[Chem. Pharm. Bull. 19(2) 395—400 (1971)]

UDC 547.457.1.02.03

The Nuclear Magnetic Resonance Spectra of Acetylated Di- and Poly-D-glucopyranoses. Differentiation between Anomers by 1,3-Diaxial Deshielding Effect

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(Received September 10, 1970)

The nuclear magnetic resonance spectra of anomeric pairs of acetylated D-glucopyranosylglucopyranoses and 1→4 and 1→6 linked D-glucans were measured in deuteriochloroform at 60 or 100 MHz. The spectral pattern difference between anomers, due to the 1,3-diaxial deshielding effect in α-anomer, proved to be an useful means to assign the configuration of glucosidic linkage.

Low and high field shifts of acetoxy signals were observed in some acetylated D-glucans.

The utility of nuclear magnetic resonance (NMR) spectroscopy for configurational and conformational analyses of carbohydrates has been well documented.[2] The assignment of their anomeric configurations is usually made from the chemical shift as well as coupling constant of the anomeric proton. Recently, Sinclair, *et al.*[3] differentiated between anomers on the basis of their chemical shift difference of the C-6 methyl proton signals after converting the hydroxymethyl group to methyl group. The deshielding effect due to an axial substituent at C-1 (α-anomer) on the axial proton at C-5 or C-3 was well observed in the glycopyranose acetates,[4] 2-acylamino-2-deoxy-glycopyranose acetates,[5] and glycopyranosyl fluoride acetates[6] in the Cl chair conformation.

In the previous communication,[7] we extended the 1,3-diaxial deshielding effect to some acetylated D-glycopyranosides to differentiate between anomers from their spectral patterns as well as from their anomeric proton signals, measured in deuteriochloroform at 60 and 100 MHz. In the acetylated D-glucopyranoside series, the α-anomer gave the following typical pattern: the H-2, H-3, and H-4 protons appeared in the range of 1.0 ppm; the axial H-3 proton came up at the lowest field of this region; the deshielding of the axial H-5 proton caused the H-5 proton to appear in the region of the C-6 methylene protons. On the other hand, the β-anomer exhibited a quite different pattern: the H-2, H-3, and H-4 protons appeared in the narrow range of 0.5 ppm and the first-order assignment was usually difficult; the H-5 proton was well shifted from the C-6 methylene protons and appeared at the upper field as a broad multiplet. Thus the spectral pattern difference between anomers seems quite useful to assign the anomeric configuration especially when the anomeric proton signal is obscured by overlapping with other proton signals, as in the cases of acetylated derivatives of a highly oxygenated monoterpene β-D-glucopyranoside,[8] aryl β-D-glucopyranosides,[7,9,10] aryl β-D-gluco-

1) Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
2) L.D. Hall, "Advances in Carbohydrate Chemistry," Vol. 19, ed. by M.L. Wolfrom, Academic Press, New York, 1964, p. 51; L. Hough and A.C. Richardson, "Rodd's Chemistry of Carbon Compounds," Vol. 1F, ed. by S. Coffey, Elsevier Publishing Co., Amsterdam, 1967, p. 139; J.J.M. Rowe, J. Hinton, and K.L. Rowe, *Chem. Rev.*, **70**, 1 (1970).
3) H.B. Sinclair and R.T. Sleeter, *Tetrahedron Letters*, **1970**, 833.
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pyranosiduronates,[7,10] and oligo- or poly-D-glycopyranoses.[11,12] The present paper provides further utility of the aforementioned spectral difference between anomers for acetylated D-glucopyranosylglucopyranoses and D-glucans.

The NMR spectra[13] of α,α-, α,β-, and β,β-trehalose octaacetates are shown in Fig. 1. They exhibited the similar changes in pattern to those observed with acetylated alkyl D-glucopyranosides reported earlier.[7] The 60-MHz spectrum of α,α-trehalose octaacetate (Fig. 1c) showed two H-3 protons at 5.53 ppm (t), two H-1 protons at 5.30 ppm (d), and four C-6 protons and two H-5 protons at 4.15 ppm (m). In α,β-trehalose octaacetate (Fig. 1b), on the other hand, H-3 and H-1 proton intensities at 5.50 and 5.36 ppm were reducted each to one proton, and the H-1 proton signal due to β-anomer appeared at 4.70 ppm (d), while the H-5 proton signal was up-shifted from the C-6 methylene proton region and appeared as a broad multiplet at 3.80 ppm. The spectrum of β,β-trehalose octaacetate (Fig. 1a) revealed that H-3 proton signals were shifted to upper field (<5.3 ppm) and two H-5 proton signals were well shifted from four C-6 methylene protons appearing at 3.80 ppm (bm). The spectra of these trehalose octaacetates measured at 100 MHz showed some improvement in their spectral resolution (Fig. 1f, e, d).

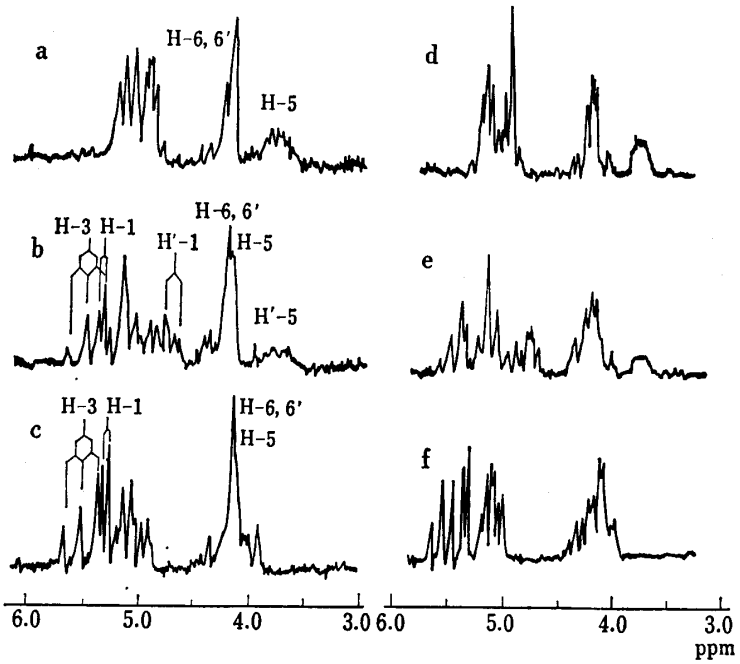

Fig. 1. NMR Spectra of Trehalose Octaacetates in $CDCl_3$

β,β-trehalose octaacetate: a, 60 MHz; d, 100 MHz
α,β-trehalose octaacetate: b, 60 MHz; e, 100 MHz
α,α-trehalose octaacetate: c, 60 MHz; f, 100 MHz

Further examination of the NMR spectra was then carried out with three anomeric pairs of D-glucopyranosylglucopyranose acetates; β-octaacetates of kojibiose (α, 1→2), sophorose (β, 1→2), nigerose (α, 1→3), laminaribiose (β, 1→3), maltose (α, 1→4), and cellobiose (β,

11) D. Gagnaire, L. Odier, and M. Vencendon, *J. Polymer Sci.*, **Part C**, 27 (1969).
12) B. Casu, M. Reggiani, G.G. Gallo, and A. Vigevani, *Carbohyd. Res.*, **12**, 157 (1970).
13) The NMR spectra were measured at room temperature in deuteriochloroform at 60 and 100 MHz, using Hitachi Model R-20A 60-MHz and JNM-4H 100-MHz spectrometers. Chemical shifts are expressed in δ (parts per million) with tetramethylsilane as internal standard. The coupling constants (J) are expressed in cycles per second: d, doublet; t, triplet; m, multiplet; bm, broad multiplet. The spectral data were usually obtained at the concentrations of 5 to 10%, while the spectra of some acetylated D-glucans were measured at the concentration of 2%, due to their slight solubility. The spin decoupling experiments were performed in several instances at 60 MHz to assign the anomeric proton.

1→4) (Fig. 2). Each disaccharide consists of reducing and nonreducing D-glucose units. The former has β-anomeric configuration while the latter has α- or β-anomeric one, depending on its glucosidic configuration. Therefore, their spectra could reasonably be interpreted by the combination of the spectra of penta-O-acetyl-β-D-glucopyranose[4,7] (reducing unit) and acetylated α- or β-D-glucopyranoside[7] (nonreducing unit). The proton signals of the reducing unit showed H′-1 proton at 5.64—5.75 ppm (d, J=7.5—8.0), H′-5 proton at 3.80—3.90 ppm (bm), and H′-6,6′ protons at 4.15—4.20 ppm (m), respectively. The proton signal involved in the glucosidic linkage was overlapped with the H′-5 proton of the reducing unit. The nonreducing unit having α-anomeric configuration (Fig. 2b, d, f) showed H-3 proton at 5.38 ppm (t), H-1 proton at 5.28—5.48 ppm (d), and H-6, 6′ and H-5 protons at 4.10—4.20 ppm (bm). The integral of the signals revealed that the relative proton intensities at 3.5—4.0 and 4.0—4.5 ppm regions were in a ratio of 2:5 as expected. In contrast to this, the nonreducing unit having β-anomeric configuration (Fig. 2a, c, e) exhibited a different spectral pattern. Thus the H-3 proton signal was shifted to upper field (→5.30 ppm) and overlapped with other methine proton signals. The anomeric proton appeared at 4.52—4.61 ppm (d), and the H-5 proton was up-shifted from C-6 methylene proton signals appearing at 3.80 ppm (bm). The integration of the proton signals of β-anomers at 3.5—4.0 and 4.0—4.5 ppm, excluding the anomeric proton, revealed the ratio of 3.5:3.5 which was very close to the expected one (3:4).

The anomeric pair of β-octaacetates of 6-O-D-glucopyranosylglucopyranose (isomaltose, gentiobiose), on the other hand, has somewhat different feature from the above anomeric pairs.

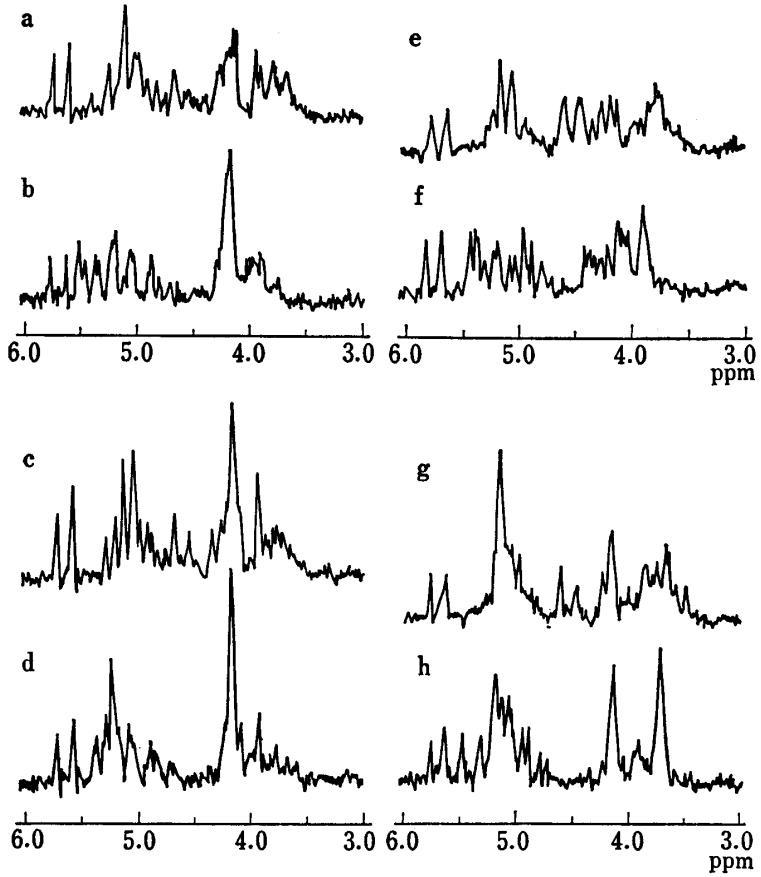

Fig. 2. 60-MHz NMR Spectra of β-Octaacetates of D-Glucopyranosyl-glucopyranoses

a: sophorose b: kojibiose c: laminaribiose d: nigerose e: cellobiose f: maltose g: gentiobiose h: isomaltose

The H′-6, 6′ protons involved in the glucosidic linkage appeared at 3.70 ppm (m∼bm). In β-isomaltose octaacetate (α, 1→6) (Fig. 2h), H-3 proton appeared at 5.50 ppm (t), H-1 proton at 5.18 ppm (d), H-5 proton at 4.10 ppm (bm), and H-6, 6′ protons at 4.15 ppm (m), respectively. The integral of the signals showed that the relative proton intensities at 3.5—4.0 and 4.0—4.5 ppm regions were in a ratio of 3.5:2.5, which was close to the expected ratio of 3:3. In β-gentiobiose octaacetate (β, 1—6) (Fig. 2g), H-3 proton shifted to upper field (<5.3 ppm) and overlapped with other methine proton signals. The H-1 proton appeared at 4.54 ppm (d), H-5 proton at 3.80 ppm (bm), and H-6,6′ protons at 4.20 ppm (m). The integral of the signals showed that the relative proton intensities at 3.5—4.0 and 4.0—4.5 ppm, excluding the anomeric proton, were in a ratio of 4:2 as expected.

Thus, the above results summarized in Table I reveal that the spectral pattern difference between anomers is quite useful to assign the anomeric configuration and further that trehaloses and 6-O-D-glucopyranosylglucopyranoses are easily distinguishable from other types of glucopyranosylglucopyranoses.

Table I. The NMR Parameter for Acetylated D-Glucopyranosylglucopyranoses[a,13]

Acetylated derivatives	Nonreducing unit				Reducing unit		
	H-1	H-3	H-5	H-6,6′	H-1′	H′-5	H′-6,6′
α,α-Trehalose	5.30 (d, J=3.5)	5.53 (t, J=9.5)	4.10 (bm)	4.15 (m)			
α,β-Trehalose	5.36 (d, J=3.5)	5.50 (t, J=9.5)	4.20 (bm)	4.20 (m)			
	4.70 (d, J=7.0)	<5.30	3.80 (bm)				
β,β-Trehalose	4.80——5.30[b]		3.80 (bm)	4.20 (m)			
β-Kojibiose	5.48 (d, J=4.0)	5.38 (t, J=9.5)	4.10 (bm)	4.20 (m)	5.69 (d, J=8.0)	3.90 (bm)	4.20 (m)
β-Sophorose	4.61 (d, J=7.5)	<5.30	3.80 (bm)	4.20 (m)	5.68 (d, J=8.0)	3.80 (bm)	4.20 (m)
β-Nigerose	5.28 (d, J=4.0)	5.37 (t, J=9.5)	4.15 (bm)	4.15 (m)	5.64 (d, J=8.0)	3.80 (bm)	4.15 (m)
β-Laminaribiose	4.60 (d, J=7.5)	<5.30	3.80 (bm)	4.15 (m)	5.64 (d, J=8.0)	3.80 (bm)	4.15 (m)
β-Maltose	5.40 (d, J=4.0)	5.38 (t, J=9.5)	4.20 (bm)	4.20 (bm)	5.75 (d, J=7.5)	3.90 (bm)	4.20 (bm)
β-Cellobiose	4.52 (d, J=7.5)	<5.30	3.80 (bm)	4.20 (bm)	5.67 (d, J=8.0)	3.80 (bm)	4.20 (bm)
β-Isomaltose	5.18 (d, J=3.5)	5.50 (t, J=9.5)	4.10 (bm)	4.15 (m)	5.71 (d, J=8.0)	3.90 (bm)	3.70 (m)
β-Gentiobiose	4.54 (d, J=7.5)	<5.30	3.80 (bm)	4.20 (m)	5.71 (d, J=8.0)	3.80 (bm)	3.70 (bm)

a) All the data were obtained from 60-MHz spectra, measured in $CDCl_3$.
b) The range of the chemical shift of H-1, H-2, H-3, and H-4 was shown.

The applicability of the spectral pattern difference between anomers was then examined with some acetylated D-glucans (Fig. 3). The spectrum of amylose (α, 1→4) triacetate (Fig. 3d) showed the peaks at 5.30 ppm (bm) and 3.95 ppm (bm), corresponding to H-1 and H-3 protons and to H-4 and H-5 protons respectively. These peaks are in good agreement with those reported by Casu, *et al.*[12] measured at 100 MHz as well as with those observed on β-maltose octaacetate (Fig. 2f). In cellulose (β, 1→4) triacetate (Fig. 3c), H-3 and H-1 signals were up shifted (<5.2 ppm) while H-4 and H-5 protons appeared at 3.6 ppm (bm), which were consistent with the data reported by Gagnaire, *et al.*[11] The NMR spectra of amylopectin and dextrin acetates were essentialy similar to that of amylose triacetate while the spectrum of lichenin (β, 1→3 & 1→4) triacetate was analogous to cellulose triacetate, thus indicating the similarity

of their main glucosidic configurations respectively. The NMR spectra of these acetates are not given therefore in Fig. 3. The NMR spectrum of dextran (α, 1→6) acetate (Fig. 3b) showed a shoulder at 5.5 ppm (bm) corresponding to H-3 proton and a peak at 5.1 ppm (bm). Moreover, two peaks appeared at 4.2 and 3.7 ppm, which correspond to H-5 and H-6, 6′ protons respectively. These signals were consistent with β-isomaltose octaacetate. In the pustulan (β, 1→6) triacetate (Fig. 3a), a peak was observed at 5.1 ppm (bm), while no distinct peaks were noticed at 3.5—4.5 ppm. This may be due to the dispersion of the C-6 methylene proton signals in this area.

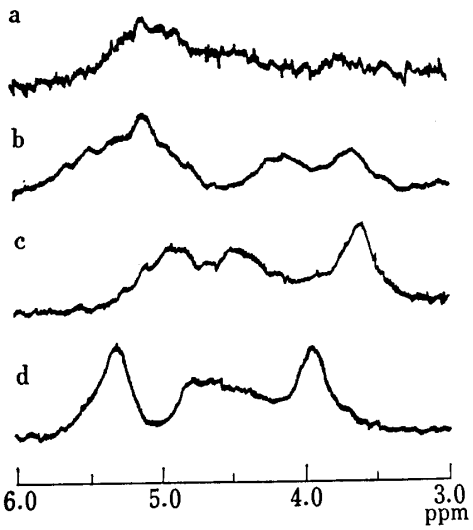

Fig. 3. 60-MHz NMR Spectra of Triacetates of D-Glucans

a: pustulan c: cellulose
b: dextran d: amylose

In D-glucan series, the proton signals were not well resolved and showed just broad peaks. Gagnaire, *et al.*[11] demonstrated the further resolution of the spectrum of cellulose triacetate by using a CAT (computer of averaged transients) method. The positions of the peaks, however, clearly enabled us to assign the configuration and position of the main D-glucosidic linkages, as far as 1→4 and 1→6 linked D-glucans are concerned.

A brief mention should be made here as to unusual low or high field shifts of acetoxy signals. In the acetylated D-glucopyranosylglucopyranoses the acetoxy signals appeared in the normal range (1.97—2.14 ppm), while the low field shift of acetoxy singal (2.20 ppm) was observed in amylose, amylopectin, and dextrin acetates. The low field shift of acetoxy signal (2.16 ppm) in amylose and α-cyclodextrin triacetates had been observed by Casu, *et al.*[1,2] which is typical of axially oriented acetoxy groups in monosaccharides.[2] We observed further that lichenin, dextran, and pustulan triacetates exhibited the high field signal at 1.60—1.75 ppm. These unusual phenomena could be interpreted by the non-bonded interactions between adjacent units which may restrict the conformation and hence affect the magnetic shielding of acetoxy groups in the macromolecules.

Experimental[14]

Materials——α,α-Trehalose, cellobiose, and amylose were purchased from Tokyo Kasei Co. Maltose, amylopectin, and dextrin were obtained from Wako Pure Chemical Co., Nagase Sangyo Co., and Difco Co., respectively. Dextran was kindly provided by Metio Sangyo Co. β-Kojibiose octaacetate (mp 117°), β-nigerose octaacetate (mp 150°), β-isomaltose octaacetate (mp 144°), and β-gentiobiose octaacetate (mp 194°) were generously supplied by Dr. Kazuo Matsuda of Tohoku University. Lichenin triacetate and pustulan triacetate were kindly offered by Dr. Yoshihiro Nishikawa of University of Tokyo.

***α,β*- and *β,β*-Trehalose Octaacetate**——The procedure of Helferich, *et al.*[16] was modified and column chromatography was employed to obtain α,β-trehalose octaacetate. Thus a mixture of tetra-O-acetyl-α-D-glucopyranosyl bromide (8 g), 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose[17] (7 g), mercuric cyanide (7 g),

14) Melting points were determined on a Kolfer block and are uncorrected. Thin-layer chromatography (TLC) was carried out on silica gel H (Merck) with multiple development. The spots were detected by ammonium metavanadate-sulfuric acid reagent.[15] Infrared (IR) spectra were determined in potassium bromide disks on Hitachi EPI-32 spectrophotometer.

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

16) B. Helferich and K. Weis, *Chem. Ber.*, **89**, 314 (1956).

17) P.Z. Allen, "Methods in Carbohydrate Chemistry," Vol. 1, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, p. 372.

and mercuric bromide (7 g) in dry benzene (100 ml) was stirred at room temperature for 2 days. The reaction mixture was filtered and then poured into water. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give a sirup, which was crystallized from ether. The crystals (1.1 g) were recrystallized from $CHCl_3$–ether–pet. ether to give 900 mg of β,β-trehalose octaacetate, mp 182—183°. The mother liquor was evaporated *in vacuo* to give a sirup (8 g), which was chromatographed on silica gel (Merck, 250 g) using $CHCl_3$–acetone (95:5) as solvent. α,β-Trehalose octaacetate was eluted in the fraction (400 ml) after washing the column with 1200 ml of the solvent. Evaporation of the solvent gave a sirup (2 g), which was crystallized from $CHCl_3$–ether–pet. ether to afford 1.0 g of α,β-trehalose octaacetate, mp 139—141°. Recrystallization from the same solvent yielded crystals melting at 143—144°. Examination by TLC with two step development using $CHCl_3$–acetone (9:1) as solvent showed that α,β-anomer moved faster than β,β-anomer.

Sophorose and Laminaribiose——Sophorose was prepared by the procedure of Coxon, *et al.*[18] Laminaribiose was prepared as described by Bächli, *et al.*,[19] while gentiobiose was formed as well due to removal of the protecting group during the reaction. They were separated by column chromatography on silica gel (Merck) using *n*-BuOH–AcOH–ether–H_2O (9:6:3:1) as solvent. Laminaribiose appeared after D-glucose, followed by gentiobiose.

β-Acetates of D-Glucopyranosylglucopyranoses——β-Sophorose octaacetate (mp 189—191°),[20] β-laminaribiose octaacetate (mp 162—163°),[19] β-maltose octaacetate (mp 163—164°),[20] β-cellobiose octaacetate (mp 198—200°),[21,22] and α,α-trehalose octaacetate (mp 80—82°/100—102°)[21,23] were prepared with acetic anhydride and sodium acetate as described in the literatures cited.

Acetates of D-Glucans——Amylose triacetate[24] and cellulose triacetate[25] were prepared with acetic anhydride and pyridine as reported previously. Amylopectin acetate, dextrin acetate, and dextran acetate were prepared by the same procedure as for amylose triacetate. The completion of acetylation was checked by IR spectra. The acetylation was repeated when the reaction was incomplete.

Acknowledgement The authors wish to express their gratitude to Dr. Kazuo Matsuda of Tohoku University for his kind gift of D-glucosylglucoses and to Dr. Yoshihiro Nishikawa of University of Tokyo for his generous offer of D-glucans. The authors' thanks are due to Prof. Shoji Takitani of Science University of Tokyo for providing them the 100-MHz spectra.

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