,4}$.[2] However, the addition of various amounts of Eu(FOD)$_3$(10—50 mg; 1.0—5.0×10^{-5} mole) to the β-anomer (25 mg, 6.3×10^{-5} mole) gave rise to downfield shifts of the proton signals to various extents as shown in Fig. 2b—2e. The addition of 50 mg (5.0×10^{-5} mole) of Eu(FOD)$_3$ permitted the assignment of all the ring protons from the first-order analysis, which was confirmed by spin

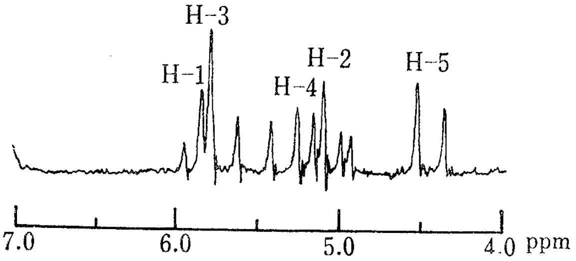

Fig. 1. 60 MHz NMR Spectrum of Methyl (Phenyl 2,3,4-Tri-O-acetyl-α-D-glucopyranosid) uronate in CDCl$_3$

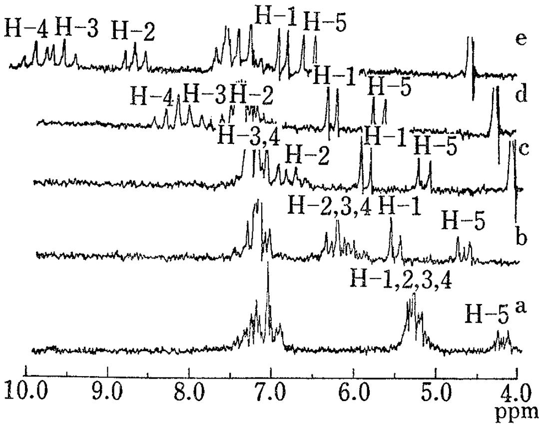

Fig. 2. 60 MHz NMR Spectra of Methyl (Phenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranosid) uronate (25 mg, 6.3×10^{-5} mole) in CDCl$_3$ (0.5 ml) Containing 0—50 mg (5.0×10^{-5} mole) of Eu(FOD)$_3$

a: 0 mg, b: 10 mg, c: 20 mg, d: 30 mg, e: 50 mg

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11) R.E. Rondeau and R.E. Sievers, *J. Am. Chem. Soc.*, **93**, 1522 (1971).

12) I. Armitage and L.D. Hall, *Chem. Ind.* (London), **1970**, 1537; *idem, Can. J. Chem.*, **49**, 2770 (1971).

13) R.F. Butterworth, A.G. Pernet, and S. Hanessian, *Can. J. Chem.*, **49**, 981 (1971).

14) The NMR spectra were measured at room temperature in deuteriochloroform (CDCl$_3$) at 60 MHz, using Hitachi Model R-20A 60 MHz spectrometer. Chemical shifts are expressed in δ (ppm) with tetramethylsilane (TMS) as internal standard. The coupling constant (J) and the chemical shift difference ($\Delta\nu$) are expressed in Hz: d, doublet; t, triplet; m, multiplet; bm, broad multiplet. The spectral data were usually obtained at the concentration of 5% in 0.5 ml of CDCl$_3$. The appropriate amount (10 to 160 mg) of Eu(FOD)$_3$ was added to the sample solution and the spectral change was recorded. The spin decoupling experiments were performed in the frequency sweep mode.

15) J.I. Musher and E.J. Corey, *Tetrahedron*, **18**, 791 (1962).

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decoupling experiments (Table I). From Fig. 2b, it was evident that 10 mg of $Eu(FOD)_3$ was enough to distinguish the anomeric proton at 5.51 ppm as a doublet with 7.0 Hz of spacing, indicating the β-configuration. As the resolution between the H-3 and H-4 proton signals was improved by increasing concentration of the shift reagent, the multiplet due to the H-5 proton turned into a sharp doublet *via* an intermediary triplet. Thus the above observations gave definite evidence that the H-5 proton was virtually coupled with the H-3 proton owing to the large $J_{3,4}$ as compared with $\Delta\nu_{3,4}$ to give the multiplet instead of the expected doublet, measured at 60 MHz without the shift reagent.

Based on the chemical shift changes brought about by $Eu(FOD)_3$, it became apparent that the proton signals attaching to the carbon atom involved in the ester linkage (H-2, H-3,

Table I. The NMR Parameter for Methyl (Aryl 2,3,4-Tri-O-acetyl-D-glucopyranosid)uronates

Aryl	Configuration	$Eu(FOD)_3$ added (mg)	H-1	H-2	H-3	H-4	H-5	Acetoxy methyl	Methyl (ester)
Phenyl	α	0	4.82 (d, J=3.5)	5.05 (q, J=3.5, 9.5)	5.79 (t, J=9.5)	5.26 (t, J=9.5)	4.45 (d, J=10.0)	2.06 (3)[a]	3.71
	β	0	5.00	—	—	5.50[b]	4.18 (m)	2.05 (3)	3.72
	β	10	5.51 (d, J=7.0)	5.90	—	6.35	4.67 (t)	2.29, 2.32, 2.35	3.90
	β	50	6.87 (d, J=7.0)	8.70 (t, J=8.0)	9.56 (t, J=9.0)	9.92 (t, J=9.0)	6.55 (d, J=9.0)	3.17, 3.30, 3.60	4.57
p-NO_2-Phenyl	α	0	5.92 (d, J=3.5)	5.06 (q, J=3.5, 9.5)	5.75 (t, J=9.5)	5.26 (t, J=9.5)	4.33 (d, J=10.0)	2.07 (3)	3.72
	β	0	5.20	—	—	5.50	4.25 (m)	2.06 (3)	3.71
	β	10	5.60 (d, J=7.0)	5.75	—	6.30	4.68 (q)	2.30 (2), 2.36	3.86
	β	70	7.31 (d, J=7.0)	9.49 (t, J=8.0)	10.80	11.10	7.10 (d, J=8.5)	3.49, 3.57, 4.09	4.65

a) Numbers in parenthesis indicate number of acetyl groups.
b) overlapping resonances

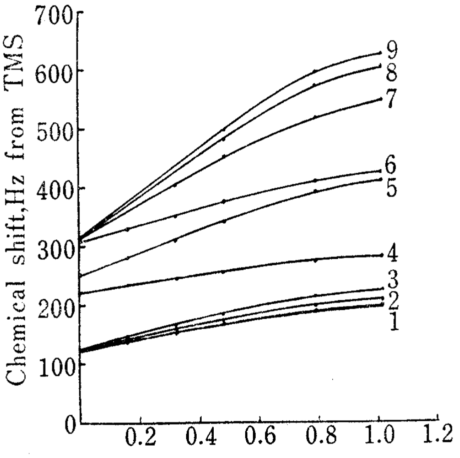


Fig. 3. Chemical Shift Changes Induced by $Eu(FOD)_3$ in the 60 MHz NMR Spectra of Methyl (Phenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranosid)uronate (25 mg, 6.3×10^{-5} mole) in $CDCl_3$ (0.5 ml)

1, 2, and 3: acetoxy methyl, 4: methyl, (ester), 5: H-5, 6: H-1, 7: H-2, 8: H-3, 9: H-4

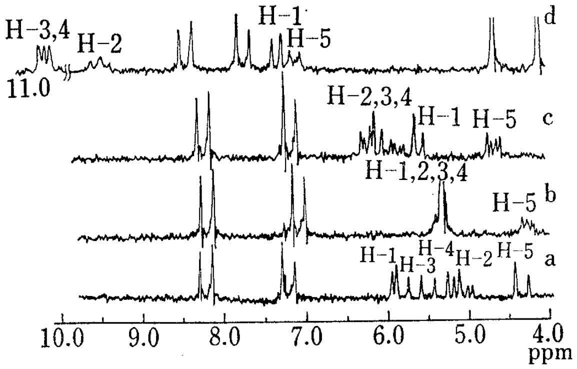


Fig. 4. 60 MHz NMR Spectra of Anomeric Pair of Methyl (p-Nitrophenyl 2,3,4-Tri-O-acetyl-D-glucopyranosid)uronates (25 mg, 5.5×10^{-5} mole) in $CDCl_3$ (0.5 ml) Containing 0—70 mg (7.0×10^{-5} mole) of Eu $(FOD)_3$

α-anomer: a, 0 mg
β-anomer: b, 0 mg; c, 10 mg; d, 70 mg

and H-4) shifted more to downfield than those of the proton signals attaching to the carbon atom involved in the ether linkage (H-1 and H-5) (Fig. 3). Therefore, the addition of the shift reagent made the H-1 proton remain at relatively upper-field and it possible to assign the anomeric configuration from its coupling constant, coupled with the spectral pattern difference between anomers.[2] The acetoxy signals were well separated with one another by the shift reagent (Table I). The aromatic protons of the aglycone appeared at 6.90—7.50 ppm shifted only 0.2 ppm even by the addition of 50 mg of $Eu(FOD)_3$.

The NMR spectra of the anomeric pair of methyl (*p*-nitrophenyl 2,3,4-tri-O-acetyl-D-glucopyranosid)uronates revealed the similar aspect to those of phenyl glucosiduronates described above (Fig. 4, Table I). However, even addition of 70 mg of the shift reagent to the β-anomer did not afford a sharp doublet due to the H-5 proton, because of the poor resolution between the H-3 and H-4 protons in this case. These results further support that the ratio of $\Delta\nu_{3,4}$ to $J_{3,4}$ is the critical parameter determining the appearance of the H-5 proton signal.

Examination of the NMR spectra was then carried out with four anomeric pairs of aryl 2,3,4,6-tetra-O-acetyl-D-glucopyranosides indicated in Table II. As a typical example, the NMR spectra of the anomeric pair of phenyl 2,3,4,6-tetra-O-acetyl-D-glucopyranosides are shown in Fig. 5. The ring protons of the α-anomers were well assigned from the first-order analysis except the H-5, H-6, and H-6′ protons which were overlapped at 3.90—4.40 ppm (Table II). In the β-anomers, on the other hand, the H-1, H-2, H-3, and H-4 protons appeared in the narrow range of 0.5 ppm and their assignment was not possible, while the H-5 proton was well up-shifted from the H-6 and H-6′ protons and appeared as a broad multiplet.

From the chemical shift changes caused by the shift reagent, it became evident that the proton signals attaching to the carbon atom involved in the ester linkage (H-2, H-3, H-4,

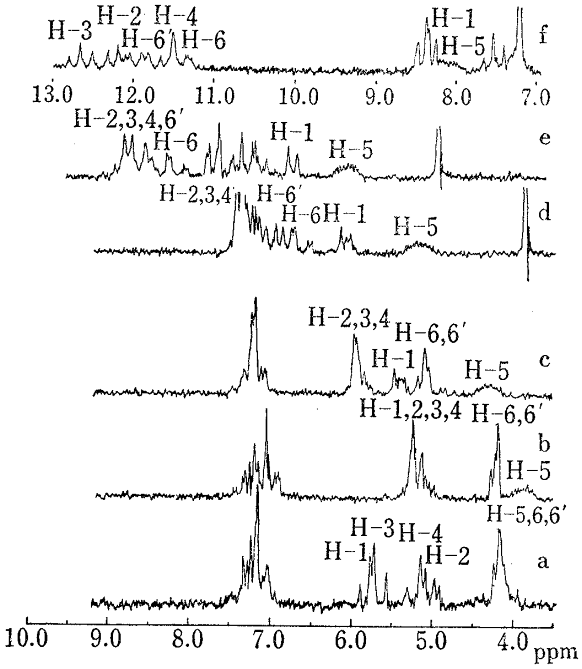
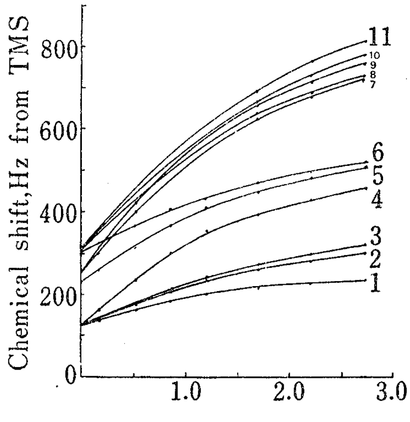


Fig. 5. 60 MHz NMR Spectra of Anomeric Pair of Phenyl 2,3,4,6-Tetra-O-acetyl-D-glucopyranosides (25 mg, 5.9×10^{-5} mole) in $CDCl_3$ (0.5 ml) Containing 0—130 mg (1.3×10^{-4} mole) nf $Eu(FOD)_3$

α-anomer: a, 0 mg
β-anomer: b, 0 mg; c, 10 mg; d, 30 mg; e, 50 mg
f, 130 mg



Molar ratio, $En(FOD)_3$/Sugar

Fig. 6. Chemical Shift Changes Induced by $Eu(FOD)_3$ in the 60 MHz NMR Spectra of Phenyl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (25 mg, 5.9×10^{-5} mole) in $CDCl_3$ (0.5 ml)

1,2,3, and 4: acetoxy methyl, 5: H-5, 6: H-1, 7: H-6, 8: H-4, 9: H-6′, 10: H-2, 11: H-3

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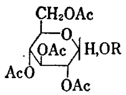
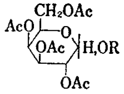
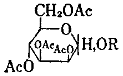
TABLE II. The NMR Parameter for Aryl 2,3,4,6-Tetra-O-acetyl-D-glycopyranosides

Sugar	Aryl (R)	Configuration	Eu(FOD)$_3$ added (mg)	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	Acetoxy methyl
	phenyl	α	0	5.75 (d, J=3.5)	5.04 (q, J=3.5, 9.5)	5.72 (t, J=9.5)	5.15 (t, J=9.5)	4.10(bm)	4.17(m)	4.17(m)	2.04(4)[a]
		β	0	4.90——			——5.40[b]	3.85(bm)	4.20(m)	4.20(m)	2.07(4)
		β	50	6.80 (d, J=7.0)	8.40——		——9.00	6.10(bm)	8.20 (q, J=3.0, 12.5)	8.50—8.80	3.09, 3.45 3.51, 5.01
		β	130	8.30 (d, J=7.0)	12.18 (t, J=8.0)	12.65 (t, J=9.0)	11.50 (t, J=9.0)	8.10(bm)	11.40 (q, J=3.0, 12.5)	11.94 (q, J=5.0, 12.5)	3.80, 4.73 5.03, 7.20
	p-NO$_2$-phenyl	α	0	5.86 (d, J=4.0)	5.06 (q, J=4.0, 9.5)	5.72 (t, J=9.5)	5.19 (t, J=9.5)	4.10(bm)	4.15(m)	4.15(m)	2.04(2) 2.07(2)
		β	0	5.00——			——5.40	3.90(bm)	4.20(m)	4.20(m)	2.08(4)
		β	30	6.39 (d, J=7.0)	—[c]	—	—	5.60(bm)	—	—	2.86, 3.01 3.15, 4.19
	1-naphthyl	α	0	5.89 (d, J=4.0)	5.15 (q, J=4.0, 9.5)	5.92 (t, J=9.5)	5.27 (t, J=9.5)	4.20(bm)	4.20(m)	4.20(m)	2.05(2) 2.10(2)
		β	0	5.10——			——5.60	3.95(bm)	4.25(m)	4.25(m)	2.05, 2.07(3)
		β	30	6.15 (d, J=7.0)	—	—	—	5.30(bm)	—	—	2.60, 2.74 2.94, 4.04
	2-naphthyl	α	0	5.88 (d, J=3.5)	5.08 (q, J=3.5, 9.5)	5.79 (t, J=9.5)	5.20 (t, J=9.5)	4.20(bm)	4.20(m)	4.20(m)	2.04 2.07(3)
		β	0	5.00——			——5.40	3.90(bm)	4.15(m)	4.25(m)	2.09(4)
		β	30	6.41 (d, J=7.0)	—	—	—	5.50(bm)	—	—	2.80, 3.06(2) 4.07
	phenyl	α	0	5.78 (d, J=3.5)	5.10——		——5.70	4.15(bm)	4.15(m)	4.15(m)	1.93, 2.02 2.06, 2.16
		β	0	4.95——			——5.65	4.15(bm)	4.15(m)	4.15(m)	2.01, 2.07(2) 2.19
		β	10	5.34 (d, J=7.0)	6.16 (q, J=7.0, 9.5)	5.80 (q, J=3.5, 9.5)	6.45 (d, J=3.5)	4.50(bm)	4.90(m)	4.90(m)	2.27, 2.38 2.47, 2.50
	phenyl	α	0	5.30——			——5.70	4.15(bm)	4.18(m)	4.18(m)	2.02(3), 2.20
		α	10	5.84(d)	6.00——		——6.20	4.50(bm)	4.80 (q, J=2.5, 12.5)	5.19 (q, J=5.0, 12.5)	2.28, 2.31 2.46, 2.60
		α	100	8.30(d)	11.35(m)	10.80 (q, J=3.5, 9.5)	11.28 (t, J=9.5)	7.60(bm)	10.25 (q, J=2.5, 12.5)	11.69 (q, J=5.0, 12.5)	3.84, 4.50 4.60, 6.75
		β	0	5.00—5.50	5.66(m)	5.00——	——5.50	3.85(bm)	4.25(m)	4.25(m)	2.02, 2.08(2) 2.24
		β	10	5.50(d)	6.44(m)	5.82 (q, J=3.5, 9.5)	6.07 (t, J=9.5)	4.30(bm)	4.96 (q, J=3.0, 12.5)	5.26 (q, J=5.0, 12.5)	2.31, 2.45 2.52, 2.63
		β	50	6.54(d)	9.45(m)	8.64(m)	8.86 (t, J=9.5)	6.02(bm)	8.10 (q, J=3.0, 12.5)	8.68(m)	3.16, 3.65 4.09, 4.80

a) Numbers in parenthesis indicate number of acetyl groups. b) overlapping resonances c) The signal was overlapped with the aromatic protons.

H-6, and H-6′) shifted more to downfield than those of the proton signals attaching to the carbon atom involved in the ether linkage (H-1 and H-5), as were observed above with the glucosiduronates (Fig. 6). Thus, the addition of 30 or 50 mg (3.0 or 5.0×10^{-5} mole) of Eu(FOD)$_3$ to the sample solution (25 mg; 5.3—5.9×10^{-5} mole) left the H-1 proton at relatively upper-field, allowing the assignment of the β-configuration from its coupling constant (d, $J_{1,2}$= 7.0 Hz) (Table II). Moreover, the addition of 130 mg (1.3×10^{-4} mole) of the shift reagent to 25 mg (5.9×10^{-5} mole) of the β-anomer of the phenyl glucoside derivative permitted the assignment of all the ring protons, coupled with the spin decoupling experiments (Table II). The acetoxy signals were well separated with one another by the shift reagent as shown in Table II. Three acetoxy signals shifted in the similar magnitudes to those observed with the glucosiduronates. However, one acetoxy signal shifted substantially more to downfield than the others as shown in Fig. 6. This signal could be ascribed to the acetoxy group attached to the hydroxymethyl group. The similar shifts were also observed with glycopyranose pentaacetates, using Eu(DPM)$_3$ as the shift reagent.[12]

Next, the NMR spectra of the anomeric pair of phenyl 2,3,4,6-tetra-O-acetyl-D-galactopyranosides were examined (Fig. 7, Table II). They showed the entirely different spectral patterns from those of glucosides, because they possess an axial acetoxy group at C-4. In the α-anomer, the H-1 proton appeared at 5.78 ppm (d, $J_{1,2}$=3.5 Hz) which was quite consistent with those of aryl α-glucosides as indicated in Table II, while other ring protons could not be assigned. Although the H-1, H-2, H-3, and H-4 protons overlapped at 4.95—5.65 ppm in the β-anomer, the addition of 10 mg (1.0×10^{-5} mole) of Eu(FOD)$_3$ to 25 mg (5.9×10^{-5} mole) of the β-anomer permitted the assignment of the β-configuration from the spacing of the H-1 proton signal (d, $J_{1,2}$=7.0 Hz), by the analogous way described above for the glucosides. The other ring protons could be assigned from the first-order analysis as well (Table II).

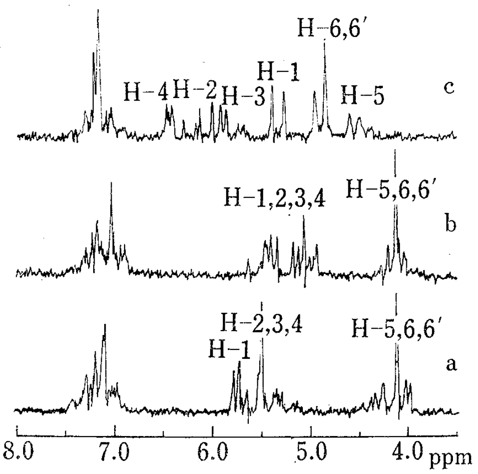


Fig. 7. 60 MHz NMR Spectra of Anomeric Pair of Phenyl 2,3,4,6-Tetra-O-acetyl-D-galactopyranosides (25 mg, 5.9×10^{-5} mole) in CDCl$_3$ (0.5 ml) Containing 0—10 mg (1.0×10^{-5} mole) of Eu(FOD)$_3$

α-anomer: a, 0 mg
β-anomer: b, 0 mg; c, 10 mg

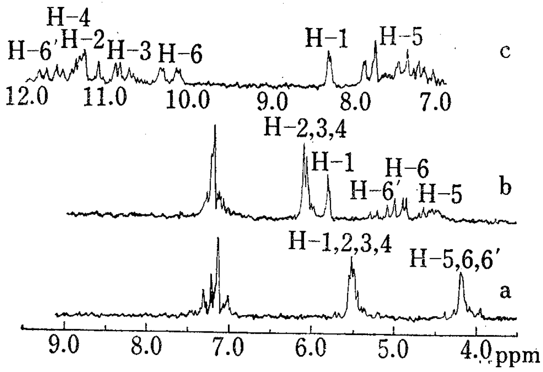


Fig. 8. 60 MHz NMR Spectra of Phenyl 2,3,4,-6-Tetra-O-acetyl-α-D-mannopyranoside (25 mg, 5.9×10^{-5} mole) in CDCl$_3$ (0.5 ml) Containing 0—100 mg (1.0×10^{-4} mole) of Eu (FOD)$_3$

a: 0 mg, b: 10 mg, c: 100 mg

Finally, the NMR spectra of the anomeric pair of phenyl 2,3,4,6-tetra-O-acetyl-D-mannopyranosides were examined (Fig. 8,9; Table II). They gave the different spectral patterns from those of glucosides and galactosides. Thus, the H-1 and H-2 protons of the α- and β-anomers are both in gauche relationship, and the spacings of the anomeric protons do not serve as key signals for the elucidation of the glycosidic linkages, as shown in Fig. 8b and 9b. Since the addition of 10 mg (1.0×10^{-5} mole) of the shift reagent to 25 mg (5.9×10^{-5} mole) of the α- or β-anomer could distinguish the H-1 protons, the anomeric configuration could be deduced by comparing the relative chemical shifts of the H-1 protons of the anomeric pair, assigning the one with the H-1 proton signal at lower field to the α-anomer (α-anomer, 5.84 ppm; β-anomer, 5.50 ppm). Furthermore, the spectral pattern difference between anomers seems to give the definite evidence. Lemieux, *et al.*[16] employed the relative positions of the H-5 and C-6 methylene protons to assign the anomeric configuration of D-mannopyranose pentaacetates. In the α-anomer of the phenyl mannoside derivatives, the H-1, H-2, H-3, and H-4 protons appeared in the narrow range at 5.30—5.70 ppm and the H-5 proton overlapped with the C-6 methylene protons at 3.90—4.40 ppm, while the addition of 100 mg (1.0×10^{-4} mole) of Eu(FOD)$_3$ to 25 mg (5.9×10^{-5} mole) of the α-anomer allowed the assignment of the ring protons with the aid of spin decoupling experiments. In the β-anomer, on the other hand, the H-2 proton signal appeared as a narrow multiplet at the lowest field (5.66 ppm) among the ring protons, which seemed to be typical for the β-anomer. The H-1, H-3, and H-4 protons overlapped in the region of 5.00—5.50 ppm, while the H-5 proton appeared at 3.85 ppm (bm), well up-shifted from the C-6 methylene protons at 4.25 ppm (bm). In this case, the addition of 10 mg (1.0×10^{-5} mole) of the shift reagent to 25 mg (5.9×10^{-5} mole) of the β-anomer was enough to premit the assignment of the ring protons. The addition of 50 mg of Eu(FOD)$_3$ resulted in overlapping of the H-3 and H-6′ protons as shown in Fig. 9c. It became apparent from the above observations that the different spectral pattern between anomers should prove of value in the elucidation of the anomeric configuration of mannosides. The analogous spectral pattern difference between anomers was observed with the anomeric pairs of acetylated D-mannopyranoses[16] and methyl D-mannopyranosides.[17]

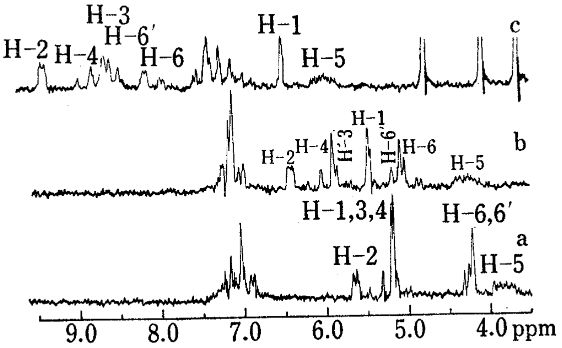


Fig. 9. 60 MHz NMR Spectra of Phenyl 2,3,4,6-Tetra-O-acetyl-β-D-mannopyranoside (25 mg, 5.9×10^{-5} mole) in CDCl$_3$ (0.5 ml) Containing 0—50 mg (5.0×10^{-5} mole) of Eu(FOD)$_3$

a: 0 mg, b: 10 mg, c: 50 mg

Experimental[18]

Materials——The anomeric pairs of methyl (phenyl tri-O-acetyl-D-glucopyranosid)uronates (α, mp 115—116°; β, mp 123—125°),[20] phenyl tetra-O-acetyl-D-glucopyranosides (α, mp 116—117°; β, mp 125—126°),[21] *p*-nitrophenyl tetra-O-acetyl-D-glucopyranosides (α, mp 112—113°; β, mp 176—177°),[22] 1-naphthyl

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16) R.U. Lemieux and J.D. Stevens, *Can. J. Chem.*, **43**, 2059 (1965).

17) M. Matsui and M. Okada, unpublished results.

18) Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography (TLC) was carried out on 5×20 cm glass plates coated with a 0.25 mm layer of silica gel H (Merck) with the detection by ammonium metavanadate-sulfuric acid reagent,[19] and column chromatography on silica gel (Merck). Infrared (IR) spectra were determined in potassium bromide disks on Hitachi EPI-32 spectrometer. Optical rotations were determined on Hitachi PO-B polarimeter in 10 cm tubes of 1 ml capacity.

19) M. Ishidate, M. Matsui, and M. Okada, *Anal. Biochem.*, **11**, 176 (1965).

20) G.N. Bollenback, J.W. Long, D.G. Benjamin, and J.A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).

21) B. Helferich and E. Schmitz-Hillebrecht, *Chem. Ber.*, **66**, 378 (1933).

22) E.M. Montgomery, N.K. Richtmyer, and C.D. Hudson, *J. Am. Chem. Soc.*, **64**, 690 (1942).

tetra-O-acetyl-D-glucopyranosides (α, mp 112—113°; β, mp 175—176°),[21,23] phenyl tetra-O-acetyl-D-galactopyranosies (α, mp 129—131°; β, mp 124—125°),[21] and phenyl tetra-O-acetyl-D-mannopyranosides (α, mp 79—81°; β, mp 168—170°),[25] were prepared according to the procedures described in the literatures.[26] Methyl (*p*-nitrophenyl tri-O-acetyl-β-D-glucopyranosid)uronate (mp 151—152°)[27] was prepared from *p*-nitrophenyl β-D-glucopyranosiduronic acid which was obtained from Chugai Pharmaceutical Co., Tokyo, by treating it with CH_2N_2 followed by acetylation with pyridine–Ac_2O in the usual way. The shift reagent $Eu(FOD)_3$ was purchased from Pierce Chemical Co., Illinois.

Methyl (*p*-Nitrophenyl Tri-O-acetyl-α-D-glucopyranosid)uronate——To a methanolic solution (2 ml) of *p*-nitrophenyl α-D-glucopyranosiduronic acid (mp 208—210° (decomp.))[28] (100 mg) was added ethereal CH_2N_2 solution portionwise until yellow color persisted. Evaporation of the solvent gave a residue, which was dissolved in pyridine (5 ml) and Ac_2O (3 ml), and stood at room temperature overnight. The reaction mixture was poured into ice water and extracted with AcOEt. The organic layer was washed with H_2O, 1N HCl, H_2O, 5% $NaHCO_3$, and H_2O, then dried over anhyd. Na_2SO_4. Evaporation of the solvent afforded a residue, which was triturated in pet. ether under ice water cooling to give fine amorphous powders (135 mg). $[\alpha]_D^{25}$ +170° (c=1.00, $CHCl_3$). *Anal.* Calcd. for $C_{19}H_{21}O_{12}N$: C, 50.11; H, 4.65; N, 3.08. Found: C, 49.95; H, 4.57; N, 3.18.

2-Naphthyl Tetra-O-acetyl-α- and -β-D-glucopyranosides——β-D-Glucose pentaacetate[29] (3.2 g) and 2-naphthol (2.0 g) were melted at 125°, then powdered $ZnCl_2$ (0.5 g) was added. The mixture was heated at 120—130° under reduced pressure for 60 min. Each 0.1 g of $ZnCl_2$ was added to the reaction mixture at 30 and 45 min. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O, 1N NaOH, and H_2O, then dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* afforded a sirup (3.0 g), which was chromatographed on a silica gel (150 g) column using cyclohexane–AcOEt (7:3) as solvent. The α-anomer was eluted in the fractions between 700 and 1000 ml. Evaporation of the solvent gave crystals (1.2 g), which was recrystallized from EtOH to give needles of the α-anomer melting at 88—89°.[30] $[\alpha]_D^{25}$ +177° (c=1.21, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{26}O_{10}$: C, 60.75; H, 5.52. Found: C, 60.88; H, 5.45. The β-anomer was then eluted in the fractions between 1100 and 1400 ml. Evaporation of the solvent gave crystals (1.1 g), which was recrystallized from EtOH to afford needles of the β-anomer melting at 133—135°.[21] Examination by TLC was performed with cyclohexane–AcOEt (7:3) as solvent. The plate was developed twice with the same solvent. The α-anomer had higher *Rf* value than that of the β-anomer.

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23) C.D. Hurd and W.A. Bonner, *J. Org. Chem.*, **10**, 603 (1945). Kariyone, *et al.*[24] reported mp 178—179.5° for the α-anomer and mp 177—178.5° for the β-anomer, which were purified by repeated recrystallizations, without presenting optical rotatory data. Hurd, *et al.* described mp 114.5° for the α-anomer and mp 178—179° for the β-anomer, which were in good agreement with those of the anomers prepared by us.

24) T. Kariyone, M. Takahashi, and K. Takaishi, *Yakugaku Zasshi*, **72**, 13 (1952).

25) B. Helferich and S. Winkler, *Chem. Ber.*, **66**, 1556 (1933).

26) The separation of α- and β-anomers was performed by column chromatography in the similar way as described for the preparation of 2-naphthyl glucosides. The solvents used were cyclohexane–AcOEt (1:1) or (7:3), except the mannosides, which were eluted with $CHCl_3$–acetone (95:5). Examination by TLC was carried out with the same solvent system used for column chromatography. The plate was developed two to three times with the same solvent. The α-anomer had higher *Rf* value than that of the β-anomer so far examined.

27) K. Kato, K. Yoshida, H. Tsukamoto, M. Nobunaga, T. Masuya, and T. Sawada, *Chem. Pharm. Bull.* (Tokyo), **8**, 239 (1960).

28) C.A. Marsh and G.A. Levvy, *Biochem. J.*, **68**, 617 (1958).

29) M.L. Wolfrom and A. Thompson, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, p. 211.

30) Kariyone, *et al.*[24] reported mp 128—128.5° for the α-anomer and mp 134—135° for the β-anomer, which were purified by repeated recrystallizations, without presenting the optical rotatory data.