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Syntheses of 6*N*(*N'*-formyl-D-phenylalanyl)-deoxyamino-β-cyclodextrin and 6*N*(*N'*-formyl-L-phenylalanyl)-deoxyamino-β-cyclodextrin and Their Inclusion Behavior

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Abstract. 6*N*(*N'*-formyl-D-phenylalanyl)-deoxyamino-β-cyclodextrin and 6*N*(*N'*-formyl-L-phenylalanyl)-deoxyamino-β-cyclodextrin (**I** and **II**) were prepared. These new hosts formed 'intramolecular host–guest complexes' and included naphthyl derivatives preferentially with a stoichiometry of 1 : 1. The inclusion behavior was different between **I** and **II** because the cavity shape formed with the CD cavity and the phenylalanine moiety of **I** was different from that of **II**.

Key words. Cyclodextrin, diastereomer, intramolecular complex.

1. Introduction

Cyclodextrins (CD) include various organic molecules in their hydrophobic cavities and, in some cases, catalyze the reactions of included guest molecules. Chemical modifications of CDs have been studied extensively with a view to improving their complexing and catalytic abilities [1]. Guest molecule specificities for CDs have been reported using modified CDs consisting of two or more functional groups which adopt specific arrangements [2]. On the other hand, in order to characterize the inclusion behavior, the orientation of a guest molecule in the CD cavity and molecular dynamics of the inclusion complexes have been investigated in detail [3]. The orientations of guest molecules in the CD cavity were governed by the molecular shape of the guest or by the interactions between the guest and the CD, for example van der Waals forces and/or hydrogen bonding. Thus, a modified CD to which an aromatic moiety is introduced *via* a flexible arm should include its aromatic moiety to form an intramolecular complex. The intramolecular complexation causes changes in the shape and size of the cyclodextrin cavity, and an inclusion behavior characteristic of the modifications has been observed [4].

We report here the preparation and purification of two mono-substituted cyclodextrin diastereomers, 6*N*(*N'*-formyl-D-phenylalanyl)-deoxyamino-β-cyclodextrin (**I**) and 6*N*(*N'*-formyl-L-phenylalanyl)-deoxyamino-β-cyclodextrin (**II**) and the differences in the inclusion behavior between these two host molecules.

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2. Experimental

Modified CDs **I** and **II** were prepared in the same manner; 6-monodeoxyamino-β-CD was prepared from 6-monotosyl-β-CD [5] according to a previous report [6]. An amount of 0.5 g of *N*-formyl-phenylalanine and 3 g of 6-monodeoxyamino-β-CD were dissolved in 10 mL of dry DMF, and the solution was treated with DCC at 5°C for 3 h. After removing *N,N*-dicyclohexylurea, the reaction mixture was evaporated to dryness. The precipitate was washed with acetone and recrystallized with water to give 1 g of **I** (yield 30%); (Found: C, 46.55; H: 6.51; N, 1.94. $C_{52}H_{80}O_{36}N_2 \cdot 2\,H_2O$ requires C, 46.43; H, 6.29; N, 2.08%) δH (90 MHz; D_2O) 2.7–4.5 (42 H, cyclodextrin CH except for C1H), 4.8–4.9 (7 H, C1H of cyclodextrin), 7.1–7.4 (5H, phenyl ring), 8.1 ppm (1H, *s*, CHO). CD **II** was obtained in a yield of 1.2 g (35%); Found: C, 45.88; H, 6.49; N, 2.08. $C_{52}H_{80}O_{36}N_2 \cdot 3\,H_2O$ requires C, 45.81; H, 6.36; N, 2.06%) ^{1}H NMR spectrum of **II** was identical to that of **I**; formyl and phenyl ring proton signals as well as other characteristic signals of cyclodextrin were observed in the expected intensity ratios.

The association constants (K) between three hosts, **I**, **II** and β-CD and various guest molecules were estimated by drawing Benesi–Hildebrand plots. The K values for the guest molecules with a naphthyl moiety were obtained from fluorescence spectra originating from their naphthyl moieties. The K values for other non-fluorescent guests were obtained by a previously reported method, using an ANS as fluorescence probe [7].

3. Results and Discussion

3.1. STRUCTURES OF THE NEW HOSTS

The new host molecules were obtained by the same procedure and in almost the same yield (Figure 1). The recrystallization behavior, however, was different between **I** and **II**. Compound **II** crystallized more readily than **I**. The structures of **I** and **II** we propose are based on an analysis of their 500 MHz ^{1}H NMR spectra (Figures 2 and 3). Since one phenylalanine molecule is bound to CD at the C-6 position in the glucose ring, seven doublet signals due to the C-1 protons in the CD ring were observed at 4.85–5.10 ppm. The other proton signals of CD were also different from the signals of the native β-CD, but the spectrum was so complicated that each signal could not be assigned to individual CD protons. This result

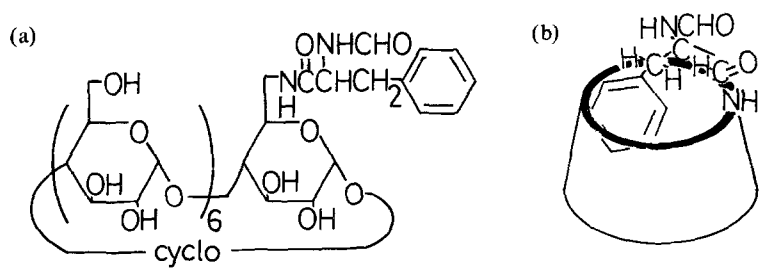

Fig. 1. Structure of new hosts **I** and **II** (a) and intramolecular complex formation (b).

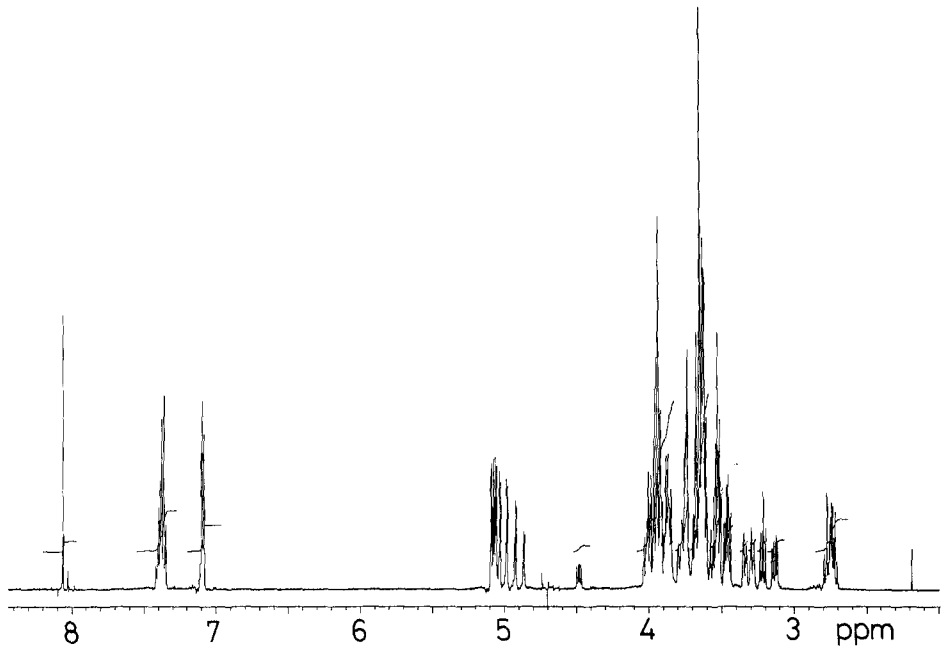

Fig. 2. 500 MHz ^{1}H NMR spectrum of *N*-formyl-D-phenylalanyl-β-CD (**I**) in D_2O.

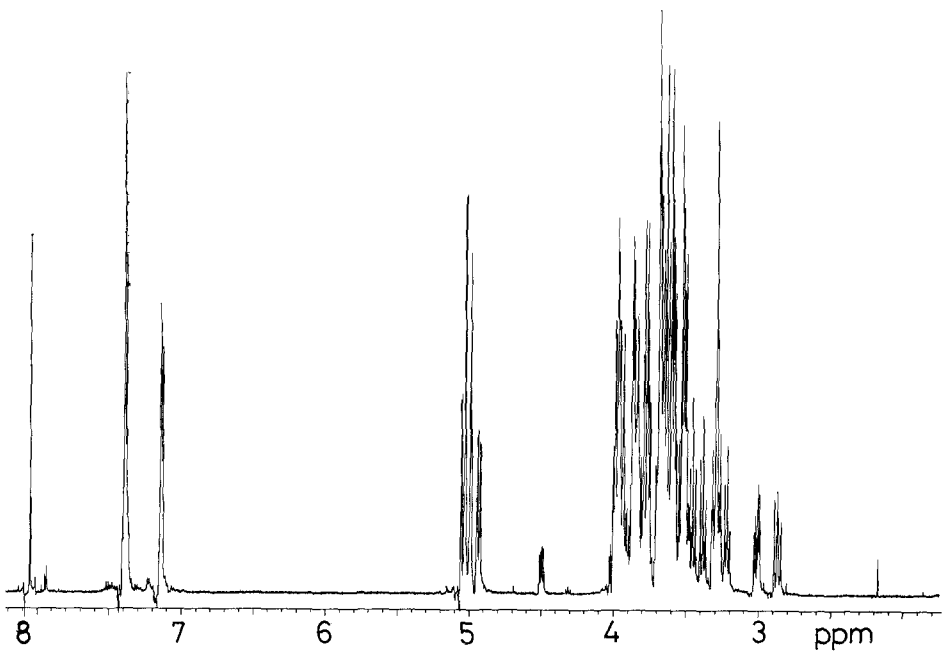

Fig. 3. 500 MHz ^{1}H NMR spectrum of *N*-formyl-L-phenylalanyl-β-CD (**II**) in D_2O.

suggests that the NMR environments of the seven glucose rings are not equivalent. In other words, the CD cavity shape is unsymmetrical. The change in cavity shape must be caused by the phenyl ring of the phenylalanine moiety. Further evidence for a change of cavity shape was obtained from the signals of the phenyl protons. Inoue and coworkers have reported that the large chemical shift change of the phenyl proton resonances was caused by complex formation between phenylalanine and CD[3]. The phenyl proton signals of **I** are found in two groups located at 7.06–7.12 ppm, corresponding to 2 protons (2′ and 6′ protons) and 7.34–7.42 ppm for 3 protons (3′, 4′ and 5′ protons), while the signals of native formyl-L-phenylalanine are found in three groups at 7.26–7.30 (2′ and 6′ protons), 7.30–7.34 (4′ proton) and 7.36–7.39 ppm (3′ and 5′ protons) (Figure 4). These chemical shift changes of the phenyl proton resonances suggest that the formylphenylalanine moiety is partly incorporated into each CD cavity, forming an intramolecular host–guest complex (Figure 1). The NMR spectrum of **II** also indicates signal splitting for the CD protons and a large chemical shift change of the phenyl protons. But there are differences between **I** and **II**, especially in the splitting pattern of the signals due to protons at the C-1 position and the phenyl proton resonances. Five doublet signals of the protons at the C-1 position in the CD, containing two overlapped signals, are observed at 4.91–5.76 ppm and the phenyl proton signals of **II** are found in two groups at 7.12–7.16 ppm, corresponding to 2 protons, and at 7.36–7.41 ppm for 3 protons. From the above results, it appears that the phenylalanine moiety of **II** is also included in the CD cavity, but the orientation of the *N*-formylphenylalanine moiety in the CD cavity is not the same as it is in **I**. It has been reported that no enantiomeric differences caused by complexation of native L and D-Phe in α- and β-CDs [8] have been observed in NMR spectra. This seems reasonable from an

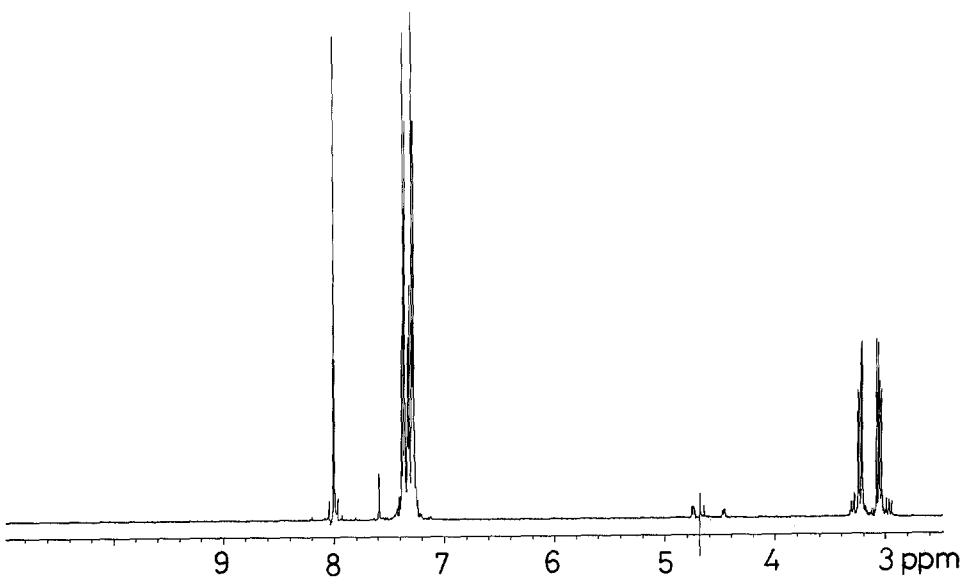

Fig. 4. 500 MHz ^{1}H NMR spectrum of *N*-formyl-L-phenylanine in D_2O.

examination of molecular models since no differences in structural features, other than chirality, can be seen. In the present study, however, since the phenylalanine moiety is attached to CD *via* a covalent bond, the above differences in NMR spectra, which are the result of structural features, are indeed observed. The formyl moiety attached to the amino group of Phe seems to increase the differences between **I** and **II**.

3.2. INCLUSION BEHAVIOR OF NEW HOSTS

The above structural differences are also reflected in the inclusion behavior. The results are summarized in Table I. The molecular formulas of the guest molecules are indicated in Figure 5. The values of guest molecules of a common

Table I. Comparison of association constants between **I** and **II**.

host	guest (k)						
	ANS[a]	TNS[a]	dansyl-[a] L-alanine	dansyl-[a] D-alanine	*N*-formly-L[b] phenylalanine	L-mandelic[b] acid	D-mandelic[b] acid
I	78 ± 11	167 ± 27	54 ± 10	42 ± 13	–[c]	–[c]	–[c]
II	68 ± 19	207 ± 29	95 ± 17	113 ± 18	–[c]	–[c]	–[c]
β-CD	79 ± 8	–[d]	144 ± 13	179 ± 13	35 ± 1	17 ± 0	18 ± 0
$K_{\mathbf{I}}/K_{\mathbf{II}}^{e}$	1.1	0.8	0.57	0.37	–	–	–

[a]Determined from fluorescence intensity at 540 nm (excited around 350 nm), pH 7.0 solutions (1/15 mol/L phosphate buffer), 25°C with 5.0×10^{-5} mol/L of guest molecule.
[b]Determined with fluorescence intensity of ANS, [ANS] = 2.0×10^{-5} mol/L, [cyclodextrin] = 1.0×10^{-3} mol/L, [guest] = $0.0 - 5.0 \times 10^{-2}$ mol/L.
[c]The value was too small to be determined.
[d]H. Kondo and coworkers reported that β-CD and TNS formed a 2:1 host–guest complex [7].
[e]The ratio of association constants of **I** and **II**.

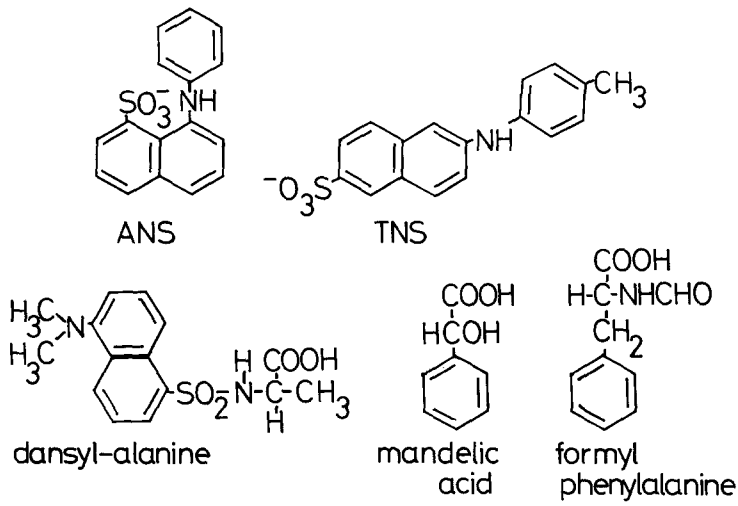

Fig. 5. Various guest molecules.

size – mandelic acid and *N*-formylphenylalanine – were too small to be determined. It is interesting that the guest molecules of a common size are hardly included at all in **I** and **II**, while the *K* values of naphthyl derivatives are comparable with inclusion in β-CD. From the NMR study, the phenyl ring seems to be inserted in the CD cavity, forming an intrusive floor. In this geometry, the substrate would be expected to penetrate less deeply, but only the naphthyl derivatives can approach the narrow cavity consisting of the CD rims and the phenyl ring, assisted by hydrophobic interactions; thus leading to the larger *K* values than those obtained for the phenyl derivatives. Since the shape of the hydrophobic cavity of **I** or **II** is not the same, their association constants are also different. In other words, the diastereomeric differences between **I** and **II** have a direct influence on cavity shape. These results indicate that even one point substitution with a flexible segment can create a characteristic change in cavity shape; and the modification in the cavity shape is reflected in the inclusion behavior.

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