CHEMISTRY LETTERS, pp. 579-582, 1994.

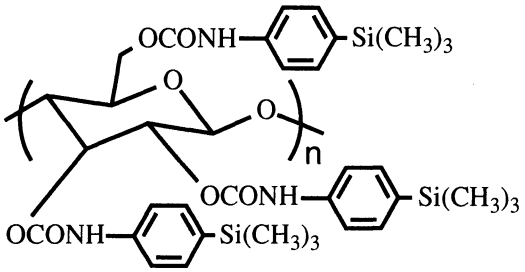
An NMR Study of Chiral Recognition Relevant to the Liquid Chromatographic Separation of Enantiomers by a Cellulose Derivative

Eiji YASHIMA, Makiko YAMADA, and Yoshio OKAMOTO*

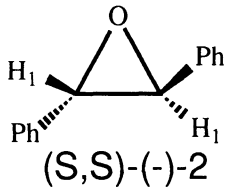
Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-01

Chiral discrimination of enantiomers by cellulose tris(4-trimethylsilylphenylcarbamate) (CTSP) was studied by ^{1}H NMR spectroscopy. The ^{1}H NMR signals of *trans*-stilbene oxide and 2-butanol were enantiomerically separated into two sets of peaks in the presence of CTSP in $CDCl_3$. The competition experiment with acetone suggests that *trans*-stilbene oxide may be adsorbed on the NH proton of carbamate residues of CTSP. Chemical shift non-equivalence in enantiotopic methyl groups of 2-propanol was observed in the presence of CTSP.

Phenylcarbamate derivatives of cellulose and amylose are the widely used chiral stationary phases (CSPs) for HPLC separation of enantiomers and can resolve a broad range of racemates.[1,2] However, the chiral recognition mechanism in a molecular level on phenylcarbamate derivatives of the polysaccharide is still unclear, although qualitative explanation has been given on the basis of chromatographic enantioseparation.[1] NMR spectroscopy may be the most powerful tool to reveal chiral recognition in a molecular level.[3] However, most phenylcarbamate derivatives of the polysaccharides with high chiral resolving power as a CSP are soluble only in polar solvents such as tetrahydrofuran, acetone, and pyridine, but not soluble in chloroform. In these polar solvents, the phenylcarbamate derivatives show low chiral recognition for enantiomers because the solvents preferentially interact with the polar carbamate residues which are the most important binding site for chiral recognition. Recently, we have found that cellulose tris(4-trimethylsilylphenylcarbamate) (CTSP) (**1**)[4] is soluble in chloroform, showing high chiral recognition ability as a CSP.[5] This, for the first time, permits us to investigate the chiral interaction occurring in solution between cellulose trisphenylcarbamates and chiral compounds such as *trans*-stilbene oxide and 2-butanol by NMR spectroscopy.



1: CTSP



(S,S)-(-)-2

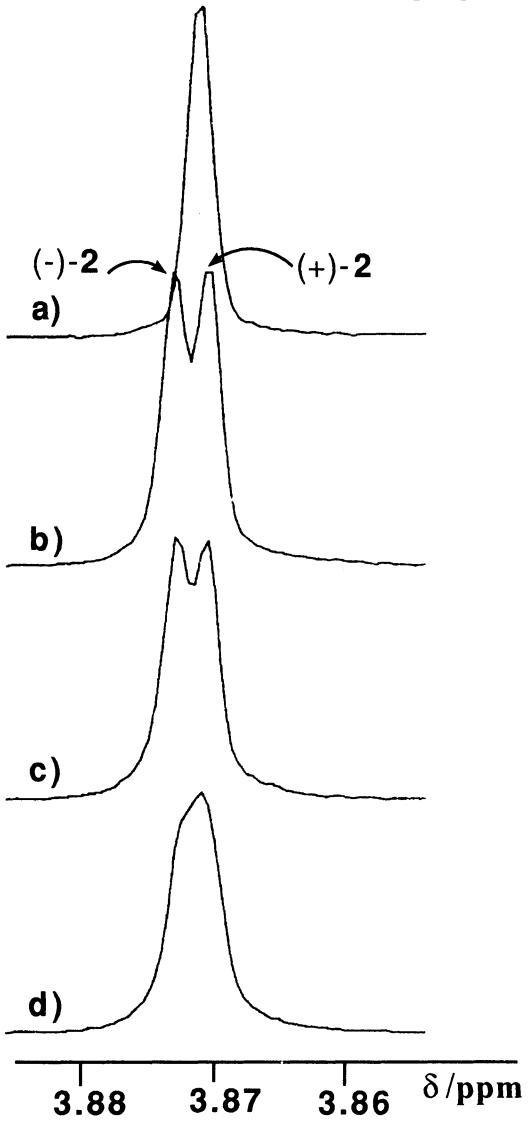


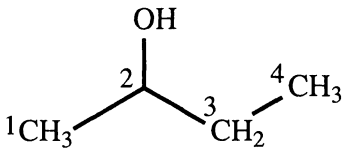
Fig. 1. 500 MHz ^{1}H-NMR spectra of **2** (5 mg) in $CDCl_3$ (1.0 ml) at 22 °C (TMS). CTSP: 0 (a), 20 mg (b, c, and d). acetone: 0 (a and b), 5 μl (c), 15 μl (d).

Figure 1 shows 500 MHz ^{1}H NMR spectra of (±)-*trans*-stilbene oxide (**2**) (5 mg, 0.025 mmol) in the presence and absence of CTSP (20 mg, 0.027 mmol of glucose unit) in CDCl3 (1.0 ml). The methine proton (H1) of **2** was enantiomerically separated into two singlet peaks in the presence of CTSP (b) ($\Delta\delta$ = 1.0 Hz). This apparently indicates that CTSP can recognize the enantiomers even in solution. The measurement using enantiomerically pure (+)- and (-)-**2** indicated that the (S,S)-(-)-H1 proton slightly shifted downfield in the presence of CTSP, whereas the (R,R)-(+)-H1 proton scarcely changed. In the chromatographic enantioseparation of (±)-**2** on the CSP, (+)-isomer eluted first followed by (-)-isomer and complete baseline separation was achieved.[6] This indicates that (-)-isomer adsorbs more strongly on CTSP. This elution order of (±)-**2** on CTSP must be correlated with the downfield shift of (-)-isomer observed in NMR. Although a considerable number of designed chiral hosts, chiral solvating agents, and chiral lanthanide shift reagents have been synthesized for recognition in solution by NMR,[7] only a few reports describe the chiral recognition with optically active polymers by NMR spectroscopy,[8] because most polymers are soluble only in the solvents which prevent to investigate chiral discrimination with small molecules.

From our previous studies on chromatographic enantioseparation of a variety of racemates on a series of cellulose tris(alkyl- or halogen-substituted phenylcarbamate)s,[1] the most important adsorbing sites for effective chiral separation have been considered to be the carbamate residues, which interact with racemates mainly through hydrogen bonding.[1] In the case of **2**, the cyclic ether oxygen can interact with the NH proton of the carbamate residue via hydrogen bonding formation. If this is the case, an addition of a compound capable of hydrogen bonding with the NH proton will prevent the interaction between **2** and CTSP. This was confirmed from the change in ^{1}H NMR of the methine proton (H1) of **2** by the addition of achiral acetone (15 μl, Fig. 1 (d)). The separated methine peaks of **2** became a singlet by the addition of acetone.[9]

Analogous change (no splitting) in the ^{1}H NMR of the methine proton (H1) of **2** was also induced by the addition of 2-propanol (5 μl) in place of acetone. Interestingly, methyl groups of 2-propanol shifted upfield from original signal (1.211 ppm, J = 6.0 Hz) and appeared as a pair of doublets (1.184 and 1.189 ppm, $\Delta\delta$ = 2.5 Hz, J = 6.0 Hz). This indicates that the two methyl groups are magnetically non-equivalent in the presence of CTSP. The chirality of the CTSP seems to force 2-propanol to bind in a diastereotopic environment, allowing the recognition of enantiotopic methyl groups. It is not clear whether 2-propanol binds to either NH or C=O of CTSP. Probably, hydrogen bond formation on the both sites participates in chiral recognition. Chemical shift non-equivalence in enantiotopic methyl groups of 2-propanol has also been observed by using chiral lanthanide shift reagents,[7,10] but, to the best our knowledge, any chiral organic host molecules and chiral solvating agents can not discriminate the methyl groups of 2-propanol.

Enantiomers of 2-butanol (5 μl) were also recognized by CTSP (20 mg in 1.0 ml $CDCl_3$),[11] and only the methyl (H4) and methylene (H3) protons were separated into a set of enantiomers ($\Delta\delta$ = 3.0 and 1.5 Hz, respectively), whereas the methyl (H1) proton was not split. These results indicate that CTSP discriminates between methyl and ethyl groups in binding, and the ethyl group may be located more closely to the chiral CTSP.



In summary, we report the first chiral recognition of enantiomers in solution with optically active polymers derived from cellulose by using ^{1}H NMR spectroscopy. The present results imply a potent applicability of phenylcarbamate derivatives of polysaccharide as a chiral shift reagent. Further studies are in progress to propose the rationale model of chiral recognition by using nuclear Overhauser effect studies and molecular mechanics calculation.

This work was financially supported in part by Grant-in-Aids for Scientific Research on Priority Areas No. 235 from the Ministry of Education, Science and Culture, and the Sumitomo Fundation.

References

1) Y. Okamoto, M. Kawashima, and K. Hatada, J. Chromatogr., **363**, 173 (1986); Y. Okamoto, Y. Kaida, R. Aburatani, and K. Hatada, "Chiral Separations by Liquid Chromatography," ed by S. Ahuja, ACS Symposium Series 471, American Chemical Society, Washington, D. C. (1991), Chap. 5.; Y. Okamoto and Y. Kaida, J. Chromatogr., in press.

2) Commercialized as CHIRALCEL and CHIRALPAK by Daicel Chemical Industries, Ltd., Tokyo, Japan.

3) For NMR studies of chiral recognition relevant to chromatography using optically active small molecule CSPs, see: W. H. Pirkle and T. C. Pochapsky, J. Am. Chem. Soc., **108**, 5627 (1986); B. Feibush, A. Figueroa, R. Charles, K. D. Onan, P. Feibush, and B. L. Karger, ibid., **108**, 3310 (1986); W. H. Pirkle and T. C. Pochapsky, Chem. Rev., **89**, 347 (1989); G. U.-Barretta, C. Rosini, D. Pini, and P. Salvadori, J. Am. Chem. Soc., **112**, 2707 (1990).

4) CTSP was prepared according to the same procedure for phenylcarbamate derivatives of polysaccharide[1)] by the reaction of cellulose and an excess of 4-trimethylsilylphenyl isocyanate in pyridine. Elemental analysis and ^{1}H NMR spectra indicate that all hydroxy groups were converted into carbamate moieties.

5) The chiral stationary phase coated on silica gel was prepared in a similar manner[1)] and the chiral recognition ability was evaluated by HPLC using hexane-2-propanol (98 : 2) as an eluent; detailed results of enantioseparation on the CTSP column will be published elsewhere.

6) Separation factor (α), $(t_2-t_0) / (t_1-t_0)$, of (±)-**2** is 1.55, where t_2 is the retention time of more retained enantiomer, t_1 is the retention time of less retained enantiomer, and t_0 is dead time.

7) For reviews see: P. L. Rinaldi, Prog. Nucl. Magn. Reson. Spectrosc., **15**, 291 (1982); "Asymmetric Synthesis," ed by J. D. Morrison, Academic Press, New York, (1983), Vol. 1, pp 153-196.

8) DNA as a chiral polymer (^{1}H NMR): J. P. Rehmann and J. K. Barton, Biochemistry, **29**, 1701 (1990). Polypeptide as a chiral polymer (^{2}H NMR): J.-L. Canet, A. Fadel, J. Salaün, I. Canet-Fresse, and J. Courtieu, Tetrahedron Asymm., **4**, 31 (1993).

9) Acetone is expected to form hydrogen bond with NH of the carbamate residues more strongly than **2**, since acetone elutes slower than **2** on a CSP derived from CTSP in chromatographic analysis.

10) W. B. Jennings, Chem. Rev., **75**, 307 (1975) and references cited therein.

11) 2-Butanol was also recognized with chiral solvating agents; C. von dem B.-Hünnefeld, A. K. Beck, U. Lengweiler, and D. Seebach, Helv. Chim. Acta, **75**, 438 (1992).

(Received December 10, 1993)