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Fluorometric Studies on Inclusion Complexation of L/D-Tryptophan by β-Cyclodextrin 6-*O*-Pyridinecarboxylates

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Novel β-cyclodextrin (β-CD) derivatives, bearing a nicotinic or isonicotinic moiety, have been synthesized by a convenient method in 21 and 25% yields, respectively. The stability constants (K) and Gibbs free energy changes ($-\Delta G^{\circ}$) for the inclusion complexation of β-cyclodextrin 6-*O*-mono(3-pyridinecarboxylate) (**1**), 6-*O*-mono(4-pyridinecarboxylate) (**2**), and 6-*O*-monobenzoate (**3**) with L- and D-tryptophan have been determined by spectrofluorometry in aqueous buffer solution (pH = 7.20) at 25.0°C. All of the modifications dramatically enhanced the original K for β-CD by a factor of 30–280 and interestingly switched the original enantiomer preference for L- to D-tryptophan, thus affording the inverted enantio-selectivities of $K_L/K_D = 2.5$ for β-CD and $K_D/K_L = 1.2–2.1$ for the modified CDs **1–3.** These results are discussed from the viewpoints of the size-fit and geometrical complementary relationship between the host and guest.

INTRODUCTION

Molecular recognition by natural and modified cyclodextrins (CDs) is currently a significant topic in chemistry and biochemistry (*1*). The nucleophilic or electrophilic group appended to the primary side of CD is known to modify the original binding ability and selectivity of natural CDs for a variety of neutral and ionic guest molecules through further ligation or stereochemical complement of the functional sidearm introduced (*2–6*). Consequently, a large number of cyclodextrin derivatives have been designed and synthesized in order to investigate their molecular recognition behavior (*7*). Recently, a great deal of effort has been devoted to the studies of modified cyclodextrins as models of the biological substrate-receptor systems (*8–12*). We also have reported some interesting results on the molecular recognition behavior of modified cyclodextrins with amino acid guests (*2*).

In the present study, we synthesized a series of β-cyclodextrin 6-*O*-mono(arenecarboxylate)s, *i.e.,* 3-pyridinecarboxylate (**1**), 4-pyridine-carboxylate (**2**), and benzoate (**3**), and investigated their inclusion complexation behavior with L- and D-tryptophan

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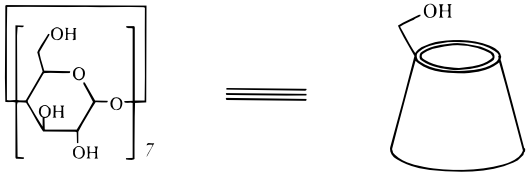
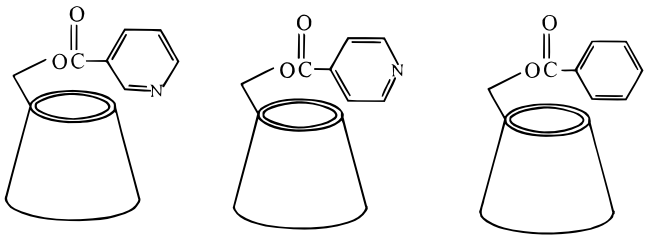
in aqueous buffer solution (pH = 7.20) at 25°C, using the fluorometric titration technique. From such investigations we can discuss the molecular binding ability and enantioselectivity of the modified CD hosts **1, 2,** and **3** for L/D-tryptophan guests from the viewpoints of the size-fit concept, the geometrical complementary relationship, and the role of cooperative weak interactions working between the receptor (host) and substrate (guest).



β-CD



(**1**) (**2**) (**3**)

EXPERIMENTAL

General

Combustion analyses were performed on a Perkin–Elmer 240 instrument. ^{1}H NMR spectra were recorded in [$^{2}H_6$]dimethyl sulfoxide (DMSO-d_6) on a Bruker AM200 spectrometer. FT-IR and UV spectra were obtained on a Nicolet FT-IR 5DX and Shimadzu UV-2401PC spectrometer, respectively. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter. Fluorescence spectra of tryptophan in the presence/absence of varying concentration of the host were obtained on a JASCO FP-750 spectrometer.

Materials

Commercially available L- and D-tryptophans (Sigma) were used without further purification. *β*-Cyclodextrin of reagent grade (Suzhou Monosodium Glutamate Works) was recrystallized twice from water and dried for 12 h *in vacuo* at 100°C. *N*,*N*-dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under reduced pressure prior to use. Commercially available nicotinic acid and isonicotinic acid of analytical reagent grade was used without further purification.

Syntheses

β-Cyclodextrin 6-*O*-mono-(3-pyridinecarboxylate) (**1**). To a solution of DMF (100 ml) containing 0.6 g of nicotinic acid and 1.65 g of dicyclohexylcarbodiimide (DCC)

was added 60 g of β-CD and 25 cm^3 of dry pyridine in the presence of 4A molecular sieves. The resultant mixture was stirred for 12 h in an ice bath and another 18 h at room temperature, and then allowed to stand for 3 days untill no more precipitation deposited. The precipitate was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of hot water and then poured into 150 cm^3 of acetone. The precipitate was collected by filtration to obtain white powder, which was washed thoroughly with a large amount of acetone. The crude product was recrystallized once from 1:1 ethanol-water and then twice from water, giving 1.5 g of pure product (**1**) as white powder in 21 % yield. MS *m/z* [FAB(NaI)]: 1241 ($M + H^+$), 1264 ($M + Na^+$); ^{1}H NMR (DMSO-d_6, TMS) δ 3.1–3.9 (m, 40H), 4.0–4.6 (m, 8H), 4.8–5.2 (m, 7H), 7.6–9.2 (m, 4H). ^{13}C NMR (DMSO-d_6, TMS) δ 164.572, 153.572, 149.949, 137.464, 125.641, 124.125, 101.965, 81.542, 74.0, 73.021, 72.369, 72.067, 68.896, 59.884. FT-IR (KBr) v 3383.5, 2914.0, 1711.5, 1625.2, 1596.1, 1570.5, 1538.8, 1444.7, 1408.2, 1364.2, 1340.6, 1311.8, 1299.7, 1266.8, 1216.4, 1192.3, 1149.6, 1074.9, 1023.9, 939.1, 858.3, 798.9, 752.8, 698.8 cm^{-1}. *Anal*. Calcd for $C_{48}H_{73}O_{36}N{\cdot}4H_2O$ C: 43.94 %, H: 6.22%, N: 1.07%; Found C: 44.10%, H: 6.27%, N: 1.43.

β-Cyclodextrin 6-*O*-mono-(4-pyridinecarboxylate) (**2**) was prepared in 25% yield from β-CD and isonicotinic acid according to the procedures similar to those employed in the synthesis of **1.** MS *m/z* [FAB(NaI)]: 1241 ($M + H^+$), 1264 ($M + Na^+$); ^{1}H NMR (DMSO-d_6, TMS) δ 3.1–3.9 (m, 40H), 4.1–4.6(m, 8H), 4.8–5.2 (s, 7H), 8.0–9.0 (m, 5H). ^{13}C NMR (DMSO-d_6, TMS) δ 164.34, 150.542, 143.085, 122.571, 101.745, 81.332, 76.64, 72.801, 72.149, 71.848, 69.77, 59.664. FT-IR(KBr) v 3374.5, 2910.0, 1717.0, 1623.5, 1567.9, 1540.3, 1443.4, 1419.7, 1364.2, 1339.7, 1291.8, 1237.5, 1148.1, 1118.3, 1073.5, 1021.9, 934.0, 849.1, 795.8, 748.8, 721.6, 638.8 cm^{-1}. *Anal*. Calcd for $C_{48}H_{73}O_{36}N{\cdot}5H_2O$ C: 43.34%, H: 6.29%, N: 1.05; Found C: 43.16%, H: 6.42%, N: 1.04%.

β-Cyclodextrin 6-*O*-monobenzoate (**3**) was synthesized in the reaction of β-CD with benzoyl chloride according to the reported procedures (*13*).

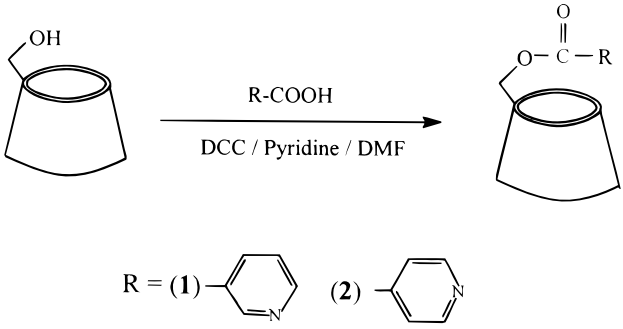
Fluorometric Experiments

The fluorometric titrations using a series of solutions containing L/D-tryptophan (2.5×10^{-6} mol dm^{-3}) and varying amounts of β-cyclodextrin derivative (**1, 2,** and **3**) (0–9.0×10^{-5} mol dm^{-3}) were carried out in aqueous buffer solution (pH = 7.20) at 25.0°C with excitation at 280 nm and observation at 358 nm.

RESULTS AND DISCUSSION

Synthesis

As shown in Scheme 1, the pyridinecarboxylated β-cyclodextrins **1** and **2** were synthesized in satisfactory yields from β-cyclodextrin and nicotinic acid or isonicotinic acid by DCC condensation technique. Although the C-6 hydroxyl is usually of the highest activity for acylation among the three hydroxyls (14), the acylation may potentially occur at C-6, C-2, and C-3 hydroxyls of β-cyclodextrin. We therefore seriously checked the position of the substituent introduced in our products by using ^{13}C NMR. In general, the ^{13}C NMR spectra of *O*-substituted sugar derivatives are



SCHEME 1.

known to show a large downfield shift of the α-carbon and a small downfield shift of the β-carbon relative to the parent sugar. Hence, we can assign the point of substitution from the ^{13}C NMR spectrum of the product. In the ^{13}C NMR spectrum of **1,** the C-6 showed a significant downfield shift up to 9.0 ppm (from 59.884 to 68.896), while a small shift of 1.9 ppm (from 72.067 to 74.0) for the C-5 (β-carbons) were observed. In contrast, if the substitution would take place at the C-2 or C-3 of a glucose unit of β-cyclodextrin, the downfield shift of C-2 or C-3 (as a α-carbon) should show large shifts. Then we conclude that the nicotinic moiety in **1** is introduced at the C-6 of β-cyclodextrin. In a similar way, the isonicotinic group in **2** is also assigned to be attached at the C-6 of β-cyclodextrin.

Circular Dichroism Spectrum

The circular dichroism spectrum of modified β-cyclodextrin **1** in aqueous solution showed a negative Cotton effect peak for the ^1L_a band at 221 nm and a small positive peak for the ^1L_b band at 275 nm. Compound **2** also showed a negative Cotton effect peak for the ^1L_a band at 215 nm and a weak positive Cotton effect for the ^1L_b band at 275 nm. Similar profile has been observed for the circular dichroism spectrum of benzoate **3** (*14*). According to the sector rule proposed by Kajtar *et al.* (*15*), these negative ^1L_a and positive ^1L_b Cotton effect peaks clearly indicate that the aromatic moiety penetrates only shallowly into the hydrophobic cavity of CD. Judging from the structural similarity of these three modified CDs, it is reasonable that the nicotinic CDs **1** and **2** show the same self-inclusion behavior as benzoate **3** (*14*).

Fluorometric Titrations

In the titration experiments using fluorescence spectrometry, the fluorescence intensity of L/D-tryptophan gradually changed with increasing concentration of natural β-CD or modified CDs **1, 2,** and **3.** Typical fluorescence spectral changes upon addition of **2** to the L-tryptophan solution are shown in Fig. 1. These results indicate the formation of inclusion complex of these CDs with tryptophan. By assuming the 1:1 complex stoichiometry, the inclusion complexation of tryptophan (G) with β-CD derivative (H) is expressed by Eq. [1].