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Reactions of Phenyl Salicylates with Cyclodextrin in Alkaline Solution[1]

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To isolate an intermediate, cyclodextrin salicylate, the reactions of phenyl salicylates with cyclodextrin were kinetically investigated in alkaline 22% acetonitrile solution. Even in the alkaline solution, the formation and accumulation of cyclodextrin salicylate was confirmed during the course of the reactions. The possible mechanism was discussed. The deacylation rate of cyclodextrin salicylate was considerably small compared with the uncatalyzed hydrolysis rates of phenyl salicylates and that of the acylation of cyclodextrin.

Keywords——cyclodextrin salicylate; cyclodextrin; kinetics and mechanism; acylation of cyclodextrin; deacylation of cyclodextrin salicylate; phenyl salicylates

Cyclodextrin (CD) has been used for the improvements of solubilities and stabilities of several drugs by the inclusion complex formations.[3–5] It has also been examined as enzyme model compound for the serine protease, in which the acyl-cyclodextrin intermediate was formed in the reaction of aromatic ester with CD.[6,7] Methyl salicylate is not now used orally, since the symptoms of poisoning such as nausea, acidosis *etc.* were known.[8] Although aspirin (acetylsalicylic acid) is widely used for a variety of diseases, the side effects, for example gastrointestinal bleeding and acid–base disturbances, are also well known.[9] Therefore, salicylic acid derivatives which have less toxicities and side effects, and which are highly water-soluble compounds are expected. Cyclodextrin salicylate may be considered to be one of those drugs.

Although there would be little complication in the case of esters synthesized from monofunctional alcohol and acid halide, cyclodextrin salicylates in this case may be obtained as mixture consisting of different molar ratios of CD to salicylic acid because of lots of the hydroxyl groups on CD. Consequently, the purifications and identifications of them would be very difficult. Meantime, cyclodextrin cinnamate which was the ester of cinnamic acid and CD in the molar ratio of 1:1, was recently synthesized from CD and *m*-nitrophenyl cinnamate in alkaline solution containing about 50% acetonitrile.[10] The similar reactions and conditions may be applied to the cyclodextrin salicylate (CDSAL) synthesis. In the alkaline solution, however, the hydroxyl groups of phenyl salicylates ionize to the phenoxide ions. It is reported that CD forms more strongly the inclusion complex with non-ionized guest molecule than the ionized one[11,12] and acylation of CD occurs due to the nucleophilic

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attack to the carbonyl carbon atom of ester by the alkoxide ion of the hydroxyl group on CD.[7] Furthermore, the hydrolysis of CDSAL might be facilitated by the intramolecular catalysis of the phenoxide ion of the salicylate residue[13] and/or of the adjacent hydroxyl group residues,[7] and if this is so, CDSAL might not be accumulated in the reaction mixture.

As the preliminary experiments to obtain such CDSAL, the reactions of phenyl salicylates with CD were kinetically studied and the convenient conditions for the isolation of CDSAL were explored. The formation and accumulation of CDSAL was confirmed during the reactions and the possible mechanism was discussed.

Experimental

Materials——The synthesis of *m*-nitrophenyl salicylate (*m*-NPSAL) was followed to the method of Tozer and Smiles[14] and the melting point was 107—108° (literature value, 105°[15]). Phenyl salicylate (PHESAL) and α-cyclodextrin (α-CD) were commercially obtained. β-Cyclodextrin (β-CD) was a gift of the Teijin Co. Ltd. (Tokyo). All other chemicals used were reagent grade.

Determination of Apparent First Order Rate Constants for Acylation of CD——Because of the low solubilities of the substrates, *m*-NPSAL and PHESAL, acetonitrile was added to the carbonate buffer and the concentration of the solvent became 22% (v/v). The apparent pH values were measured at the end of the reactions by Hitachi–Horiba pH meter.

The appearance rate of *m*-nitrophenol (*m*-NP) from *m*-NPSAL in both the presence and absence of CD was measured at 400 nm by Hitachi UV-124 spectrometer. The pseudo-first order analysis could be applied for the determination of the apparent rate constant.

The reaction rate constant of PHESAL with β-CD was determined from the UV spectral changes at 340 nm by the Guggenheim method.[16] This method can be used for the determination of the apparent rate constant on the first order reaction of which the end point was not known. The preliminarily experimental results showed that the apparent rate of the reaction of PHESAL with β-CD at around pH 10 was 5 to 10 times faster than that of subsequent reaction.

Determination of First Order Rate Constants for Deacylation of CDSAL——The ultraviolet (UV) spectral changes at 340 nm was used for the determination of the deacylation rate of CDSAL. The UV spectral changes due to the acylation of CD by *m*-NPSAL did not interfere with the determination of the deacylation rate constants by the first order analysis, since the deacylation rate constants were about 20 to 50 times slower than the apparent rate constants for the acylation of CD.

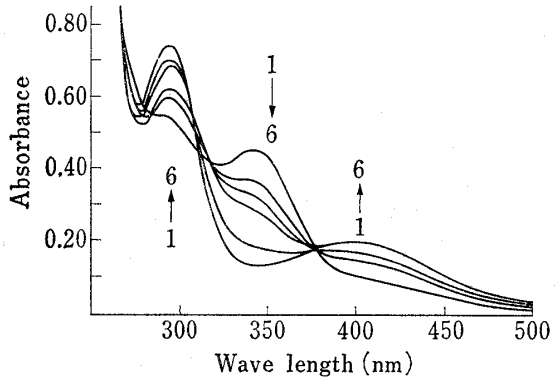

Fig. 1. UV Spectral Changes with Time by Reaction of *m*-NPSAL with β-CD

Time in hours after reaction was initiated; 1: 0.00, 2: 0.083, 3: 0.167, 4: 1.50, 5: 6.25, 6: 23.5. pH 11.3 carbonate buffer containing 22% acetonitrile and 25°, $[m\text{-NPSAL}]_0=1.00\times10^{-4}$ M and $[\beta\text{-CD}]_0=5.00\times10^{-3}$ M.

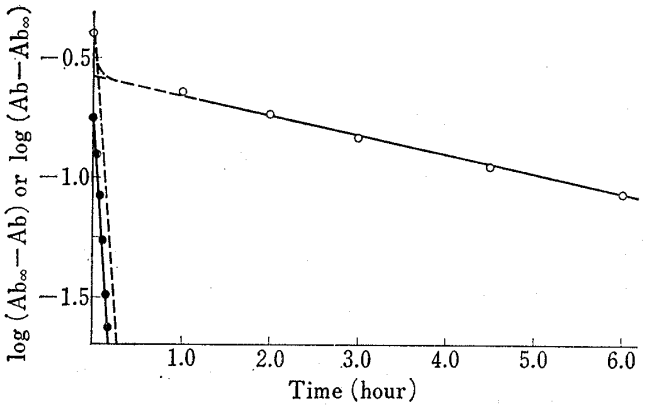

Fig. 2. First Order Plots for Reaction of *m*-NPSAL with β-CD

pH 12.6 carbonate buffer containing 22% acetonitrile and at 25°, $[m\text{-NPSAL}]_0=1.00\times10^{-4}$ M and $[\beta\text{-CD}]_0=1.00\times10^{-2}$ M. ●; monitored at 400 nm, ○; monitored at 340 nm.

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Results and Discussion

Aspect of Reactions of Phenyl Salicylates with CD

UV spectral changes with time for the reaction of *m*-NPSAL with β-CD are shown in Fig. 1. The reaction seemed to occur with two steps. The spectra of the fast reaction showed two isosbestic points at near 317 nm and 378 nm during the first one and half hour, after which the spectra beyond about 380 nm remained unchanged with time. The shape and intensity of the absorption spectrum from about 380 nm to 500 nm were identical to that of the separately prepared solution of *m*-NP and β-CD having the same molar concentrations as the initial concentrations of *m*-NPSAL and β-CD. After attainment to the constant absorbance at near 400 nm, the absorbances at around 340 nm still decreased and those at 290 nm increased with time. The slow UV changes showed the newly additional isosbestic point at near 304 nm. The final UV spectrum of the reaction solution agreed with that of the mixture of *m*-NP, salicylic acid (SAL) and β-CD, equivalent to the initial molar concentrations of *m*-NPSAL and β-CD.

Figure 2 shows the first order plots for the reaction of *m*-NPSAL with β-CD, based upon the appearance of *m*-NP monitored at 400 nm. Meantime, monitored at 340 nm, another good linear relationship between log ($Ab-Ab_\infty$) and time was also obtained after the initial fast changes of UV spectra, as shown in Fig. 2. At the other pH region, the similar plots to those in Fig. 2 were obtained. For the reaction of PHESAL with β-CD, the reaction also seemed to occur with two steps. The apparent rate of the slow step agreed with that in the reaction of *m*-NPSAL with β-CD.

VanEtten *et al.* also reported that in the reactions of *meta*-substituted phenyl benzoates with CD the acyl portion was transferred to a hydroxyl group of CD, forming a cyclodextrin benzoate which underwent hydrolysis *via* subsequent reaction at a rate independent of the nature of the phenolic group.[7] The UV spectral changes and the two fast and slow rates for the present study indicate that an intermediate, β-cyclodextrin salicylate (β-CDSAL), is formed in the reaction of *m*-NPSAL with β-CD and also in that of PHESAL with β-CD. The fast reaction at the initial stage was denoted as the acylation of CD and the second one as the deacylation of CDSAL.

Reaction Parameters for the Acylation of CD

Effects of CD concentrations on the apparent first order rate constants of *m*-NP appearances are shown in Fig. 3. The rates increased hyperbolically and appeared to reach the respective maximal value of k_{obs} with the concentrations of CD. By decreasing the concentration of acetonitrile in the buffer (2% acetonitrile), the rate was more accelerated with the concentration of β-CD. These results suggest that the reaction of *m*-NPSAL proceeds through the inclusion complex formation and acetonitrile used to solubilize the substrate seems to prevent the complex formation between *m*-NPSAL and CD.

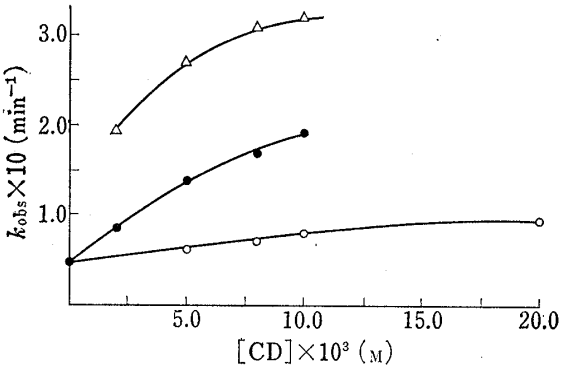

Fig. 3. Effect of CD Concentration on Apparent Rate Constant of *m*-NP Appearance at 25°

○; α-CD, pH 10.2 carbonate buffer containing 22% acetonitrile.
●; β-CD, pH 10.2 carbonate buffer containing 22% acetonitrile.
△; β-CD, pH 11.0 carbonate buffer containing 2% acetonitrile.
$[m\text{-NPSAL}]_0=1.00\times10^{-4}$ M.

According to all of the results mentioned above, the reaction scheme is generally shown as Chart 1 for the reaction of *m*-NPSAL with CD. In Chart 1, *m*-NPSAL·CD is the

$$\begin{array}{ccccc} m\text{-NPSAL} + \text{CD} & \overset{K_{diss}}{\rightleftarrows} & m\text{-NPSAL}\cdot\text{CD} & \xrightarrow{k_{cat}} & m\text{-NP} + \text{CDSAL} \\ \downarrow k_{un} & & & & \downarrow k_{deacyl} \\ m\text{-NP} + \text{SAL} & & & & \text{CD} + \text{SAL} \end{array}$$

Chart 1

Michaelis–Menten type complex between *m*-NPSAL and CD. CDSAL is cyclodextrin salicylate and k_{deacyl} is the first order rate constant of CDSAL hydrolysis. The first order rate constants for the uncatalyzed reaction and the *m*-NPSAL·CD reaction are expressed as k_{un} and k_{cat}, respectively. K_{diss} is the apparent dissociation constant of *m*-NPSAL·CD.

The values of k_{cat} and K_{diss} can be obtained by the use of the following equation (1) known as Lineweaver–Burk equation.[17]

$$\frac{1}{k_{obs}-k_{un}} = \frac{K_{diss}}{(k_{cat}-k_{un})[CD]_0} + \frac{1}{k_{cat}-k_{un}} \quad (1)$$

where k_{obs} is the apparent first order rate constant for the appearance of *m*-NP in the presence of CD and $[CD]_0$ is the initial concentration of CD. Figure 4 shows the plots of $1/(k_{obs}-k_{un})$ *versus* $1/[CD]_0$ for the data of Fig. 3. The K_{diss} value was obtained from the slope divided by the intercept, and the k_{cat} value was calculated from the values of the intercept and k_{un}. The rate and dissociation constants for the reactions of phenyl salicylates with CD are listed in Table I. The ratio of the k_{cat} value to the k_{un} value for the reaction of *m*-NPSAL with β-CD was larger than that for the reaction with α-CD. The value of K_{diss} for β-CD is smaller than that for α-CD. These may be ascribed to the difference in the diameters of the cavities between α-CD and β-CD.

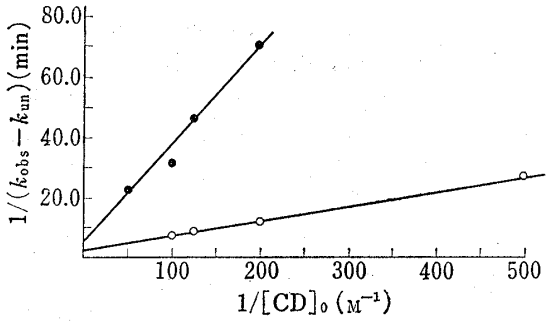

Fig. 4. Plots of $1/(k_{obs}-k_{un})$ *versus* $1/[CD]_0$ for Data of Fig. 3 in the Cases of 22% Acetonitrile

○; for α-CD, ●; for β-CD.

Table I. Rate and Dissociation Constants for Reaction of Phenyl Salicylates with CD in the Absence and Presence of CD[a]

Compounds		k_{un} (min^{-1})	k_{cat} (min^{-1})	K_{diss} (M)
m-NPSAL	α-CD	4.81×10^{-2}	2.48×10^{-1}	6.54×10^{-2}
	β-CD	4.81×10^{-2}	6.04×10^{-1}	2.78×10^{-2}
PHESAL	β-CD	4.13×10^{-3}	1.80×10^{-2}	2.32×10^{-2}

a) pH 10.2 carbonate buffer containing 22% acetonitrile and at 25°.

pH-Rate Profiles for the Acylation of Cyclodextrin

Figure 5 shows the pH–rate profiles for the appearances of *m*-NP in the presence and absence of 1.00×10^{-2} M CD. The apparent first order rate constants in the presence of CD were larger than those in the absence of CD. All the three profiles were of similar shapes. The pH–rate profiles showed that above pH about 11 the rates stayed constant and below pH around 9.5 the slopes of the profiles were approximately unity. Bender *et al.* reported that the hydrolysis of *p*-nitrophenyl 5-nitrosalicylate in the alkaline 34.4% dioxane solution proceeded by the reaction of water with the ionized ester, indicating the intramolecular general basic catalysis by the phenoxide ion.[13] Thus for the pH–rate profile for *m*-NPSAL

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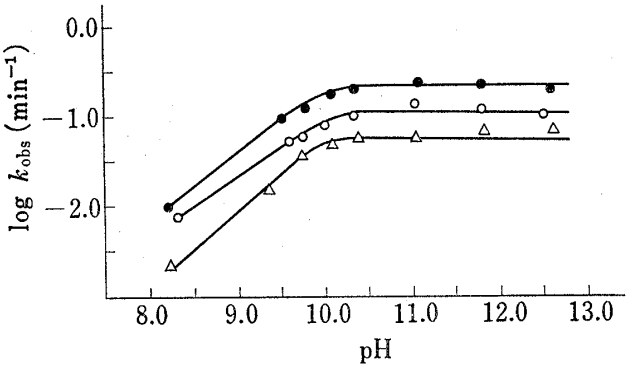

Fig. 5. The pH–Rate Profiles for Appearance of *m*-NP from *m*-NPSAL in the Presence and Absence of CD at 25°

△; uncatalyzed reaction of *m*-NPSAL.
○; in the presence of *α*-CD (1.00×10^{-2} M).
●; in the presence of *β*-CD (1.00×10^{-2} M).
$[m\text{-NPSAL}]_0=1.00\times10^{-4}$ M

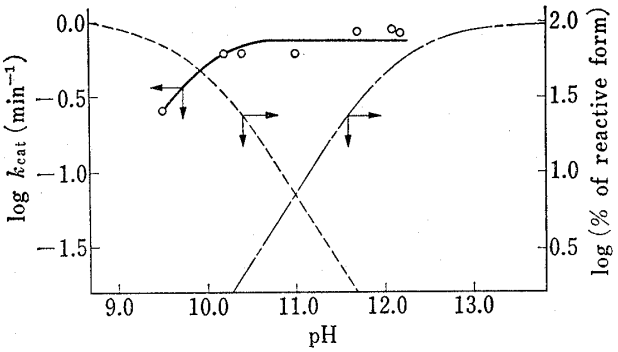

Fig. 6. The pH–Rate Profile for Acylation of *β*-CD and the log (% of reactive form) *versus* pH Curves for *m*-NPSAL and *β*-CD

——; pH–log k_{cat} profile.
----; theoretical curve for the proportion of *m*-NPSAL reactive form.
—·—; theoretical curve for the proportion of *β*-CD reactive form.

in the absence of CD (k_{un}), the similar explnation may be possible. The apparent pK_a value of *m*-NPSAL in the presence of 22% acetonitrile was estimated as 9.9 from this pH–rate profile.

On the other hand the pH–rate profiles in the presence of CD were complicated, since the apparent first order rate constants depended on the other parameters as expressed in equation (1). To survey the k_{cat} dependency on pH, the k_{cat} values were determined from the plots based on the equation (1). The plots of the log k_{cat} against pH are shown in Fig. 6. Again the k_{cat} values in the range of pH about 11 to 12 remained approximately unchanged and the values seemed to decrease below pH around 10. For the interpretation of this pH–rate profile there are two possibilities, in which one is the reaction of the non-ionized *m*-NPSAL with the ionized CD (alkoxide ion of CD) and the other that of the ionized *m*-NPSAL with the non-ionized CD. However, the former reaction should be predominant in this case, since the alkoxide ion of CD was involved for the acylation of CD.[7] The per cents of both reactive forms at each pH were calculated using the pK_a values of 9.9 and 12.1[7] for *m*-NPSAL and CD, respectively. The theoretical log (% of reactive form) *versus* pH curves were pictured in Fig. 6. In considering the pH–rate profile from the pH dependences of the reactive forms predicted by these theoretical curves, the k_{cat} values at around pH 12 may have to be slightly smaller. For the theoretical curve of CD, however, the pK_a value used was 12.1 which was estimated from kinetic data in 0.5% acetonitrile solution.[7] The kinetic results of this study was obtained in 22% acetonitrile solution, and then the pK_a value of CD in this condition might be shifted to the larger value. If the k_{cat} values were successively determined in more alkaline region, the values would decrease continuously with pH.

Table II. Comparisons of Three Rate Constants related to *β*-CDSAL Accumulation at Various pH

pH	k_{cat} (min^{-1})	k_{deacyl} (min^{-1})	k_{un} (min^{-1})	k_{cat}/k_{un}	k_{cat}/k_{deacyl}	k_{un}/k_{deacyl}
9.5	2.55×10^{-1}	1.50×10^{-4}	1.99×10^{-2}	1.28×10	1.70×10^{3}	1.33×10^{2}
10.2	6.04×10^{-1}	4.48×10^{-4}	4.81×10^{-2}	1.26×10	1.35×10^{3}	1.07×10^{2}
11.7	8.60×10^{-1}	2.43×10^{-3}	5.20×10^{-2}	1.65×10	3.54×10^{2}	2.14×10
12.6	—	3.10×10^{-3}	7.09×10^{-2}	—	—	2.29×10

Comparisons of the Rate Constants for the Reaction of *m*-NPSAL with β-CD

Table II lists the three rate constants related to the CDSAL accumulation on the process of the reaction of *m*-NPSAL with β-CD and the ratios of the each other. It is expected that the larger the values of k_{cat}/k_{un} and k_{cat}/k_{deacyl}, the more the accumulation occurs in the reaction. The values of k_{deacyl} were considerably small compared with those of k_{un} and k_{cat} and the ratios of about 130 and 1700, for example at pH 9.5, were observed, respectively. This supports the accumulation of β-CDSAL in the reaction mixture. It will be possible to isolate the intermediate, CDSAL, under the condition of pH nearby or slightly below the pK_a value of *m*-NPSAL.