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TWO-DIMENSIONAL ^{1}H-N.M.R. STUDIES OF CELLO-OLIGOSACCHARIDES: THE UTILITY OF MULTIPLE-RELAY CHEMICAL-SHIFT-CORRELATED SPECTROSCOPY

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ABSTRACT

500-MHz, ^{1}H-n.m.r. spectra of cello-oligosaccharides were studied. The resonance assignments for cellotriose were made by combined use of multiple-relayed, coherence-transfer chemical-shift-correlated spectroscopy (multiple-RELAY-COSY). Spectra of a mixture of the α and β anomers of D-glucose were completely separated into the respective spectra by four-fold-RELAY-COSY. Resonance assignments for cellulose were made on the basis of the results for cello-oligosaccharides.

INTRODUCTION

^{13}C-Nuclear magnetic resonance (n.m.r.) studies of cello-oligosaccharides and their acetylated derivatives have been reported to some extent[1–3]. There have, however, been few studies of the ^{1}H-n.m.r. spectra of these compounds; this is because the ^{1}H-n.m.r. spectra of cello-oligosaccharides are too complex to be analyzed by such conventional methods as double-resonance experiments and comparison of the spectrum with those of related compounds. Two-dimensional (2D) n.m.r. spectroscopy has been developed in this decade, and has become a powerful technique for analyzing the structure of complex molecules. The ^{1}H-n.m.r. spectrum of cellobiose has been analyzed by using two-dimensional *J*-resolved and ^{13}C–^{1}H chemical-shift-correlated spectroscopies[4].

Relayed coherence-transfer chemical-shift-correlated spectroscopy (RELAY-COSY) was proposed as a useful method for analyzing spin networks in complex ^{1}H-n.m.r. spectra[5] and for identifying neighbouring nuclei in ^{13}C-n.m.r. spectra[6]. Double-RELAY-COSY (2-RELAY-COSY) was described by Eich[7], and the feasibility of its use was demonstrated by Bax and Drobny[8] for a cyclic peptide and an oligonucleotide.

We now show the usefulness of multiple-RELAY-COSY for analysis of the complex spin-networks of ^{1}H-n.m.r. spectra of cello-oligosaccharides. Detailed

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assignments of ^{1}H-n.m.r. resonances of cellotriose were achieved by this method. Vicinal and geminal ^{1}H–^{1}H coupling-constants for cellotriose were determined by two-dimensional, *J*-resolved spectroscopy. The ^{1}H-n.m.r. spectrum of cellulose was compared with those of cello-oligosaccharides.

EXPERIMENTAL

D-Glucose and cellobiose were obtained from Wako Pure Chemical Industries, Ltd. Cello-triose, -tetraose, -pentaose, and -hexaose, prepared by acid hydrolysis of cellulose[9], were presented by Dr. M. Yoneyama of Hokkaido University. Cellulose was a gift from Dr. K. Kamide of Asahi Chemical Ind. Co., Ltd.

N.m.r. measurements were performed on JEOL GX-500 and GX-400 spectrometers. All measurements were made by using 5 mm-diameter sample tubes. D-Glucose and cello-oligosaccharides (10–20 mg) were each dissolved in 0.4 mL of D_2O. Cellulose was dissolved, at a concentration of 5 wt%, in alkaline D_2O solution (NaOD, 10 wt%) at 4°. A residual, HDO peak was suppressed by the gated-decoupling method. Multiple-RELAY-COSY spectra were obtained by using pulse sequences based on the original idea of Eich *et al.*[5,7], and the phase-cycling method to suppress possible artifacts followed that reported by Bax and Drobny[8]. Phase-cyclings, 8-step, 16-step, 32-step, and 64-step, were used for 1-RELAY-COSY, 2-RELAY-COSY, 3-RELAY-COSY, and 4-RELAY-COSY, respectively. COSY[10,11] and multiple-RELAY-COSY spectra were measured with 256 × 512 data matrices. By zero filling in the F1 dimension, a 512 × 512 data matrix was obtained. *J*-Resolved[12,13] spectra were measured with 128 × 1024 data matrices. The mixing time of all multiple-RELAY-COSY experiments was 30 ms. The total measurement time for each multiple-RELAY-COSY was ~10 h. The contour display of spectra is presented in the absolute-value mode.

RESULTS AND DISCUSSION

Resonance assignments of cellotriose. — A normal, ^{1}H-n.m.r. spectrum of cellotriose in D_2O is shown in Fig. 1. Whereas a large number of resonances overlap in the high-field region of 3.4–4.0 p.p.m., four well-resolved, doublet resonances appear in a lower-field region. They are assignable to H-1 of the α anomer of ring A, H-1 of the β anomer of ring A, H-1 of ring B, and H-1 of ring C, in increasing order of the field from the left-hand end of the spectrum. These assignments are based on empirical rules of chemical shifts and ^{1}H–^{1}H coupling-constants for anomeric protons, and comparison of the spectrum with those of cellobiose, cellotetraose, cellopentaose, and cellohexaose.

The strategy for the resonance assignment of cellotriose was based on combined use of multiple-RELAY-COSY and the advantage of well-separated H-1

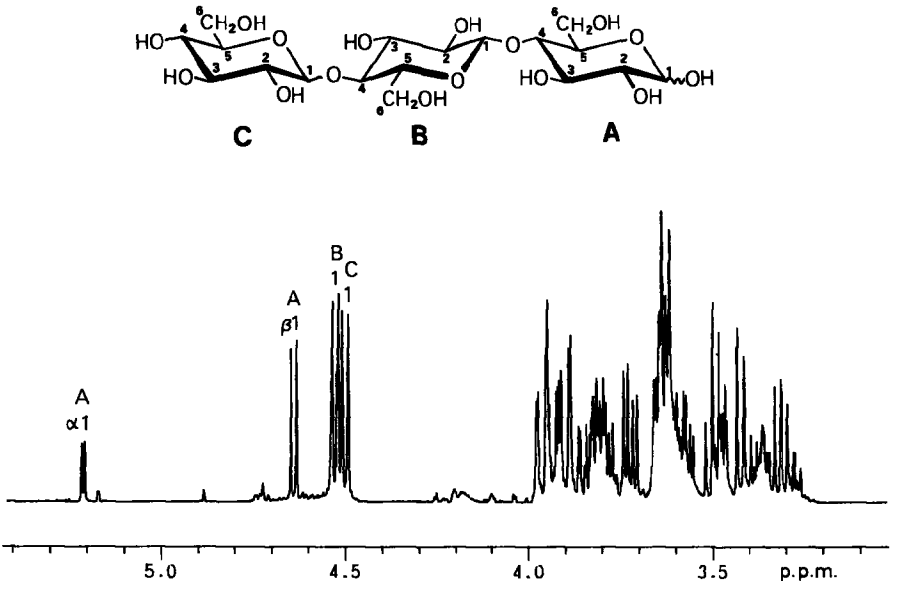

Fig. 1. 500-MHz, ^{1}H-n.m.r. spectrum of cellotriose in D_2O at 80°.

resonances. Fig. 2 shows a series of multiple-RELAY-COSY spectra of cellotriose: (a) is the normal COSY spectrum, and (b), (c), (d), and (e) are partial spectra of 1-, 2-, 3-, and 4-RELAY-COSY, including the auto peaks of H-1 (α anomer of ring A) and corresponding J-cross-correlation peaks. A cross peak of this H-1 in COSY (Fig. 2a) informs us of the chemical shift of H-2 of the same ring. The 1-RELAY-COSY spectrum (Fig. 2b) shows two cross-peaks; one is the same as in the COSY spectrum, and the other is due to relayed magnetization transfer from H-1 to H-3 *via* H-2. Thus, this new cross-peak indicates the chemical shift of H-3. The 2-RELAY-COSY spectrum (see Fig. 2c) gives rise to one more cross-peak, due to doubly relayed magnetization transfer from H-1 to H-4 *via* H-2 and H-3. This cross peak tells us the chemical shift of H-4. Similarly, chemical shifts of H-5 and H-6 can be determined by 3-RELAY-COSY (see Fig. 2d) and 4-RELAY-COSY (see Fig. 2e), respectively. Thus, resonances of the α anomer of ring A were assigned by such a combined use of multiple-RELAY-COSY experiments. Resonances of the β anomer of ring A, ring B, and ring C of cellotriose were analyzed in the same manner. The chemical-shift data for cellotriose at 25° are summarized in Table I.

Multiple-RELAY-COSY has two experimental limitations. The first is due to the decay of magnetization with the time constant T_2 during the mixing period, which makes higher-order multiple-RELAY-COSY less useful for large molecules. The second is due to the difficulty in optimization of mixing time for an unknown sample. In this experiment, we used 30 ms for all mixing times ($1/4\,J$ with $J \sim 8$ Hz),

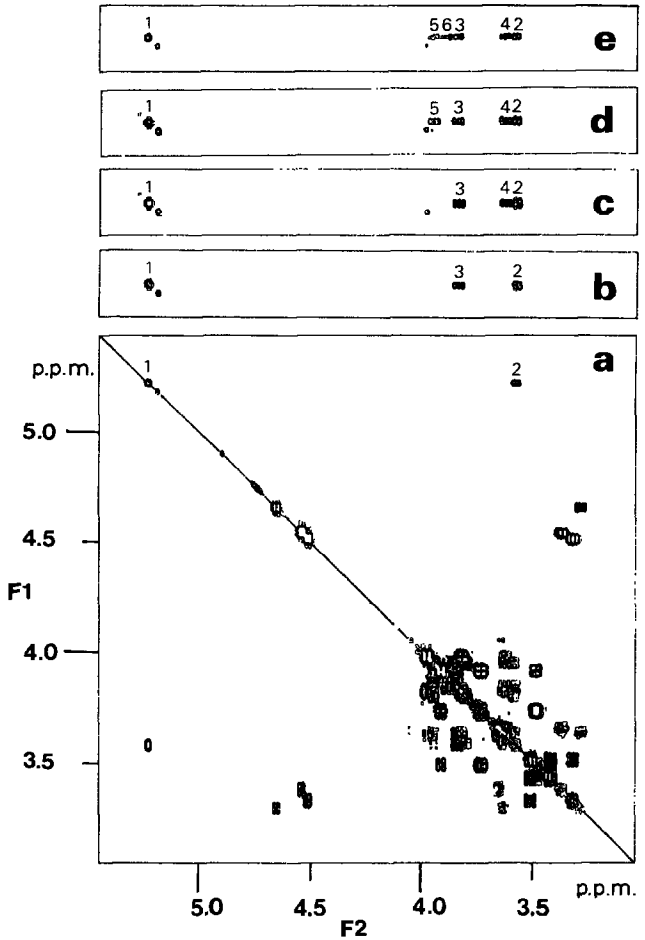

Fig. 2. 500-MHz, 2D ^{1}H-n.m.r. spectrum of cellotriose under identical conditions to those of Fig. 1. (a) COSY, (b) 1-RELAY-COSY, (c) 2-RELAY-COSY, (d) 3-RELAY-COSY, and (e) 4-RELAY-COSY.

TABLE I

CHEMICAL SHIFTS[a] OF ^{1}H-N.M.R. RESONANCES OF CELLOTRIOSE

Ring	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*
A,α	5.22[b]	3.57[b]	3.83[c]	3.63[c]	3.95[c]	3.87[c]	3.87[c]
A,β	4.66[b]	3.28[b]	3.65[c]	3.65[c]	3.62[c]	3.95[b]	3.81[b]
B	4.53[b]	3.36[b]	3.66[c]	3.66[c]	3.63[c]	3.98[b]	3.82[b]
C	4.51[b]	3.31[b]	3.51[b]	3.42[b]	3.49[c]	3.92[b]	3.73[b]

[a]Chemical shifts (p.p.m.) at 25°, relative to sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-2-silapentanoate (TSP). [b]The accuracy is within ±0.01 p.p.m. [c]The accuracy is within ±0.03 p.p.m.

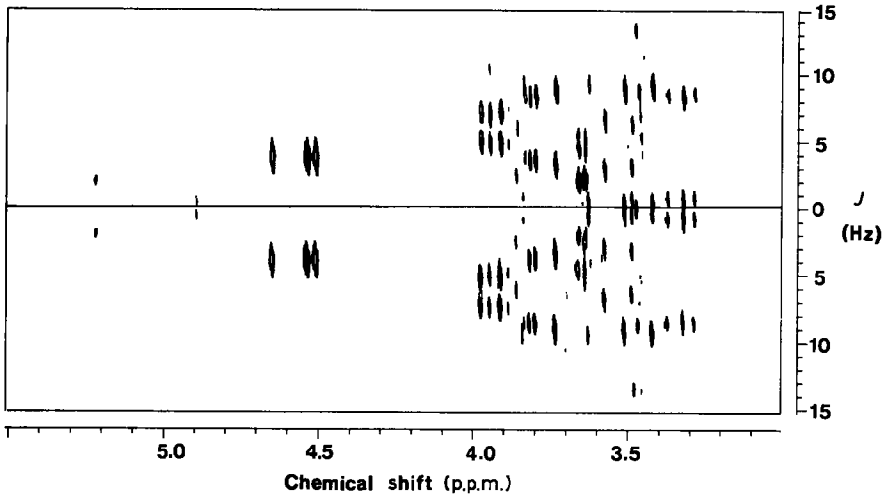

Fig. 3. 400-MHz, 2D *J*-resolved spectrum of cellotriose under identical conditions to those of Fig. 1.

and we obtained satisfactory results for cellotriose. Despite these limitations, multiple-RELAY-COSY could become one of the most useful techniques, especially for the structural analysis of oligosaccharides.

^1H–^1H Coupling constants of cellotriose. — Coupling constants of assigned protons of cellotriose were determined by two-dimensional, *J*-resolved spectroscopy (see Fig. 3). The projection onto the F2 axis along the F1 axis shows a so-called "broad-band decoupled spectrum", and the F1 cross-section displays a multiplet spectrum of a given proton (data not shown). *J* values were obtained from the F_1 cross-section, and are summarized in Table II. Some could not be determined due to the strongly coupled multiplet structure and severe overlapping in the range of 3.55–3.65 p.p.m. The values remained unchanged in a temperature range of 25–80°. These coupling constants of cellotriose are in agreement with those of cellobiose[4].

TABLE II

^1H–^1H COUPLING CONSTANTS[a] OF CELLOTRIOSE

Ring	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
A,α	4.0	9.8	n.d.[b]	n.d.	m[c]	m	m
A,β	7.8	9.4	m	m	2.4	5.0	12.2
B	7.8	9.4	m	m	2.0	4.8	12.2
C	7.8	9.2	9.0	9.4	2.4	5.6	12.2

[a]Coupling constants (Hz, sign not determined) in absolute value at 25°. The accuracy is within ±0.4 Hz. [b]Not determined. [c]Strongly coupled multiplet.

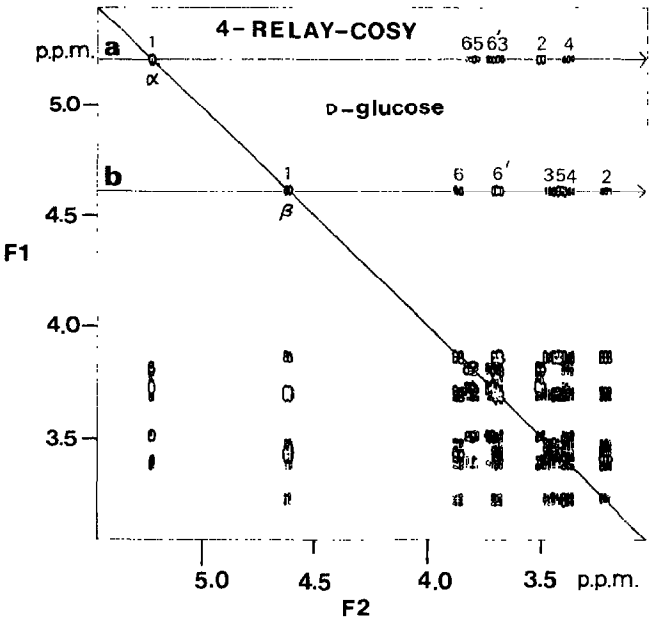

Fig. 4. 4-RELAY-COSY spectrum of D-glucose in D_2O at 80°. The resonance assignments were made by the procedure shown in Fig. 2, and are indicated in this Figure.

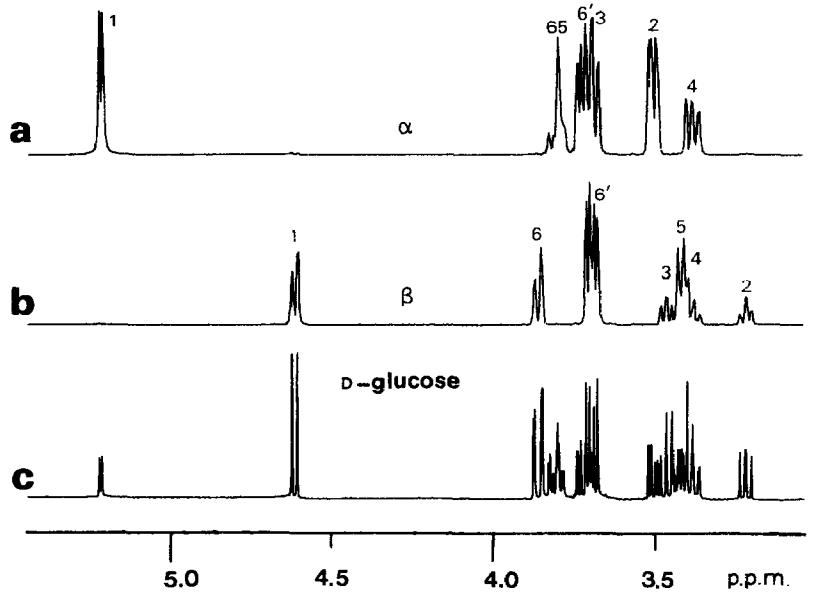

Fig. 5. Cross sections parallel to the F1 axis of 4-RELAY-COSY of Fig. 4, taken at the F2 frequencies of α-H-1 (a) and β-H-1 (b). The normal 1D spectrum of D-glucose is shown in (c)

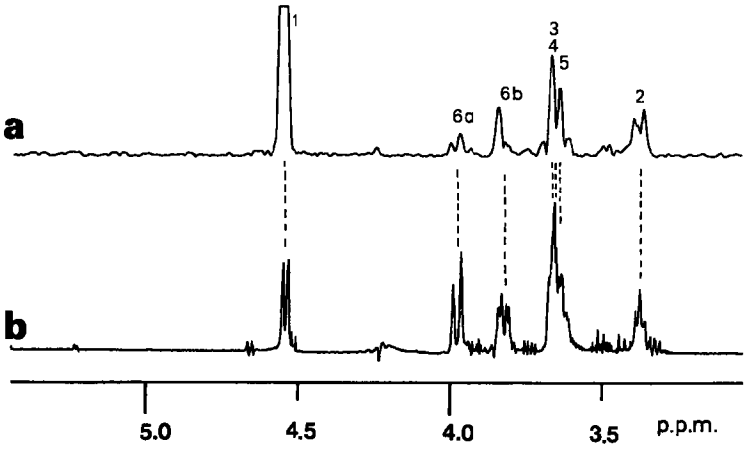

Fig. 6. (a) A cross-section parallel to the F1 axis of 4-RELAY-COSY of cellotriose, taken at the F2 frequency of H-1 of ring B. The resonance assignments are indicated. (b) A difference spectrum obtained by subtracting the normal spectrum of cellobiose from that of cellohexaose.

Spin chromatography of D-*glucose.* — The 4-RELAY-COSY spectrum of D-glucose is shown in Fig. 4, which demonstrates one of the features of multiple-RELAY-COSY. 4-RELAY-COSY of the spin system of the ring of D-glucopyranose provides all possible cross correlations among H-1, H-2, H-3, H-4, H-5, H-6a, and H-6b. This is comparable with the TOCSY experiment reported by Braunschweiler and Ernst[14], and the HOHAHA experiment reported by Davis and Bax[15]. The cross-section containing the auto peak of α-H-1 and the cross-section containing auto peaks of β-H-1 are shown in Fig. 5a and b, respectively. These cross sections pick up resonances of the α and the β anomer from a mixture of the two (see Fig. 4c). This method, so-called[16] "spin-chromatography", can be applied to the separation of spectra, not only of a mixture of anomers but also of different residues of oligosaccharides.

Resonance assignments of cellulose. — Fig. 6a shows a cross section of 4-RELAY-COSY containing the auto peak of H-1 of ring B of cellotriose, and Fig. 6b is a difference spectrum obtained by subtracting the normal spectrum of cellobiose from that of cellohexaose. Spectra (a) and (b) are very similar to each other, indicating that all internal pyranosyl residues of cello-oligosaccharides show identical spectra. This was confirmed by a comparison of the normal spectra of cellotetraose, cellopentaose, and cellohexaose, and also by multiple-RELAY-COSY spectra of these oligosaccharides. Thus, chemical shifts of internal residues of these cello-oligosaccharides can refer to those of ring B of cellotriose (see Table I). Furthermore, chemical shifts of all protons of reducing and nonreducing end-groups of cellotetraose, cellopentaose, and cellohexaose are also similar to those of ring A and ring C, respectively, of cellotriose.

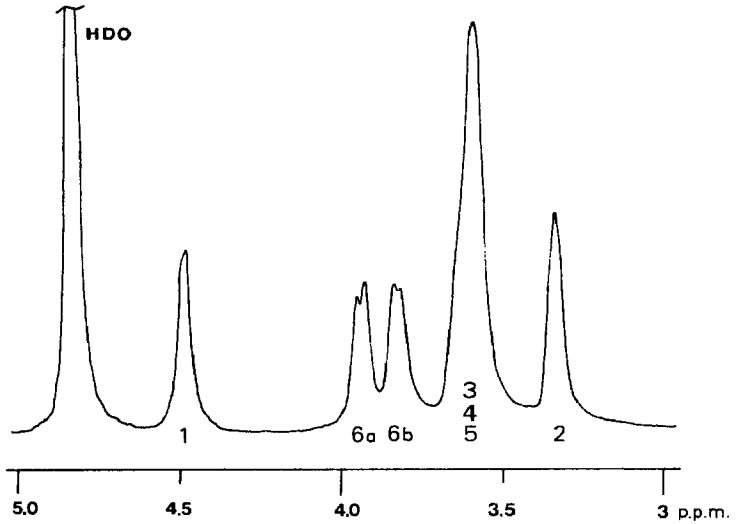

Fig. 7. 400-MHz, ^{1}H-n.m.r. spectrum of 5 wt% cellulose in D_2O containing 10 wt% of NaOD, at 25°.

Fig. 7 shows the spectrum of cellulose in 10 wt% NaOD solution. It should be noted that this spectrum markedly resembles those shown in Fig. 6. Thus, the spectrum of cellulose can be assigned by a comparison with the results of ring B in cellotriose. The assignment of each resonance line of cellulose is also shown in Fig. 7.

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