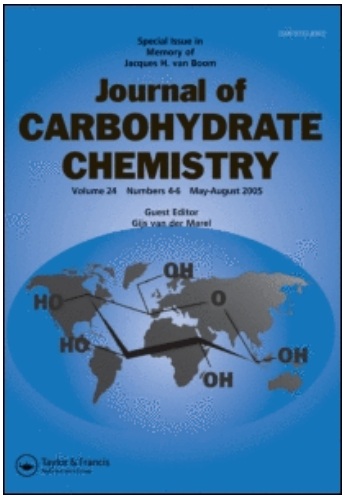

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^{1}H NMR SPECTRA OF OLIGOSACCHARIDE SUBSTITUTED CYCLODEXTRINS[1]

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ABSTRACT

^{1}H NMR spectra of some oligosaccharide substituted cyclodextrins composed of only α-D-glucose units are analysed. Chemical shifts of protons of each glucosyl group of the chain were determined by experiments with the HOHAHA pulse technique. In spite of the similar kinds of protons, dispersion of chemical shifts is observed. The most dispersed proton is the anomeric proton, and the largest change in the chemical shifts is 0.5 ppm.

INTRODUCTION

To obtain information about molecular recognition phenomena of oligosaccharide chains, ^{1}H and ^{13}C NMR spectroscopy provides valuable methods. From these methods, in principle, we can obtain the chemical shifts of all H and C atoms of interacting molecules in the molecular recognition reactions. To date, many inclusion phenomena of cyclodextrins have been studied with NMR methods.[2-8] ^{1}H NMR signals of

oligosaccharide chain protons are concentrated in the range of 3-6 ppm, and sometimes overlap. Consequently, it is very difficult to assign the chemical shifts of all H and C signals of oligosaccharide chains. In this report, we present the results of the determination of the chemical shifts of protons in cyclodextrins substituted with oligosaccharide chains composed of only glucosyl groups by using the NMR HOHAHA(Homonuclear Hartmann-Hahn cross polarization) pulse technique.[9,10]

EXPERIMENTAL

CHEMICALS Oligosaccharide substituted cyclodextrins (CD) are 6-O-α-gluco-pyranosyl-α-CD(1), 6-O-α-maltopyranosyl-α-CD(2), 6-O-α-maltotriosyl-α-CD(3), 6-O-α-maltotetraosyl-α-CD(4), 6-O-α-maltopentaosyl-α-CD(5), 6-O-α-maltohexaosyl-α-CD(6), 6-O-α-glucopyranosyl-β-CD(7), and 6-O-α-maltopyranosyl-β-CD(8). These substituted CDs were synthesized by a fermentative method with pullulanase and glucoamylase, and were purified by gel filtration chromatography.[11,12] Amylose (Hayashibara Biochem. Lab. Inc., M.W. Approx. 16000) was used without any purification for chemical shift comparison with the oligosaccharide substituted cyclodextrins.

NMR MEASUREMENTS ^{1}H and ^{13}C NMR spectra were measured with a JEOL GSX400 (399.65 MHz for ^{1}H and 100.40 MHz for ^{13}C) NMR spectrometer at 30 °C in the deuterium oxide solution. External sodium-2,2-dimethyl-2-silapentonate-d_4 (DMSP) was used as a reference. A JEOL FX200 (50.10 MHz for ^{13}C) NMR spectrometer was used for the measurements of relaxation times of ^{13}C signals under similar conditions to those described above.

RESULTS AND DISCUSSION

ASSIGNMENTS OF ANOMERIC PROTONS Anomeric protons of the core cyclodextrins in the oligosaccharide substituted cyclodextrins were observed at almost the same chemical shifts as α-CD(5.060 ppm) and β-CD(5.084 ppm) as an average, respectively. The effects of the addition

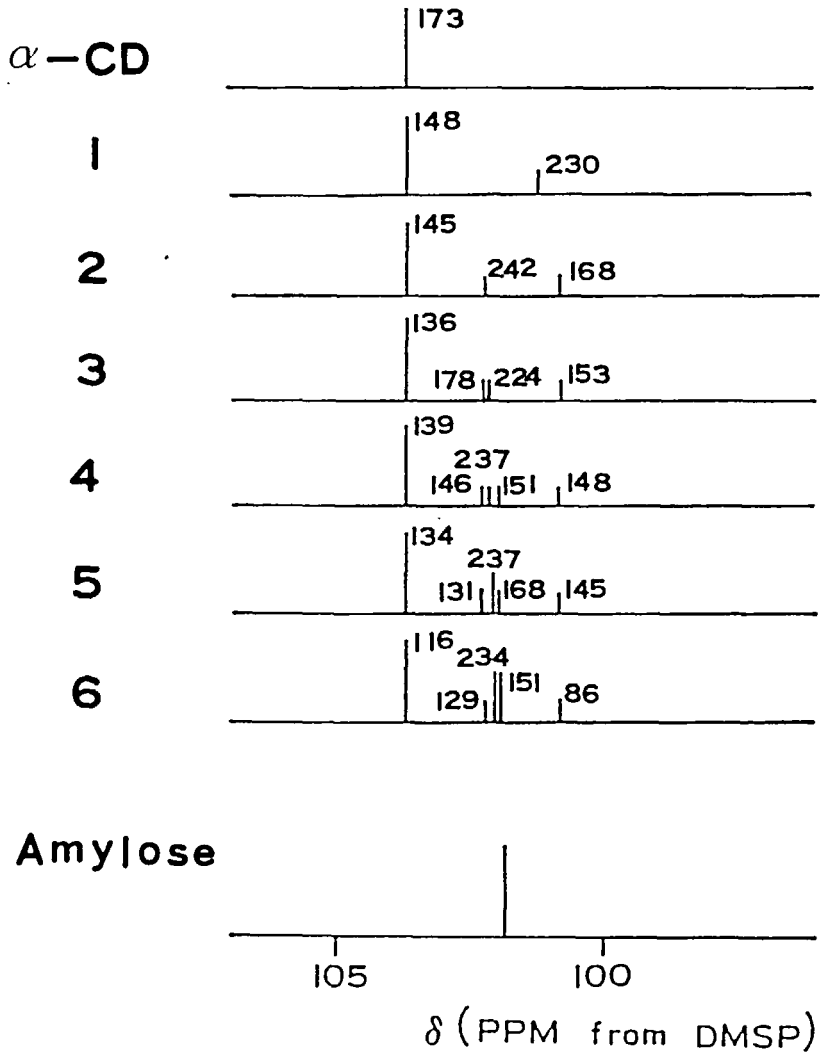

Fig. 1. ^{13}C Chemical shifts and relaxation time of C-1 signals. Tall bows show C-1 signals of core cyclodextrins and low bows show C-1 signals of branched glucosyl groups.

of the oligosaccharide chains were not notable. The signals of the anomeric protons of the core cyclodextrins were observed as a slightly broadened doublet or asymmetric triplet. On the other hand, anomeric protons of the chain part had different chemical shifts from those of

Table 1. Chemical Shifts (ppm) and Relaxation Time (ms) of C-1

	Assignment		α-CD		1		2		3		4		5		6	
			δ	T_1	δ	T_1	δ	T_1	δ	T_1	δ	T_1	δ	T_1	δ	T_1
α-CD	core	B,C	103.62	173	103.70	148	103.62	145	103.62	136	103.60	139	103.62	134	103.65	116
	branch	1A			101.22	230	100.82	168	100.82	153	100.82	148	100.84	145	100.80	86
		2A					102.21	242	102.24	178	102.25	131	102.24	131	102.19	129
		3A							102.12	224	101.98	151	101.92	168	101.90	151
		4A									102.20	237	102.04	237	101.90	151
		5A											102.09	237	102.01	234
		6A													102.01	234

	Assignment		β-CD		7		8	
			δ	T_1	δ	T_1	δ	T_1
β-CD	core	B,C	104.11	169	104.65	129	104.61	127
	branch	1A			101.87	192	104.61	120
		2A					102.79	232

Amylose	δ	T_1
	101.80	-

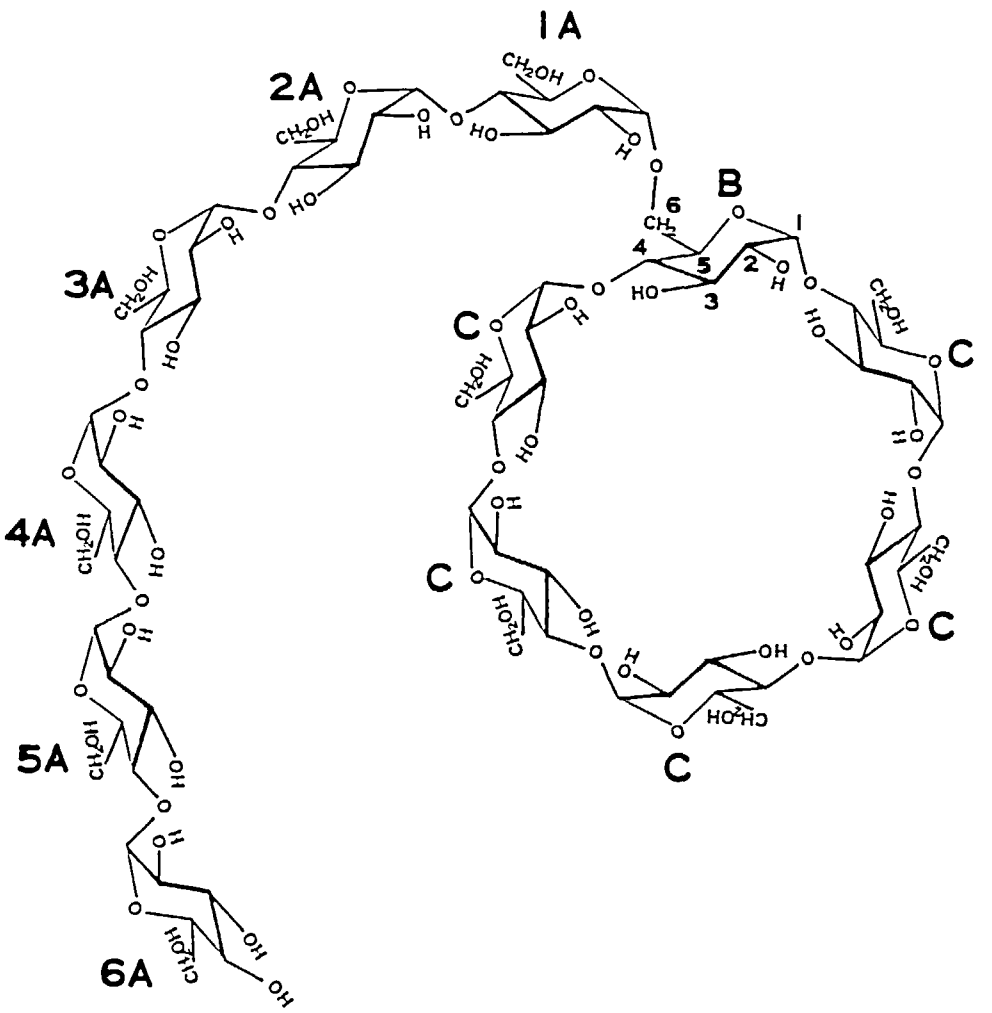

Fig. 2. 6-O-α-D-Maltohexaosyl-α-CD(6)
labelled according to Yamamoto et al.[14]

the core, and separated signals were observed. These chain signals were found at a lower field than core anomeric protons except for the glucosyl residue combined directly to the core cyclodextrin. These anomeric protons were assigned with the aid of C-H COSY spectra. Here, the carbon signals were reasonably assigned from the relaxation times of the signals.[13] Fig. 1 shows the T_1 and the chemical shifts of the C-1

carbons of substituted CDs. The relaxation times of the core C-1 carbons were shortened as the chain became longer. These data were reasonably understood, since the relaxation times were generally expected to be short when the molecular weight increased.[13] Furthermore, as shown in the case of maltohexaosyl-α-CD(6), the relaxation times of C-1 signals of branch glucosyl group were not the same, and changed with the relative position from the core cyclodextrin. The relaxation times of the C-1 signals of branched glucosyl groups near the core cyclodextrin were shorter than those of the glucosyl groups distant from the core. These changes in the relaxation times were interpreted as originating from the large freedom of mobility of the glucosyl group in the branch part, thus effecting the long relaxation time. These effects were also observed with other carbon signals in addition to those of C-1 atoms. Table 1 shows the chemical shifts, relaxation times and assignments of C-1. Here, we used the notations shown in Fig. 2, following ones described by Yamamoto et al.[14]

Fig. 3 shows the C-H COSY spectrum of maltopentaosyl-α-CD(5), magnifying the anomeric proton region. These determined anomeric proton chemical shifts were not in the order of their distances from the core cyclodextrin. However, the chemical shifts of anomeric protons seemed to approach the one of amylose, after the reciprocating change. The determined anomeric proton chemical shifts are listed in the table 2.

CHEMICAL SHIFTS FROM HOHAHA EXPERIMENTS The 1D HOHAHA experimental mode edited by JEOL was used for these experiments. The mixing time of this experimental mode corresponded to the spin lock time and determined the network of the ^{3}J connectivities. In a 1D HOHAHA spectrum, by changing the mixing time, the signals of one glucosyl group of which anomeric proton was irradiated were successively obtained. Table 2 shows the determined ^{1}H chemical shifts of each glucosyl group of the branch oligosaccharide chains. In this table, are included results in which H-4 of the terminal glucosyl group was irradiated instead of the anomeric proton. H-4 of the terminal glucosyl group was characteristically isolated in the higher field region. In Table 2, spin coupling constants and chemical shifts of core protons were

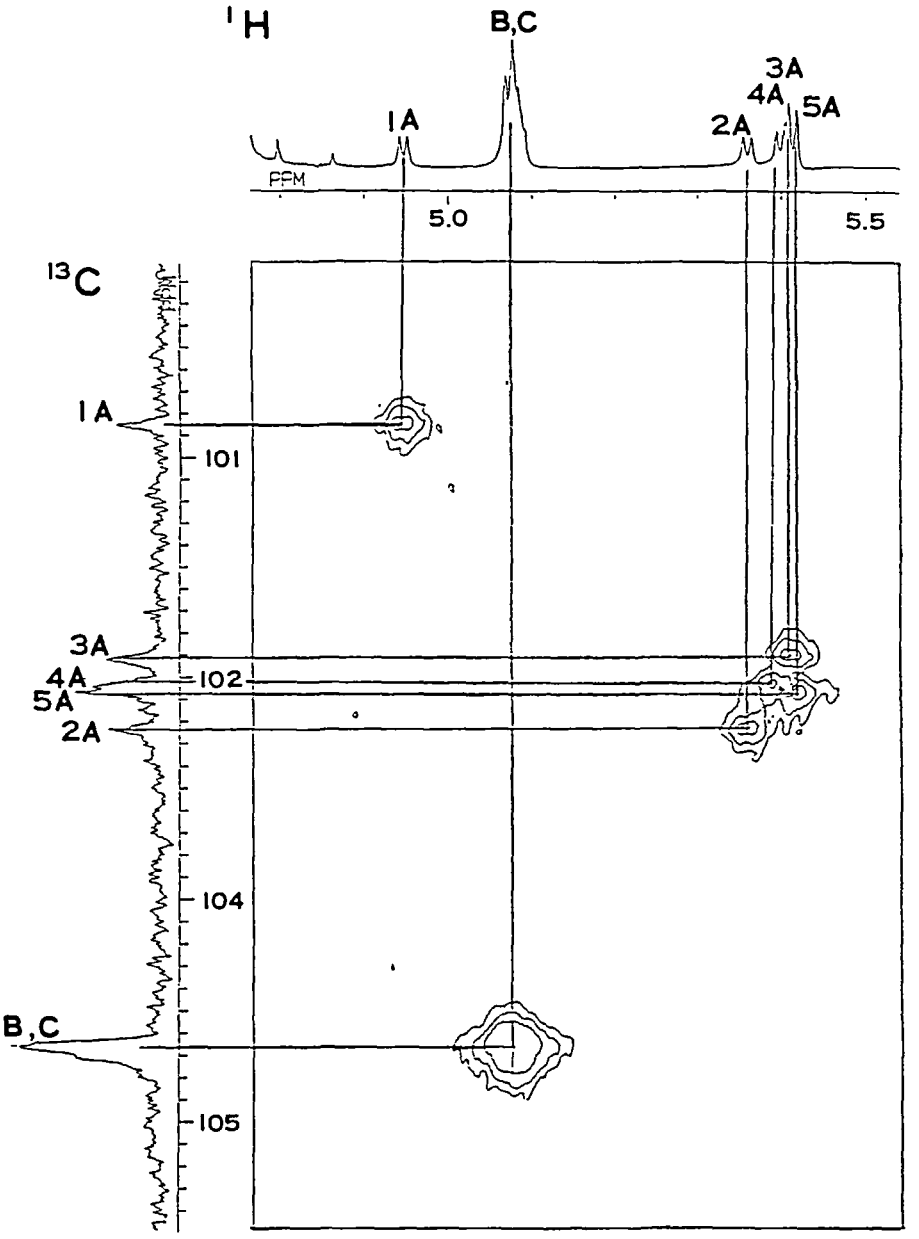

Fig.3. C-H cosy spectrum of 5.

Table 2. ^{1}H Chemical Shifts of Branched Glucosyl Residues

	Gluco-pyranosyl residue	Chemical shifts (ppm from DMSP)						
		H-1	H-2	H-3	H-4	H-5	H-6	
1	1A	4.947	3.550	3.751	3.443	3.798	3.854	3.785
2	1A	4.935	3.588	4.005	3.651	3.839	3.873	-
	2A	5.365	3.583	3.687	3.423	3.747	3.853	3.797
3	1A	4.904	3.558	3.976	3.618	3.809	-	-
	2A	5.305	3.596	3.924	3.623	3.824	3.810	-
	3A	5.375	3.577	3.687	3.409	3.711	3.850	3.757
4	1A	4.929	3.585	4.002	3.649	3.853	-	-
	2A	5.348	3.623	3.957	3.656	3.836	-	-
	3A	5.384	3.624	3.948	-	-	-	-
	4A	5.382	3.587	3.686	3.417	3.727	3.856	3.761
5	1A	4.947	3.602	4.019	3.667	3.927	-	-
	2A	5.359	3.641	3.976	3.679	3.804	-	-
	3A	5.413	3.645	3.969	3.751	3.857	-	-
	4A	5.390	-	-	-	-	-	-
	5A	5.418	3.603	3.708	3.437	3.748	3.873	3.772
6	1A	4.940	3.595	4.011	3.658	3.865	3.884	3.827
	2A	5.354	3.635	3.963	3.660	3.853	3.851	-
	3A	5.364	3.633	3.970	3.674	3.858	4.020	3.860
	4A	5.402	3.593	3.970	3.427	3.851	3.752	-
	5A	-	-	-	-	-	-	-
	6A	5.399	3.598	3.701	3.429	3.753	3.865	3.817
7	1A	4.955	3.556	3.752	3.440	3.764	3.858	3.824
8	1A	4.958	3.609	4.023	3.657	3.887	-	-
	2A	5.371	3.598	3.702	3.439	3.764	3.868	-
Amylose		5.426	3.640	3.976	3.679	3.87 - 3.85		

neglected, because these exact values were not obtained except in a few cases. The results obtained with glucosyl-α-CD(1) showed good agreement with the previous results of DQF-COSY experiments.[14] As shown in Table 2, the chemical shifts of the glucosyl group in the chain in the cases of substituted β-CD (7 and 8) were similar to the ones of corresponding substituted α-CD. Thus, similar changes in the chemical shifts might be probably observed in the β-CD series with longer chains. Again from Table 2, the most dispersed proton among the oligosaccharide substituted cyclodextrins was the anomeric proton, the largest change in the chemical shift was 0.5 ppm. The most unchanged proton of the oligosaccharide chain was H-2, change in the chemical shifts being 0.09 ppm, at the most. Reversions in the chemical shifts were observed between H-2 and H-4, and between H-3 and H-5. Proton signals of the glucosyl residue directly attached to the core cyclodextrin and those of the end glucosyl residue of the oligosaccharide chain had characteristic chemical shifts. Proton signals of the middle glucosyl residue showed similar chemical shifts to the corresponding ones of amylose.

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