SYNTHESIS OF TWO REGIOSPECIFIC ISOMERS OF MONOTOSYL-γ-CYCLODEXTRIN

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Abstract: Tosylation of γ-cyclodextrin in an aqueous alkaline solution gave two regiospecific isomers (2-monotosyl-γ-cyclodextrin(**1**) and 6-monotosyl-γ-cyclodextrin(**2**)), that could recognize and catalyze small sized guest molecules; 2-monotosyl-γ-CD recognized small sized guest molecules more effectively than did 6-monotosyl-γ-CD.

In contrast to α- and β-cyclodextrins(CD), which form 1:1 host-guest complexes with small molecules, recent spectroscopic studies have suggested that γ-CD can include two molecules such as naphthalene derivatives, pyrene and various dyes, owing to the large size of the cavity.(Ref. 1). The characteristic property of γ-CD enables us to make use of γ-CD as "a micro reaction medium" in which two different kinds of molecules such as a catalyst and a substrate can co-operate effectively.(Ref. 2) The first step to achieve these ternary reaction systems was selective modification of γ-CD. We have already reported that α- and β-CD can be specifically monotosylated in an aqueous alkaline solution.(Ref. 3). In this original condition, the reaction proceeded accompanied by the host-guest complex formation between CD and tosyl chloride. Moreover, specificity at the modified site should be reflected in the complex formation; in the case of α-CD, a tosyl residue was attached at the secondary C-2 position and in the case of β-CD at the primary C-6 position. Treatment of γ-CD under the same aqueous condition gave two regiospecific isomers of monotosyl-γ-CD. We report herein the preparation and isolation of the regiospecific isomers of monotosyl-γ-CD and some inclusion behavior for usual-sized guest molecules.

Materials and Methods

Materials. The tosylation method was reported previously.(Ref. 3) γ-CD was sulfonated by reaction with 6 equiv. of *p*-tolylsulfonyl chloride in an aqueous alkaline solution at room temperature for 1 hr. After filtering off the exess tosyl chloride, the reaction mixture was purified through a HPLC apparatus (Toyo Soda LS-410; φ:2.5x30 cm);

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eluted with 15% acetonitrile in water; and detected by UV absorption at 230 nm and refractive index. The fractions of products **1** and **2** were evaporated and freeze-dried, respectively. and determined by analytical HPLC to have only one peak. Compounds **1** and **2** were identified by ^{1}H-nmr spectra and elemental analysis, Calcd for $C_{55}H_{89}O_{42}S$: 45.48%(C), 5.99%(H) and 2.21%(S). Found:45.73%(C), 5.75%(H) and 2.23%(S) for **1** isomer and 45.38%(C), 5.99%(H) and 2.05%(S) for **2** isomer. The yield of **1** and **2** was 4% and 5%.

Instrument. ^{1}H-nmr spectra and ^{13}C-nmr spectra were measured using a JEOL JNM-MH-100 NMR spectrometer and a JEOL FX-90Q spectrometer, respectively. The samples for ^{13}C-FT-nmr measurement were dissolved in DMSO-d_6 at 70°C with TMS as an external reference with 4000-45000 pulses. Circular dichroism was measured, using a JASCO-500C spectrometer, in pH 7.2 Tris-HCl buffering solution with a 0.1 or 0.01 dm cell respectively.

Kinetic Measurement. The hydrolysis of *p*- and *m*-nitrophenyl acetate was followed by measuring the absorbance at 400 nm with a JASCO UVIDEC-1 spectrophotometer. The reaction was initiated by addition of 15 µl of a stock solution of the ester in acetonitrile to 3.0 ml of Tris-HCl buffering solution. The pH of the solution was 9.10. The final concentration of nitrophenyl ester was 2.5×10^{-5} M. The reaction temperature was controlled at 30.0±0.5°C. Plots of $\log(A_\infty - A)$ *vs.* time for the reaction in the absence and the presence of **1**, **2** and γ-cyclodextrin gave straight lines. The pseudo-first-order rate constants were calculated from the plots. The rate of hydrolysis was measured to at least 20% completion of the reaction. The rate constants reported are averages of the values in three or four runs which agreed within 5%. After the kinetic measurement, it was determined by analytical HPLC that the tosyl moiety attached at the CD was not decomposed.

Result and Discussion

γ-CD was sulfonated by reaction with excess tosyl chloride in an aqueous alkaline solution. The pH value of the reaction mixture was changed from 13 to 8 finally. The reaction mixture was purified by preparative HPLC to obtain two pure substances(**1** and **2**). The yields of **1** and **2** were comparable, and the retention time of **2** was about two-fold longer than that of product **1**. However, both compounds were proved by ^{1}H-nmr spectra and elemental analysis to have only one tosyl moiety introduced into the glucose ring in γ-CD. The ^{13}C-nmr spectra of **1** and **2** in DMSO-d_6 solution are shown in Figure 1 and Figure 2. In order to determine whether a peak was due to the methine carbon at the C-2, C-3, C-4 and C-5 position or due to the methylene carbon at the C-6 position, a new nmr technique called the INEPT method was used.(Ref. 4). In the case of **1**, in addition to the peaks of native CD itself, there are no peaks corresponding to the shift of the C-6 carbon but four small peaks corresponding to a downfield shift of C-2' and an upfield shift of C-1', C-3' and C-4', so the tosyl moiety of **1** is introduced at the C-2 position of the glucose ring in γ-CD. In the field above 55 ppm, there were two peaks which are due to the methine carbon (Figure 3). These peaks could not be observed in the initial stage of this measurement(Figure 4). Tosylated carbohydrates usually decompose at

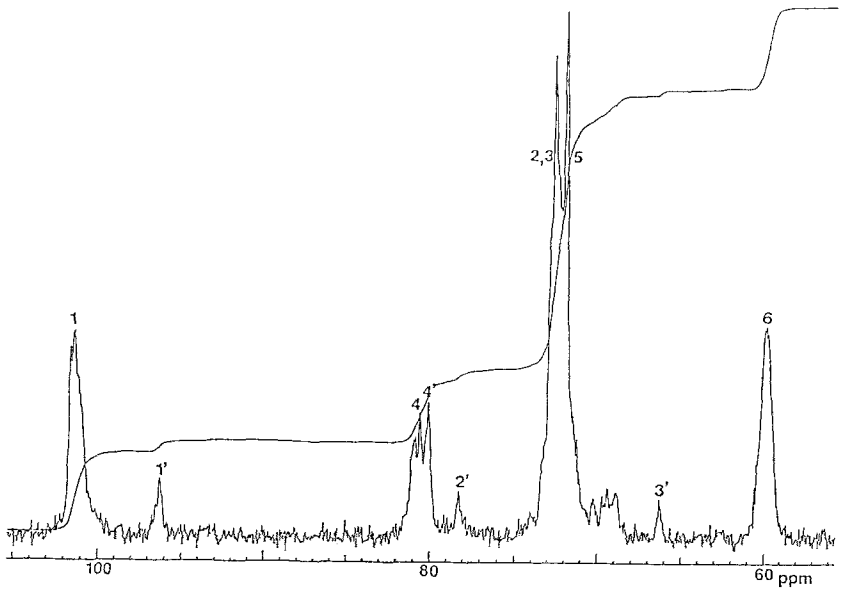


Figure 1.

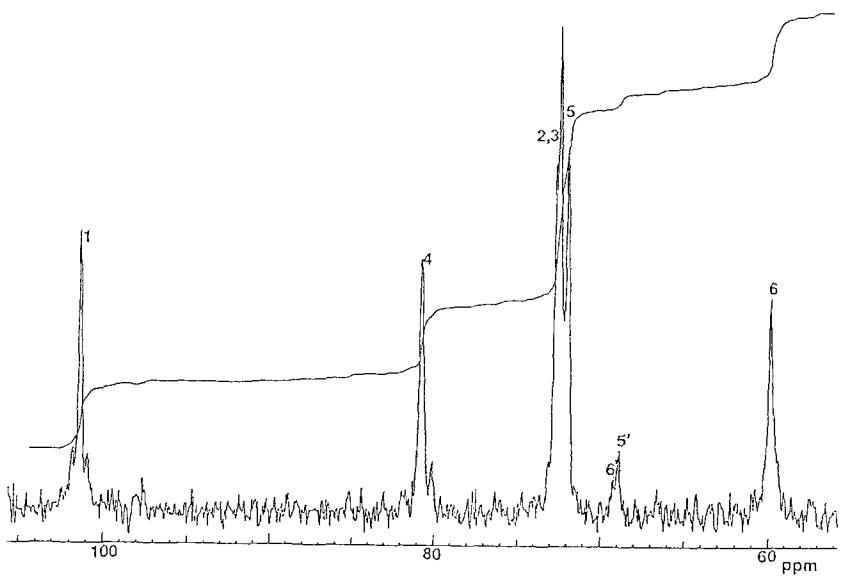


Figure 2.

^{13}C-nmr spectra of (1)(Figure 1) and of (2)(Figure 2);solvent:DMSO-d_6, reference:ext(TMS), temperature:70°C.

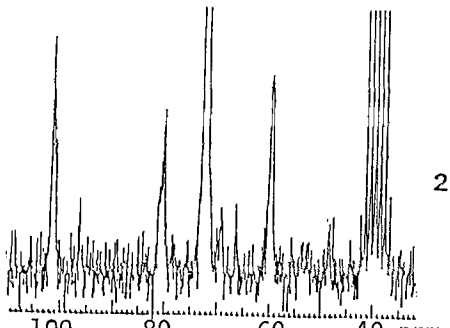


Figure 4.

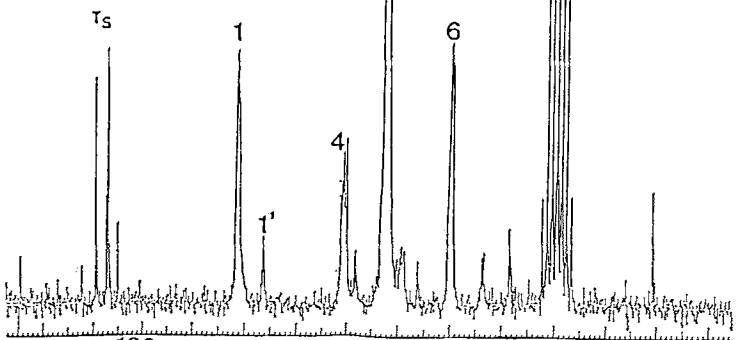


Figure 3.

^{13}C-nmr spectra of (1) with 4000 pulses(Figure 4)
and 45000 pulses(Figure 3)

high temperature and in an alkaline condition to the cyclic-anhydro derivative; 2-tosyl-CD decomposed to form 2,3-epoxy-CD. Since the measurement of ^{13}C-nmr spectra required over 24 hrs at 70°C, it seemed that a part of the tosyl moiety attached at the CD ring decomposed to 2,3-epoxy-CD. However, in the case of **2**, there are only two small peaks around 65 ppm corresponding to a downfield shift of C-6' and an upfield shift of C-5'. A tosyl moiety of **2** is introduced at the C-6 position. From these results, it may be concluded that the relation of **1** and **2** is as regiospecific isomers; one corresponds to 2-monotosyl-γ-CD and the other to 6-monotosyl-γ-CD. In order to confirm the conformational relation of the CD cavity and the tosyl moiety, the circular dichroism spectra and UV spectra in an aqueous solution were observed(Figure 5). Both **1** and **2** have negative molar ellipticity around 230 nm. The intensity of **1** and **2** was three-fold weaker than that of 6-monotosyl-β-CD. Only **1** shows weak induced circular dichroism around 270 nm. These results suggest that the hydrophobic cavity of γ-CD had some effect on the tosyl moiety. However, it was not clear whether the environment around the tosyl moiety of **1** was different from that of **2**; whether the inclusion activity of **1** was different from that of **2**. Whereas in the presence of the guest molecules, the inclusion behavior of **1** and **2** attracted much attention. Figure 6 shows the circular dichroism spectra

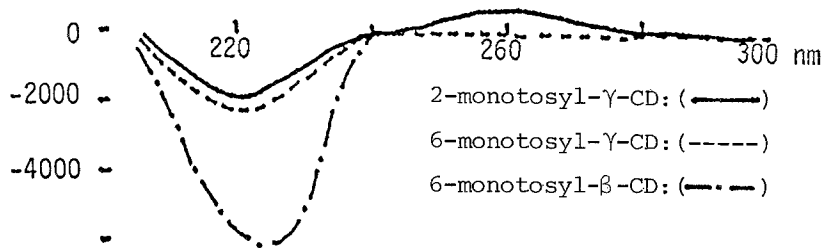


Figure 5. Circular dichroism spectra in an aqueous solution.

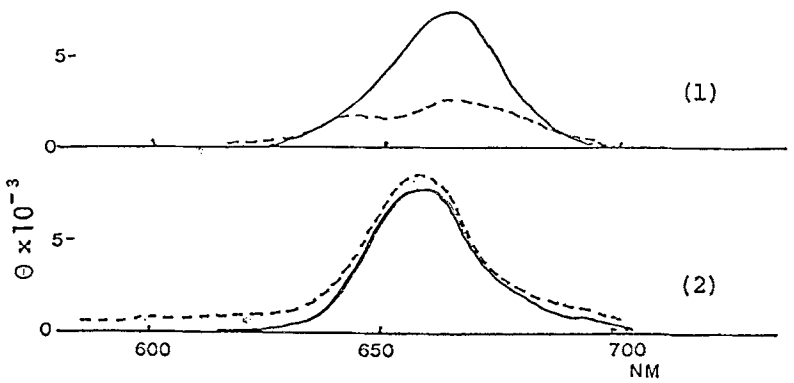


Figure 6. Circular dichroism of methylen blue induced by (1) and (2) in presence(----) and absence(——) of cyclohexanol.

of methylene blue induced by adding 1 and 2. Although native γ-CD includes two methylene blue molecules owing to the large size of the cavity, both 1 and 2 form 1:1 complexes. The complex formation with 1 was easily inhibted by cyclohexanol, but the complex with 2 was not inhibted. This result suggests that the tosyl moiety attached to γ-CD makes the cavity size of γ-CD narrow because of its inclusion into the γ-CD's cavity. In the case of the hydrolysis reaction of nitrophenyl acetate, the characteristic points were the enhancement of binding activity for a small-sized substrate compared with native γ-CD and the "*meta*-selectivity" with 1. The Lineweaver-Burk plots and the kinetic parameters in the hydrolysis reaction of *p*- and *m*-nitrophenyl acetate with compound 1, 2 and γ-CD are shown in Figure 7 and Table 1. The Michaelis-Menten constant Km suggests that compound 1 formed a complex with the substrate two fold tighter than that of compound 2 and four fold tighter than that of γ-CD. Modified-γ-CD having a tosyl group oriented inside the cavity would be able to recognize and catalyze the

substrate effectively. Compound 1 indicated "*meta*-selectivity", which was different from the selectivity of the usual capped-β-CD (Ref. 5), which also suggests that the folded group of compound 1 should not be the capping form. The hydrolysis of nitrophenyl acetate is accelerated by the attack of alkoxide anion of the secondary hydroxyl in the CD ring. It may be concluded from the results that with nitrophenyl acetate being included in the wider side (secondary hydroxyl side) of CD ring, the difference of 1 and 2 in the modified position reflected the complex formation as described in the following scheme. In the case of

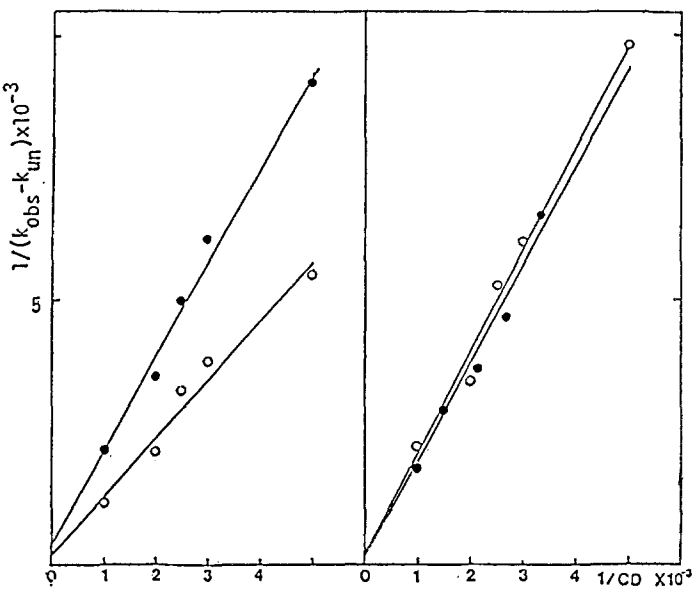
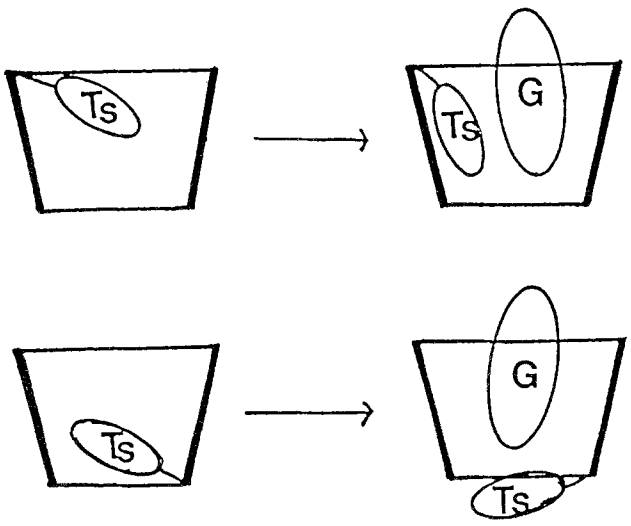


Figure 7. Lineweaver-Burk plots

TABLE 1

substrate	compound	(1)	(2)	γ-CD
p-NPA	$k_2x10^3s^{-1}$	2.4	4.1	1.9
	Km $x10^3M$	3.9	7.0	13.6
	k_2/Km	0.61	0.59	0.13
m-NPA	$k_2x10^3s^{-1}$	4.3	4.3	-
	Km $x10^3M$	4.4	8.1	-
	k_2/Km	0.98	0.53	-

Lineweaver-Burk plots(Figure 7) and the kinetic parameters (Table 1) of hydrolysis: pH 9.10, temperature 30.0±0.5°C, (●):*p*-NPA, (○): *m*-NPA



Scheme

1, the tosyl moiety is deeply included with the guest molecule in the CD cavity as a spacer; in other words, compound **1** included the guest molecules by an "induced fit" type of complex formation(Ref. 6,7); whereas, the tosyl moiety of **2** only caps the closer side of the CD ring. Breslow's group has already reported that the recognition ability of 6-monotosyl-β-CD was different from the ability of β-CD.(Ref.8) In that case the tosyl moiety should insert partly into the cavity, the guest molecules would be expected to bind less deeply. As the cavity of γ-CD was larger than that of β-CD, the tosyl moiety of **2** did not inhibit the complex formation with nitrophenyl ester. From the results of this investigation, a new character of γ-CD is suggested; 1) tosylation in an alkaline solution gave both 2-monotosyl-γ-CD and 6-monotosyl-γ-CD, 2) monotosyl-γ-CD recognized small sized guest molecules more than native γ-CD, 3) 2-monotosyl-γ-CD included the guest molecules tighter than 6-monotosyl-γ-CD.

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