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The Regiospecific Mono Tosylation of Cyclodextrins

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α- and β-Cyclodextrins were tosylated with tosyl chloride in an alkaline solution. The products were confirmed to be monotosylated compounds, and the tosyl group was introduced at the C-3 position of one glucose unit of the cyclodextrin molecule. The 1:1 complex formation of cyclodextrin with tosyl chloride was observed spectrometrically.

Cyclodextrins (CD) have been shown to form inclusion complexes with many kinds of molecules. For this reason, CD has been used to simulate an enzyme action.[1–3] Bender *et al.* showed enzyme-like actions of CD, that is, a large rate enhancement and stereospecificity in ester hydrolysis, and saturation and competitive inhibition phenomena, kinetically.[4,5]

In order to prepare a better enzyme model, several modified cyclodextrins have been synthesized. For example, Cramer and Mackensen synthesized imidazole-containing cyclodextrins by tosylation techniques in organic solvents.[6–8] In these procedures, one or more primary hydroxyl group(s) of cyclodextrins were modified, and so it was difficult to prepare regiospecific monosubstituted cyclodextrins. The introduction of an imidazole group of the primary hydroxyl side of cyclodextrins resulted in only a slight rate enhancement. It was not clearly ascertained if the formation of cyclodextrin acylate in an aqueous solution proceeded through complexation, but the benzoate group was attached on the secondary hydroxyl side.[5,9,10]

Another route to attach functionality on the secondary hydroxyl side of cyclodextrin is carboxymethylation by using the anion of dimethylsulfoxide. Bender's group introduced hydroxamic-acid derivatives on the more open face of the cyclodextrin molecule by using this method.[11,12] Imidazole-containing α-cyclodextrin, the catalytic group of which was introduced on a glucose unit, was prepared *via* monotosylated cyclodextrin in an aqueous solution.[13]

In this paper, we wish to report a simple, useful method for the preparation of regiospecific monotosylated cyclodextrins, the tosyl group of which is introduced on the secondary alcohol side of cyclodextrin accompanying 1:1 complex formation between tolylsulfonyl chloride and cyclodextrin in an aqueous solution. We also wish to suggest the mechanism of this regiospecific tosylation.

Experimental

p-Tolylsulfonyl-β-cyclodextrin. β-Cyclodextrin (1 g) was dissolved in a 0.15 M (1 M=1 mol dm^{-3}) sodium hydroxide solution (40 ml) with a pH of around 13.0. *p*-Tolylsulfonyl chloride (1 g) was dissolved in a minimum quantity of acetonitrile, after which the solution was magnetically stirred, drop by drop, into the alkaline solution. The suspension was then vigorously stirred for 1 h, adding 1 M NaOH occasionally to maintain the pH of the solution at more than 12.5. The reaction was terminated by neutralization with 1 M hydrochloric acid. The reaction mixture was filtered *in vacuo* to remove the unreacted *p*-tolylsulfonyl chloride, and then the filtrate was passed through a column with a cation-anion exchanger (Amberlite MB-3 ϕ 1.5×50 cm) and eluted with water. The desalted eluate was condensed and dried with a water bath *in vacuo*. The HPLC data, obtained using a OSD-SIL column (Tokyo Soda LS-410, ϕ 0.5×30 cm) eluted with 10% acetonitrile in water and detected by UV absorption at 254 nm, indicated there were more than 20 species in this crude product. The purity, calculated on the basis of the UV peak area, showed 70% of the main product. The present crude tosyl-CD (*ca.* 0.9 g) was dissolved in a minimum amount of water, and then the solution poured into a large amount of methanol (150 ml). The methanol solution was filtered and evaporated to dryness. The residue from the methanol (0.6 g) was dissolved again in warm water (9 ml, 60 °C) and filtered. The solution was left in a refrigerator at 5 °C for a few days. The white crystalline product was collected by filtration and dried *in vacuo* (0.4 g). In submitting the sample to elemental analysis, the present product (1 g) was further purified by a combination of various types of column chromatography. The aqueous solution was passed through porous polystyrene gel (ϕ 3×20 cm, DIAION HP-20). The column was washed with water, and the trapped component was eluated by the use of 30% aqueous methanol. The fractions containing tosylated CD were indicated by UV absorption, refractive index, and optical rotation, and were then collected and liophilized. Also, the product was purified by the use of gel chromatography (Sephadex G-15, ϕ 3×100 cm), eluated with water as well as a Lober column (ϕ 2.5×31 cm, Merck) and eluated with 10% acetonitrile in water. The yield was 0.1—0.3 g for each column chromatography. The purity was confirmed by HPLC and ^{1}H-NMR. Mp 168—170 °C (dec). Found: C, 43.66; H, 6.08%. Calcd for $C_{49}H_{76}O_{37}S \cdot 3H_2O$: C, 43.72; H, 6.14%.

p-*Tolylsulfonyl-α-cyclodextrin.* The tosylation of α-CD was carried out in the manner used for β-CD described above, except for the reaction medium. A carbonate buffer solution of pH 12 was used. For the purified sample: mp 160—162 °C; Found: C, 43.04; H, 6.28%. Calcd for $C_{43}H_{66}O_{32}S \cdot 3H_2O$: C, 43.16: H, 6.31%.

The R_f values of these two tosyl CD products in TLC on silica gel were 0.41 for α-CDOTs and 0.54 for β-CDOTs respectively (solvent: 1-butanol-dimethylformamide-water, 2:1:1; detecting reagent: iodine vapor or diphenylamine spray, followed by UV radiation). λ_{max} nm (logε: 274(2.40), 265 (2.70), and 263(2.78) for α-CDOTs, and 273(2.63), 265(2.71), and 262(2.73) for β-CDOTs, with referrence to 273(2.65), 265(2.75), and 262(2.75) for EtOTs at pH 6.50, 25 °C.

6-(Mono)-O-tolylsulfonyl-α-cyclodextrin. This compound was prepared by the method of Melton and Slessor.[14] The purification of the crude product was carried out by filtration through a Celite pad and by recrystalization from

95% ethanol. The total yield was 30%. Found: C, 43.46; H, 6.40%. Calcd for $C_{43}H_{66}O_{32}\cdot 3H_2O$: C, 43.16; H, 6.31%. Mp 155—157 °C (reported[14]) 159—162 °C); the R_f in TLC was 0.55.

NMR Measurement. The ^{13}C-NMR measurement of tosyl CD was carried out at the concentration of 0.1—0.5 g in 1.0 ml of a D_2O or DMSO-d_6 solution, referred to dioxane as an internal standard with 2700—10000 times accumulation. The ^1H-NMR was observed in a 10% (g/ml) D_2O solution, using trimethylsilyl tetradeutropropionate as the internal referrence.

Partial Hydrolysis of Monotosylated β-Cyclodextrin. The present monotosylated β-CD, prepared in an aqueous solution, was partially hydrolized in a 1 M HCl solution at 80 °C for 6 h. The reaction mixture was neutralized with 1 M NaOH and then desalted with an ion exchanger, Amberlite MB-3. In the product, two components were observed with HPLC and paper chromatography (in 1-butanol–dimethylformamide–water (2:1:1), with dimethylaniline–phtalic acid as the detecting reagent). The lower spot of the R_f value (0.55) was the same as that of authentic glucose. The hydrolyzate was purified by gel chromatography using Sephadex G-15, giving a product with a higher R_f value (0.72) in the paper chromatography; the product was confirmed as monotosylated glucose by characterization by NMR; 2.22(ppm) due to the CH_3 of the tosyl group, 3.2—4.0 due to nonanomeric C–H, 5.0 due to C_1H, and 6.8—7.3 due to benzene-ring C–H. Both the R_f value (0.72) and the spectrum of ^1H-NMR agreed with those of authentic 3-*O*-tosyl glucose, which was prepared according to Freudenberg and Ivers.[23]

Determination of Dissociation Constants. The increase in the absorbance of the aromatic chromophore in the presence of cyclodextrin was determined by the spectrophotometric method. To suppress the disturbance due to the hydrolysis of tosyl chloride and the pH change in the solution, the UV absorbance was observed within several seconds after the mixing of tosyl chloride. With a change in CD concentration from 0—0.955×10^{-2} M, the substrate concentration was held constant at 5.0×10^{-5} M. A linear relationship was observed between the spectral change and the added concentration of CD, employing the Benesi-Hildebrand plot at 246 nm with the equation: $C_{CD}\cdot C_{TsCl}/\Delta A = K_{diss}/\Delta\varepsilon + C_{CD}/\Delta\varepsilon$, where K_{diss} indicates a dissociation constant; C_{CD}, the initial concentration of CD; C_{TsCl}, the initial concentration of TsCl; ΔA, the change in the absorbance, and $\Delta\varepsilon$, the difference between the molar extinction coefficients of the free and the complexed substrate. The slope $(1/\Delta\varepsilon)$ and the intercept $(K_{diss}/\Delta\varepsilon)$ were obtained by the least-squares method.

Results and Discussion

Tosylation of α-, and β-Cyclodextrins in an Aqueous Medium. The reaction of α-, and β-CD with TsCl in an alkaline solution at room temperature for an hour gave products. The products from both α-CD and β-CD were confirmed to be monotosylated compounds (α-CDOTs and β-CDOTs) by UV estimation referred to ethyl tosylate (EtOTs) as well as by ^1H-NMR estimation by the peak area.[15—17] It is noteworthy that only one tosyl residue was introduced to a CD molecule in spite of there being many hydroxyl groups on a CD molecule. The optimum alkaline concentrations were around pH 12 for α-CD and around pH 13 for β-CD. Prolonged (12 h) and short (6 min) reactions gave a low attachment of the tosyl residue on CD. One hour was the optimum reaction time. The optimum reaction temperature was about 20 °C in the range of 0—60 °C.

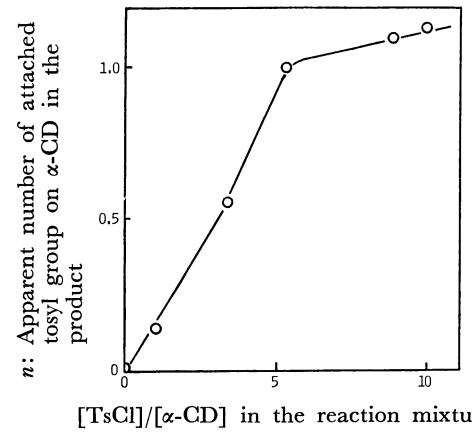


Fig. 1 The number of attached tosyl group on α-CD is plotted as a function of [TsCl]/[α-CD]. Tosylation was occured in 0.15 M NaOH solution at 25 °C for 1 h. The number (*n*) was calculated from the ^1H-NMR measurement.

As is shown in Fig. 1, when the ratio of the initial concentration of TsCl/α-CD was changed from 0 to 10, the plot of the apparent number of the tosyl group introduced on α-CD gave a saturation curve. It seemed that the number, n, increased linearly with the increase in the TsCl/α-CD ratio, which ranged 0 to 5. Hardly more than one tosyl residue to one CD molecule was introduced, even if the TsCl concentrations was 10 times larger than the CD concentration. When CD was tosylated in pyridine, in contrast, CD reacted easily with more than one TsCl and the number (*n*) would be stoichiometrically controlled. These facts suggest strongly there is 1:1 complex formation during the tosylation reaction in an aqueous solution.

The present product of tosyl-CD was compared with the tosyl-CD through stoichiometrically controlled monotosylation in pyridine, where the tosyl group was attached on a primary hydroxyl group of a glucose unit.[14] The R_f value of TLC was distinctly different between α-CDOTs prepared in pyridine and the α-CDOTs prepared in an aqueous solution. This fact suggested that the present monotosylated α-CD, prepared in an aqueous solution had a tosyl group at a secondary hydroxyl group of a glucose unit of a CD molecule.

The ^{13}C-NMR spectra of these tosyl CD's were observed and assigned according to the literature.[19—22] There was no evidence that each tosyl CD was a mixture of any isomers. The comparison of these spectra showed clearly that each of the tosylated cyclodextrins should have a single and particular structure as a tosyl CD. It had been reported that the amino group of amino-α-CD derived from the present tosylated α-CD was introduced at the C-3 position of a glucose of the CD molecule by means of ^{13}C-NMR spectroscopy.[13] Therefore, the tosyl group of the present tosylated α-CD may be introduced mainly at the C-3 position of the glucose ring of α-CD.

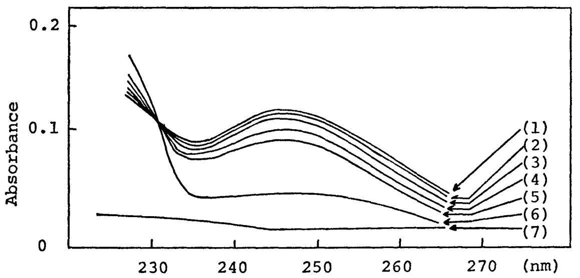


Fig. 2. Spectrum of tosyl chloride at varying α-CD concentrations; pH 6.50, 25 °C, acetonitrile/buffer= 0.5% (v/v). [TsCl] is 5.0×10^{-5} M. [α-CD] is changed at 9.55, 6.63, 3.32, 1.66, 0.332, and 0 ($\times10^{-3}$ M) from the curve 1 to 6 respectively. [TsCl] and [α-CD] are none for the curve 7.

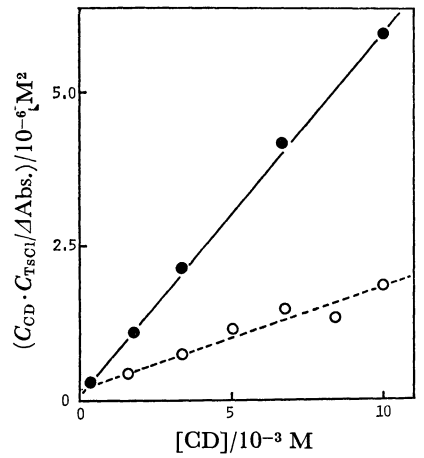
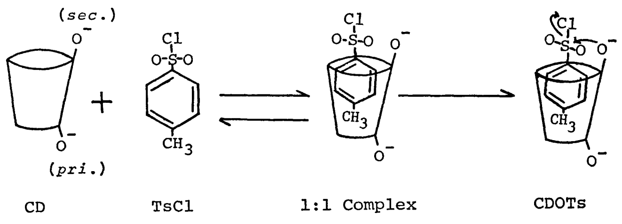


Fig. 3. Benesi-Hildebrandt plot for the mixture of CD and TsCl.
●: α-CD, ○: β-CD.

In order to get direct evidence to determine the substituted position of the tosylated β-CD group, the partial hydrolysis of β-CDOTs was carried out. The resulting hydrolyzate gave two spots in paper chromatography. The lower spot agreed with that of authentic 3-*O*-tosylglucose. The structure of the purified component with the higher R_f value was observed by ^{1}H-NMR to be monotosylglucose. The ^{1}H-NMR spectra and R_f values were coincident with the authentic sample of 3-*O*-tosylglucose. This result strongly suggested that the tosyl group of β-CDOTs was introduced at the C-3 position of glucose unit of β-CD.

Direct Evidence for a Complex Formation. The changes in the UV absorption spectra of TsCl in aqueous solutions with various amounts of the excess α-CD are shown in Fig. 2. A comparison of the UV spectra of EtOTs and TsOH with that of TsCl in an α-CD solution showed a distinct difference. Therefore, the broad absorption at around 245 nm in Fig. 2 must be due to the complex formation of CD with TsCl, and not to TsOH or tosylated CD. Moreover, the complex formation of CD with TsCl may be confirmed by the observation of the isosbestic point (Fig. 2). The Benesi-Hildebrand plots[18] for both α-CD and β-CD with TsCl gave a straight line (Fig. 3). The dissociation constants (K_{diss}) for the complexes of CD with TsCl, as calculated from the slopes of these lines, are 0.68×10^{-3} M for α-CD and 1.8×10^{-3} M for β-CD. These dissociation constants showed that the binding of α-CD to TsCl is tighter than in the case of β-CD, which may allow the alkoxide anions of α-CD more chances to attack the sulfur atom of TsCl than β-CD. This suggested that α-CD reacted with TsCl more easily than β-CD and why α-CD reacted with TsCl at a lower pH than did β-CD.



Scheme 1. The mechanism of the regiospecific monotosylation of cyclodextrin with *p*-tolylsulfonyl chloride in alkaline solution.

Mechanistic Scheme. The mechanistic scheme of the regiospecific tosylation of cyclodextrin with tosyl chloride in an alkaline solution is shown schematically in Scheme 1. As soon as tosyl chloride was dispersed in water, a cyclodextrin cavity should form a 1 : 1 complex with a tosyl chloride molecule. In that case, as the methyl group of the tosyl chloride consists of the hydrophobic part of the molecule, the methyl group should be near the primary hydroxyl group down into the cyclodextrin cavity, as is shown in the scheme. The sulfonyl chloride group should be positioned near the secondary hydroxyl group. The scale molecular model (CPK model) indicated that the distance between the sulfur atom of TsCl in the complex and the C-3 oxygen atom of the glucose unit in CD was a little shorter than the distance between the sulfur atom and the C-2 oxygen atom. This proximity effect between the sulfonyl chloride and the secondary hydroxyl group may accelerate the nucleophilic attack of a secondary hydroxyl (3-*O*) anion as the sulfur atom of the tosyl chloride molecule. This would result in a single tosylated CD on a specific secondary hydroxyl of cyclodextrin.

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