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^{13}C NMR Study on Diastereomeric Interactions between Cellulose Tris(4-methylbenzoate) and 1-Phenylethanol Enantiomers

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ABSTRACT: For investigating the mechanism of chiral recognition by cellulose derivatives, ^{13}C NMR spectra of 1-phenylethanol were measured in $CDCl_3$ in the presence or absence of cellulose tris(4-methylbenzoate) (**1**). (*R*)- and (*S*)-enantiomers of 1-phenylethanol showed different chemical shifts in the presence of **1**. The degree of chemical shift difference was large at aromatic ipso carbon (1'-C) and methine carbon (1-C) with a hydroxyl group. Spin-lattice relaxation times (T_1s) were also measured to compare mobilities of the enantiomers. In the presence of **1**, T_1 for each carbon of the (*R*)-enantiomer was shorter than that for corresponding carbon of the (*S*)-enantiomer. The presence of **1** restricts the mobility of the (*R*)-enantiomer more than that of the (*S*)-enantiomer. T_1 difference between (*R*)- and (*S*)-enantiomers was large at 1-C. The results indicate that 1-phenylethanol is chirally recognized by **1** at the point of 1-C carbon.

KEY WORDS Diastereomeric Interaction / ^{13}C NMR / Relaxation Time / Cellulose Tris(4-methylbenzoate) / 1-Phenylethanol / HPLC /

Cellulose derivatives are known as chiral column-packing materials of high performance liquid chromatography (HPLC) for optical resolution.[1] For the purpose of developing new chiral separation materials, we have been studying how cellulose derivatives recognize and separate enantiomers.

NMR is thought to be one of the most useful techniques to observe intermolecular interactions. As to NMR studies of intermolecular diastereomeric interactions between polymeric compounds and relatively small molecules, molecular recognitions by DNA[2] and cyclodextrin[3] have been reported. However, there are few NMR studies on the interactions between polymeric HPLC stationary phases and chiral compounds.[4]

In this work, we investigated intermolecular interactions between cellulose tris(4-methylbenzoate) (**1**), one of the excellent column-packing materials, and enantiomers of 1-phenylethanol (**2**), those are optically resolved by HPLC with the column packed with **1**.

EXPERIMENTAL

Materials

Polymer **1** was the product of Daicel Chemical Industries, Ltd. The degree of esterification was 2.8 and weight average molecular weight was 2×10^5, as determined by GPC in chloroform with polystyrene standard. Enantiomers [(*R*)-**2**,(*S*)-**2**] and racemate [(*R*,*S*)-**2**] of **2** were obtained from Wako Pure Chem. Ind., Ltd. The reagents were used without further purification.

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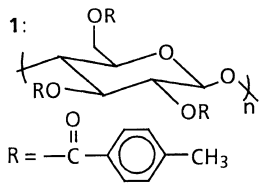

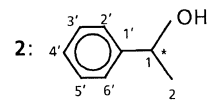

NMR

^{13}C NMR experiments were performed at a certain controlled temperature by a JEOL JNM-A500 spectrometer operating at 125.65 MHz. A 5 mm NMR sample tube was used. Tetramethylsilane (TMS) was used as the internal standard for chemical shift.

Proton-decoupled ^{13}C NMR spectra were recorded under the following conditions: spectral width, 33898.31 Hz; acquisition time, 0.9667 s; pulse delay, 1.0333 s; pulse flip-angle, 45°; scans, 200—1000 times. Spectra were collected with 32768 (32K) points to give a digital resolution of 1.03 Hz. ^{13}C NMR spectra with high digital resolution were recorded under the following conditions: spectral width, 16129.03 Hz; acquisition time, 4.0632 s; pulse delay, 0.0667 s; scans, 128 times. The spectra were obtained with 131072 (128 K) points to give a digital resolution of 0.12 Hz.

The inversion recovery method with complete proton-decoupling[5] was used to determine the T_1s. T_1s of large ($T_1 > 20$ s), middle (20 s $> T_1 >$ 8 s), and small (8 s $> T_1 >$ 3 s) values were measured separately. The following conditions were employed for T_1 measurements: spectral width, 33898.31 Hz; acquisition time, 0.4833 s; and the spectra were acquired with 16384 points to give a digital resolution of 1.03 Hz. Pulse delay of 150 s and number of scans of 32 for the large T_1 of 1′-C in **2** in the absence of **1**, 100 s and 32 times for the middle T_1s of 1′-C in the presence of **1**, and 1-C, 2′,6′-C, 3′,5′-C in the absence of **1**, and 40 s and 32 times for the small T_1s of other carbons.

NMR Samples

Mixtures of **1** and **2** were dissolved in $CDCl_3$ and the solutions were placed in NMR sample tubes. As for sole **2**, T_1s were obtained with and without degassing by several freeze-thaw cycles. Since the obtained T_1s of **2** were almost the same in both cases, T_1s for other samples were measured without degassing.

RESULTS AND DISCUSSION

^{13}C NMR Spectra

Important interactions expected in the chromatographic separation with cellulose benzoate derivatives would be hydrogen bonding with ester group and hydrophobic or π–π interaction with aromatic moiety.[1] Among many chiral compounds resolved by chiral HPLC using **1** as a stationary phase, compound **2** was chosen for our studies, because it has a simple structure of hydroxyl group and aromatic ring.

The ^{13}C NMR spectrum of a mixture of (*R*,*S*)-**2** and **1** was measured in $CDCl_3$ at 35°C. Some signals were observed separately for the (*R*)- and (*S*)-enantiomers. To facilitate signal assignments for the enantiomers, the molar ratio of (*R*)-**2** and (*S*)-**2** was set as 2 : 1. Total (*R*)-**2** and (*S*)-**2** against one glucopyranose residue in **1** was set as 3 : 1, as it is most likely that one molecule of the **2** enantiomer interacts with one 4-methylbenzoyl group, that is, three molecules of **2** interact with one glucopyranose residue of **1**. The NMR spectra measured are shown in Figure 1. For all carbon types, each enantiomer showed different signals except 2′,6′-C. The (*R*)-**2** : (*S*)-**2** ratio is 2 : 1, and thus the stronger signals in each pair of the signals in Figure 1 were assigned to (*R*)-**2**. These assignments were also confirmed by separate measurements of (*R*)-**2** with **1** and (*S*)-**2** with **1**. Table I shows the chemical shifts of (*R*)-**2** and (*S*)-**2** in the presence or absence of **1**.

Chemical shift differences between the corresponding carbons of (*R*)-**2** and (*S*)-**2** were

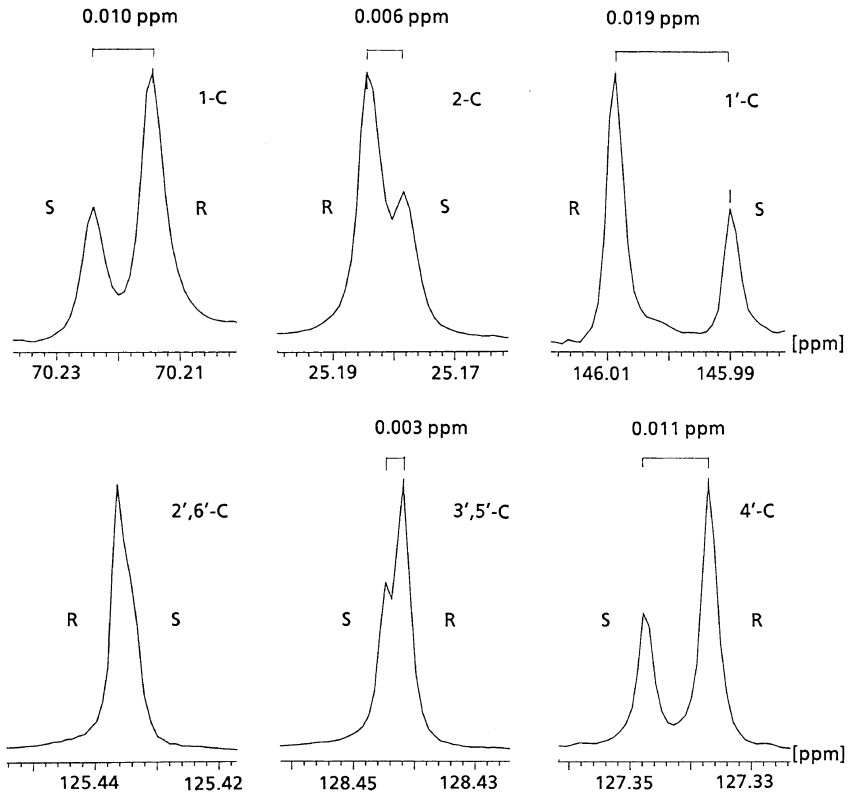

Figure 1. ^{13}C NMR spectra of **2** with high digital resolution (0.12 Hz) in the mixture of (*R*)-**2**:(*S*)-**2**:**1** = 2:1:1 in $CDCl_3$ at 35°C.

obtained by subtracting the (*S*)-**2** values from the (*R*)-**2** ones ($\Delta\delta$), shown in Figure 1 and in the right-most column in Table I. The chemical shift difference is thought to be caused from different interactions of each enantiomer with **1**. All peaks of (*R*)- and (*S*)-**2** showed downfield shifts from (*R*,*S*)-**2** upon the addition of **1** (the range of downfield shift was 0.02 to 0.12 ppm). Thus, it might be expected that the stronger the interaction between **1** and **2**, the larger the downfield shift. However, the peak order of (*R*)-**2** and (*S*)-**2** is not the same for all carbons (Figure 1); the 1-, 3′-, 4′-, and 5′-C signals of (*R*)-**2** showed smaller downfield shifts than those of (*S*)-**2**, while the 2- and 1′-C signals of (*R*)-**2** showed larger downfield shifts than those of (*S*)-**2**. The 2′,6′-C signals were not completely separated, but the 2′,6′-C signal of

Table I. ^{13}C NMR Chemical shifts (ppm) of **2** in the presence or absence of **1** in $CDCl_3$ at 35°C

Carbon	Sample			$\Delta\delta$[c]
	(*R*,*S*)-**2**[a]	(*R*)-**2**[b]	(*S*)-**2**[b]	
1-C	70.11	70.214	70.224	−0.010
2-C	25.06	25.184	25.178	+0.006
1′-C	145.92	146.009	145.990	+0.019
2′,6′-C	125.42	125.436	125.436	0[d]
3′,5′-C	128.36	128.441	128.444	−0.003
4′-C	127.26	127.337	127.348	−0.011

[a] Chemical shifts measured in the absence of **1**.
[b] Chemical shifts measured in a mixture sample with molar ratio of (*R*)-**2**:(*S*)-**2**:**1** = 2:1:1 (49 mg:23 mg:102 mg) in $CDCl_3$ (1 g).
[c] Chemical shift differences between enantiomers in the mixture of (*R*)-**2**:(*S*)-**2**:**1** = 2:1:1, δ[(*R*)-**2**]−δ[(*S*)-**2**].
[d] 2′,6′-C signal of (*S*)-**2** was observed in upper field than (*R*)-**2**'s as a shoulder.

(*S*)-**2** was observed upper in the field than (*R*)-**2**'s as a shoulder. Chemical shift differences between the enantiomers are in the order of 0–0.019 ppm. In a situation of one order smaller than those induced by the addition of **1**, the peak order would not be useful for the elucidation of the extent of interaction.

The ^{1}H NMR spectrum was also meausred for the same sample, but no chemical shift difference was observed between the enantiomers. Chemical shift of ^{13}C is greatly affected by paramagnetic shielding factor σ_P which arises from asymmetry of the *p* orbital of ^{13}C. The effects of σ_P are negligible in the case of ^{1}H chemical shift. The spectroscopic differentiation of (*R*)-**2** and (*S*)-**2** in the presence of **1** by ^{13}C NMR may be due to diastereomeric interaction between **1** and **2** that perturbs the electronic state of the *p* orbital, causing change of σ_P.[6]

^{13}C Spin-Lattice Relaxation Time (T_1) of 1-Phenylethanol

When a small molecule interacts with a large molecule, *i.e.*, a polymer, the mobility of the small molecule should be restricted as long as the interaction is strong enough and the mobility of the polymer is much lower than that of the small molecule. The ^{13}C spin-lattice relaxation time (T_1), which is sensitive to molecular motion, is thus a good measure of the interaction.[7] T_1s of (*R*)- or (*S*)-**2** were determined for mixtures of **1** and (*R*)- or (*S*)-**2** with a molar ratio of 1 : 1 or with a molar ratio of 1 : 0.5, and for sole **2** under the same conditions. T_1s are given in Table II. All carbons of (*R*)-**2** showed shorter T_1s than those of (*S*)-**2** in both molar ratios (1 : 1 and 1 : 0.5). T_1 decreases with restricted mobility within an extreme narrowing condition ($\omega_0\tau_c \ll 1$), and we can assume that such a small molecule as **2** is in the condition at room temperature.[7] Hence, it is clear that (*R*)-**2** is more restricted in mobility than (*S*)-**2** in the presence of **1**. T_1s of **1** measured under the same conditions showed minor change of values, and thus it was not informative for sites of interactions.

Table II. Spin-lattice relaxation times (T_1s/s)[a] of **2** in $CDCl_3$ at 35°C in the presence or the absence of **1**

Carbon	(*R*,*S*)-**2**[b]	1 : (*R*)-**2**[c] = 1 : 0.5	1 : (*S*)-**2**[c] = 1 : 0.5	1 : (*R*)-**2**[d] = 1 : 1	1 : (*S*)-**2**[d] = 1 : 1
1-C	12.^{3}	7.0^0	8.0^0	7.1^2	7.9^5
2-C	4.2^5	3.0^5	3.2^6	3.1^9	3.3^0
1′-C	30.^{3}	11.^{9}	13.^{5}	11.^{1}	12.^{9}
2′,6′-C	9.0^3	4.5^7	5.1^1	4.8^8	5.1^6
3′,5′-C	8.7^2	4.4^7	4.9^4	4.7^1	4.9^7
4′-C	6.0^1	3.3^6	3.6^1	3.5^0	3.6^8

[a] T_1's precision was within ±2%.
[b] Racemate (24 mg) was dissolved in $CDCl_3$ (1 g) in the absence of **1**.
[c] Enantiomer (12 mg) and **1** (100 mg) were dissolved in $CDCl_3$ (1 g).
[d] Enantiomer (24 mg) and **1** (100 mg) were dissolved in $CDCl_3$ (1 g).

Table III. Decrease in $T_1(D)$ for (*R*)-**2** and (*S*)-**2** in the presence of equimolar amount of **1**[a]

Carbon	*D*(*R*)	*D*(*S*)	*D*(*R*)–*D*(*S*)
1-C	−0.421	−0.354	−0.067
2-C	−0.249	−0.224	−0.025
1′-C	−0.634	−0.574	−0.060
2′,6′-C	−0.460	−0.429	−0.031
3′,5′-C	−0.460	−0.430	−0.030
4′-C	−0.418	−0.388	−0.030

[a] Calculated from eq 1.

To investigate which site of the **2** enantiomer was most affected by the presence of **1** in terms of mobility, decrease of $T_1(D)$ from the T_1 of sole **2** was calculated and normalized by the latter;

$$D = \frac{[(T_1 \text{ in the presence of } \mathbf{1}) - (T_1 \text{ in the absence of } \mathbf{1})]}{(T_1 \text{ in the absence of } \mathbf{1})} \quad (1)$$

D values calculated for the sample of 1 : 1 molar ratio are listed in Table III, where *D*(*R*) and *D*(*S*) represent the *D* values for (*R*)-**2** and (*S*)-**2**, respectively, together with their differences, *D*(*R*)–*D*(*S*).

As the difference of $D(R)$–$D(S)$ was most evident at 1-C (-0.067), which bears hydroxyl group, we speculate that the effects of diastereomeric interactions between **1** and **2** responsible for chiral recognition are most important around 1-C,[8] and the hydroxyl group is the site of the interaction with **1**, most probably through hydrogen bonding between OH group of **2** and ester group of **1**.

Wainer *et al.* suggest that hydrogen bonding plays an important role in chiral discrimination HPLC with polysaccharide phases.[9] This is the first report that shows the difference between recognized and unrecognized enantiomers based on spectroscopic data.

From the viewpoint of developing new uses of polysaccharide derivatives, the present results are interesting because they infer the use of polysaccharide derivatives as cheap and useful chiral shift reagents.

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