Chem. Pharm. Bull. 29(1) 213—219 (1981)

Effects of Cyclodextrins on the Hydrolysis of Prostacyclin and Its Methyl Ester in Aqueous Solution[1]

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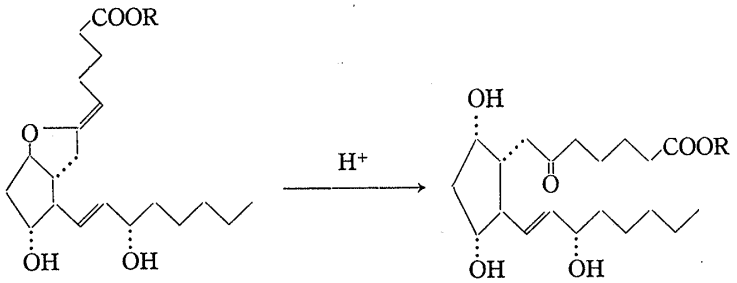
(Received July 30, 1980)

The rates of hydrolysis of prostacyclin (PGI_2) and its methyl ester (PGI_2Me) in aqueous solution were significantly retarded by α-, β-, and γ-cyclodextrins (α-, β-, and γ-CyDs), and showed characteristic saturation kinetics and competitive inhibition. The deceleration effects of CyDs on the hydrolysis of PGI_2Me were about 3 times larger than those on the hydrolysis of PGI_2. The importance of the spatial relationship between the host and guest molecules was reflected in the kinetically determined stability constant (K_c) for these inclusion complexations. To elucidate the deceleration mechanism of the CyDs, the effects of pH, solvent and temperature on the hydrolysis rate were studied. The protolytic dissociation of the terminal carboxylic acid moiety of PGI_2 was suppressed by the binding to CyDs, depending upon the magnitude of the K_c value. Thermodynamic activation parameters suggested that the deceleration mechanism of CyDs in the case of PGI_2 was somewhat different from that for PGI_2Me, which may be due to different modes of inclusion.

Keywords——prostacyclin; prostacyclin methyl ester; α-, β-, and γ-cyclodextrins; hydrolysis of prostacyclins; saturation kinetics; stability constant; activation parameter; hydrophobic interaction; deceleration mechanism

Prostacyclin (PGI_2)[2] is of interest as a potential therapeutic agent in the treatment of thrombosis because of its potent activities in inhibiting platelet aggregation and relaxing vascular smooth muscle.[3] However, PGI_2 undergoes an extremely facile hydrolysis of the vinyl ether group to yield 6-keto-$PGF_{1\alpha}$ in aqueous solution,[4] with loss of biological activities.[5] Some of the present authors have succeeded in improving the chemical stability of ONO-802,[6] a derivative of prostaglandin E_1, by the use of cyclodextrins (CyDs).[7] In these continuing investigations, the effects of α-, β-, and γ-CyDs (having different cavity sizes) on the hydrolysis of PGI_2 to 6-keto-$PGF_{1\alpha}$ were investigated in the hope of improving the chemical stability of PGI_2. Furthermore, the hydrolysis of PGI_2 methyl ester, PGI_2Me,[8] was also studied to gain insight into the mechanism of PGI_2 hydrolysis.



PGI_2 (R=H)
PGI_2Me (R=CH_3)

6-keto-$PGF_{1\alpha}$
6-keto-$PGF_{1\alpha}Me$

Chart 1

Experimental

Materials——PGI_2 sodium salt and PGI_2Me were gifts from Kaken Chemical Co., Ltd. and Ono Pharmaceutical Co., Ltd., respectively. α-, β-, and γ-CyDs were purchased from Nippon Shokuhin Kakō Co., Ltd.

and recrystallized twice from water. All other materials and solvents were of analytical reagent grade.

Kinetics——The hydrolysis rates were monitored spectrophotometrically by measuring the decrease in the absorbance of PGI_2 or PGI_2Me at 230 nm[4] where additives such as CyDs and EtOH had no significant effect on the molar absorptivities of prostacyclins ($\varepsilon_{PGI_2}=650$ and $\varepsilon_{PGI_2Me}=1750$ at 230 nm in pH 11.0 phosphate buffer). The reaction was initiated by addition of the stock solution of PGI_2 sodium salt in water or that of PGI_2Me in EtOH into a spectrophotometric cell containing phosphate buffer at constant temperature. The final concentrations of the prostacyclins and EtOH were 3.0×10^{-4} M and 1.0 v/v%, respectively. The hydrolysis followed exact first-order kinetics, and no appreciable side reactions such as intramolecular ketal formation[9] or ester hydrolysis (particularly for PGI_2Me) were observed under these experimental conditions.[10]

Results and Discussion

Effects of CyD Concentration

Figure 1 shows a typical example of the effects of α-, β-, and γ-CyD concentrations on the observed hydrolysis rate constant (k_{obs}) of PGI_2 to form 6-keto-$PGF_{1\alpha}$. In both PGI_2 and PGI_2Me systems, the reaction rates decreased hyperbolically with increasing CyD concentration, showing characteristic saturation kinetics.[11] The dependency of k_{obs} on the CyD concentration was quantitatively treated by Eq. (1)[11,12] to obtain the apparent stability constant (K_c) and rate constant (k_c) of the complex, on the basis of the following 1 : 1 complexation scheme (Chart 2), where k_o and $(CyD)_t$ are

$$\frac{(CyD)_t}{k_o-k_{obs}}=\frac{1}{k_o-k_c}\cdot(CyD)_t+\frac{1}{K_c\cdot(k_o-k_c)} \qquad \text{Eq. (1)}$$

$$\begin{array}{ccc} PGI_2 + CyD & \overset{K_c}{\rightleftarrows} & PGI_2\text{-}CyD \\ \downarrow k_o & & \downarrow k_c \\ 6\text{-keto-}PGF_{1\alpha} & & 6\text{-keto-}PGF_{1\alpha} + CyD \end{array}$$

Chart 2

the rate constant in the absence of CyDs and the total concentration of CyDs, respectively. Fignre 2 shows the plots of the data derived from Eq. (1), based on the data of Fig. 1. A linear relationship was also obtained for the PGI_2-Me–CyD systems, confirming 1 : 1 complexation

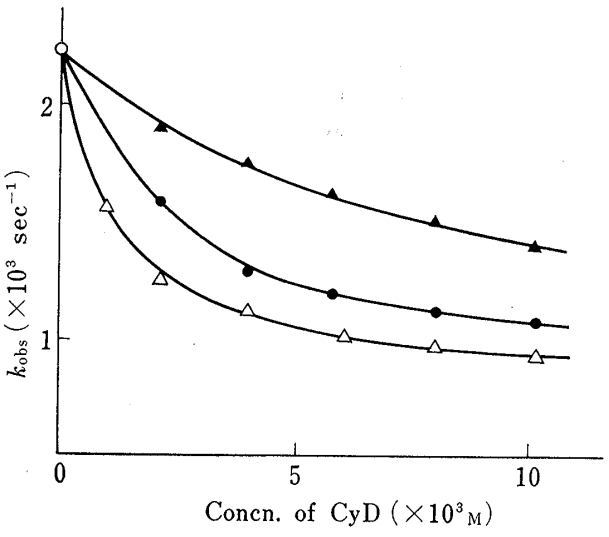


Fig. 1. Observed Rate Constants for the Hydrolysis of PGI_2 as a Function of CyD Concentration in Phosphate Buffer (pH 7.0, $\mu=0.2$) at 15°

○: PGI_2 alone,
●: PGI_2–α-CyD system,
△: PGI_2–β-CyD system,
▲: PGI_2–γ-CyD system.

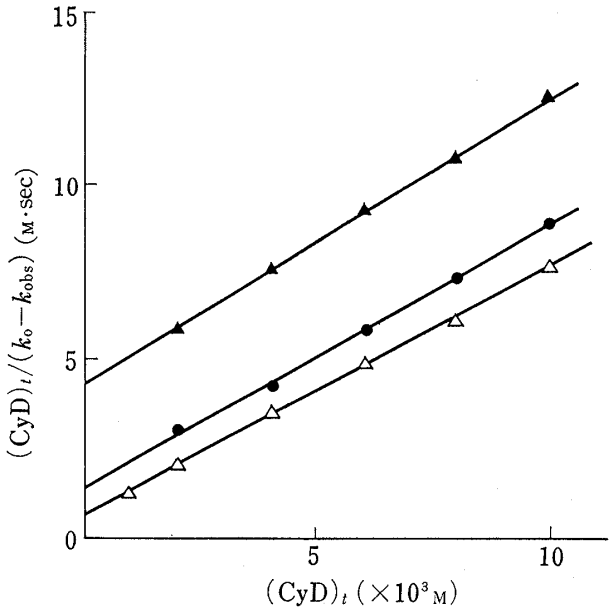


Fig. 2. Determination of K_c and k_c for PGI_2–CyD Complexes by Plotting Kinetic Data (Fig. 1) According to Eq. (1)

●: PGI_2–α-CyD system,
△: PGI_2–β-CyD system,
▲: PGI_2–γ-CyD system.

Table I. Rate Constants and Stability Constants[a] of PGI_2–CyD and PGI_2Me–CyD Systems

System	k_o ($\times 10^3$ sec^{-1})	k_c ($\times 10^4$ sec^{-1})	k_c/k_o	K_c (M^{-1})
PGI_2	2.27	—	—	—
PGI_2–α-CyD	—	8.33	0.368	461
PGI_2–β-CyD	—	7.61	0.336	902
PGI_2–γ-CyD	—	9.17	0.405	157
PGI_2Me	0.514	—	—	—
PGI_2Me–α-CyD	—	0.619	0.120	572
PGI_2Me–β-CyD	—	0.663	0.129	1100
PGI_2Me–γ-CyD	—	0.822	0.163	192

a) Accuracy of ±3%; kinetic conditions were the same as in Fig. 1.

(Chart 2). Table I summarizes the results on k_o, k_c, k_c/k_o, and K_c. The K_c values increase in the γ-, α-, and β-CyD complexes in that order in both the PGI_2 and PGI_2Me systems. In each CyD system, K_c for the PGI_2Me complex is larger than that for the PGI_2 complex. The above results indicate that the spatial relationship between the host and guest molecules, together with the hydrophobicity of the guest molecule, may play an important role in these complexations. On comparison with the k_c/k_o values, the deceleration effects of CyDs on the hydrolysis of PGI_2Me were found to be larger than those of PGI_2; a good correlation between k_c/k_o and K_c was found for each substrate system. This can be explained on the basis of the different geometries of the guest molecules within the CyD cavities, as will be described later.

Effects of Phosphate Buffer Concentration and pH

Figure 3 shows the dependences of k_{obs} for PGI_2 on total phosphate buffer concentration (pH 7.1 as an example) in the absence and in the presence of CyDs. No appreciable change in the linear dependence was observed even in the presence of CyDs, indicating that phosphate anions do not interfere with CyD complexation.[13]

Figure 4 shows the effects of pH on the hydrolysis rate constants (k) of PGI_2 and PGI_2Me extrapolated to zero buffer concentration. The pH-profiles of the PGI_2 systems were biphasic with a slope of −1 at low and high pH regions (pH<2 and pH>5.5, respectively), while for the PGI_2Me systems the linear dependence was retained over the pH range of 3—8. It is of interest to examine the protolytic dissociation behavior of the terminal carboxylic acid moiety of PGI_2 bound to CyDs, since the hydrolysis of PGI_2 is known to be subject to intramolecular carboxylate ion catalysis.[14] Thus, the biphasic pH-profiles in Fig. 4 were analyzed in terms of Eq. (2) to obtain the apparent protolytic dissociation constant (K_a) of PGI_2 on the basis of the following scheme

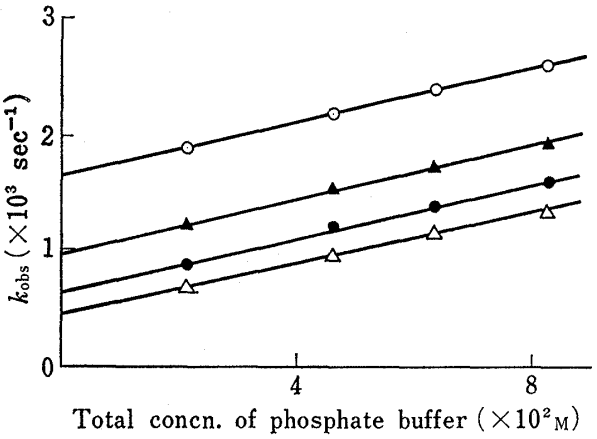


Fig. 3. Effects of Total Concentration of Phosphate Buffer (pH 7.1, $\mu=0.2$) on the Rate of Hydrolysis of PGI_2 in the Absence and in the Presence of CyDs (1.0×10^{-2} M) at 15°

○: PGI_2 alone, ●: PGI_2–α-CyD system,
△: PGI_2–β-CyD system, ▲: PGI_2–γ-CyD system.

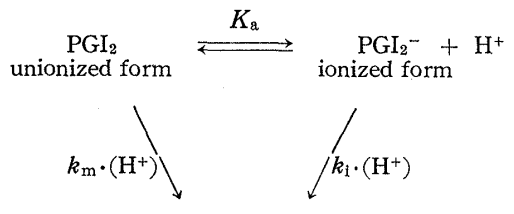


Chart 3

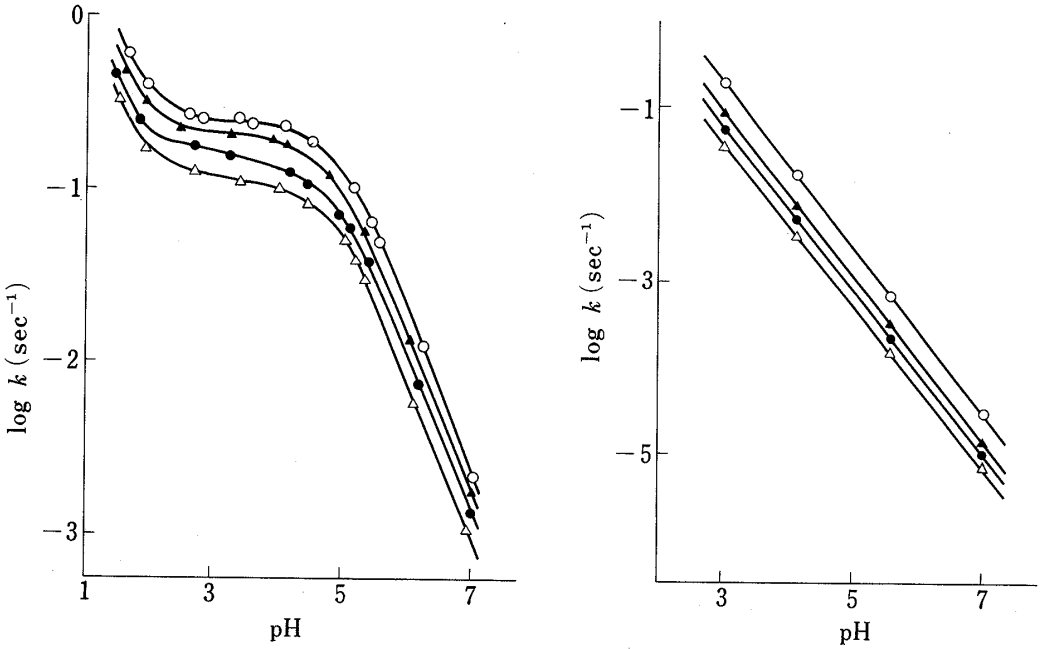


Fig. 4. pH-Profiles for the Hydrolysis of PGI_2 (left) and PGI_2Me (right) in the Absence and in the Presence of CyDs (5.0×10^{-3} M) at 15°

○: alone, ●: α-CyDsystem, △: β-CyD system, ▲: γ-CyD system.

Table II. Effects of CyDs[a] on the Apparent Protolytic Dissociation Constant of PGI_2 at 15°

System	pK_a	pK_a'[b]	ΔpK_a[c]
PGI_2	4.96	—	—
PGI_2–α-CyD	—	5.07	0.11
PGI_2–β-CyD	—	5.20	0.24
PGI_2–γ-CyD	—	4.98	0.02
in 30 v/v% EtOH	—	5.50	0.54

a) The concentration of CyDs was 5.0×10^{-3} M.
b) Apparent pK_a value in the presence of CyDs.
c) $\Delta pK_a = pK_a' - pK_a$; accuracy of ±0.07.

$$k = \frac{k_m\cdot(H^+)^2 + k_i\cdot K_a\cdot(H^+)}{(H^+)+K_a} \qquad \text{Eq. (2)}$$

(Chart 3), where k_m and k_i are the hydrolytic rate constants for unionized and ionized PGI_2 molecules, respectively, and (H^+) is hydrogen ion concentration. The refined pK_a values obtained by using a non-linear least-squares method[15] are summarized in Table II. The protolytic dissociation was found to be suppressed by the binding to CyDs, and the effect of β-CyD was larger than those of α- and γ-CyDs; this correlates well with the magnitudes of K_c. The PGI_2 molecule appears to be located within the hydrophobic environment of CyD, since the dissociation was also inhibited in a less polar solution containing 30 v/v% EtOH. Similar results have been obtained for $PGF_{2\alpha}$–CyD systems.[16] These results indicate that the deceleration of the hydrolysis of PGI_2 may be at least in part a result of the inhibition of intramolecular carboxylate ion catalysis due to the decrease in the acidity of the terminal carboxyl group.

Effects of Solvents

Figure 5 shows the effects of EtOH concentration on the hydrolysis rates of PGI_2 and PGI_2Me in the absence and presence of CyDs. Under these experimental conditions, the pH

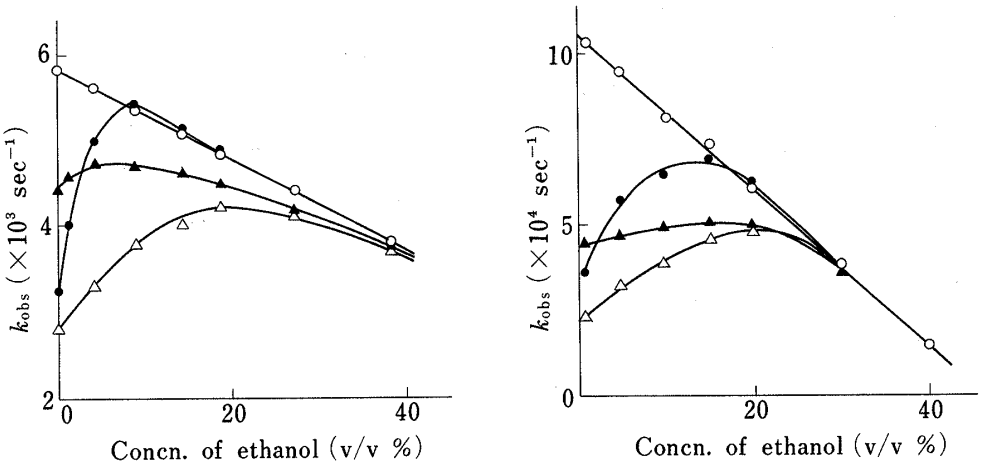


Fig. 5. Effects of Ethanol Concentration on the Rates of Hydrolysis of PGI_2 (left) and PGI_2Me (right) in the Absence and in the Presence of CyDs (1.0×10^{-2} M) in Phosphate Buffer (pH 6.8, $\mu=0.2$) at 25°

○: alone, ●: α-CyD system, △: β-CyD system, ▲: γ-CyD system.

and ionic strength were constant (pH=6.8, $\mu=0.2$), and no significant influence of EtOH on the pH of the medium was observed. In the absence of CyDs, linear relationships were obtained in both the PGI_2 and PGI_2Me systems (slopes: -5.5×10^{-5} and -2.3×10^{-5}, respectively), indicating a decrease of the rate as the polarity of the solution decreased.[17] In the presence of CyDs, however, k_{obs} increased gradually in the low EtOH concentration range, and then asymptotically approached the rate constant of the free substrates on further addition of EtOH. Similar results were obtained when dioxane was added to the reaction solution. These kinetic results can be ascribed to competitive inclusion of the solvents and guest molecules in the CyD cavities.[18] In fact, the rate change due to decomplexation of the PGI_2Me–CyD system was rather small compared to that of the PGI_2–CyD system, because of the larger stability constant of the former system (see Table I).

Effects of Temperature

The effects of temperature on the hydrolysis rates were investigated to gain further insight into the deceleration mechanism. Figure 6 shows Arrhenius plots of the hydrolysis rates of PGI_2 in the absence and presence of CyDs over a temperature range of 10—30°. Similarly, the Arrhenius relationship held well for PGI_2Me systems over the temperature range employed. Table III summarizes the thermodynamic activation parameters. It appears that the low hydrolytic reactivity of PGI_2Me compared to PGI_2 is due to an unfavorable activation entropy

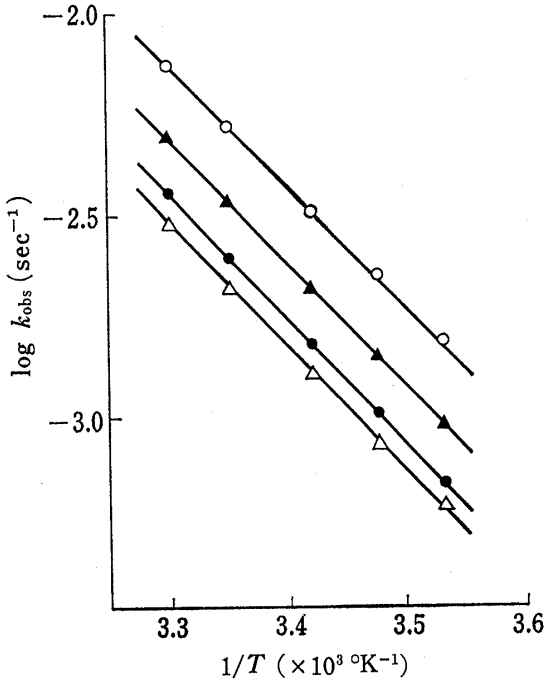


Fig. 6. Arrhenius Plots for the Hydrolysis of PGI_2 in the Absence and in the Presence of CyDs (1.0×10^{-2} M) in Phosphate Buffer (pH 7.2, $\mu=0.2$)

○: PGI_2 alone, ●: PGI_2–α-CyD system,
△: PGI_2–β-CyD system, ▲: PGI_2–γ-CyD system.

Table III. Thermodynamic Activation Parameters[a] for the Hydrolysis of PGI_2 and PGI_2Me in the Absence and in the Presence of CyDs[b] in Phosphate Buffer (pH 7.2, $\mu=0.2$)

System	$\Delta G^{\ddagger}_{298}$ (kcal/mol)	E_a (kcal/mol)	$\Delta S^{\ddagger}_{298}$ (e.u.)
PGI_2	20.6	14.0	−24.1
PGI_2–α-CyD	21.0	14.1	−25.3
PGI_2–β-CyD	21.1	14.1	−25.5
PGI_2–γ-CyD	20.8	14.0	−24.9
in 30 v/v% EtOH	20.8	14.0	−24.9
PGI_2Me	21.5	11.8	−34.7
PGI_2Me–α-CyD	22.2	16.0	−22.9
PGI_2Me–β-CyD	22.4	13.9	−30.4
PGI_2Me–γ-CyD	22.0	13.7	−29.9
in 30 v/v% EtOH	22.5	10.9	−40.9

a) Accuracy: E_a, ±0.1 kcal/mol; $\Delta S^{\ddagger}_{298}$, ±0.3 e.u.
b) The concentration of CyDs was 1.0×10^{-2} M.

term (ΔS^*). In a rate-determining transition state of the hydrolysis of vinyl ethers, the substrate is known to exist as a cationic species[19] (see "Appendix"). The positive charge being generated on the PGI_2 molecule will be dispersed by the terminal carboxylate anion through intramolecular electrostatic interaction,[14] while the charge on the PGI_2Me molecule may be localized because of the lack of such an effective interaction. Therefore, the large negative ΔS^* observed for PGI_2Me probably reflects the orientation requirements of many water molecules around the positive charge in the transition state. The entropy change of the freezing of water was reported to be −5.5 e.u.[17] If the entropy values are simply interpreted as reflecting hydration, at least two additional water molecules seem to be involved in the solvation of PGI_2Me. These considerations are supported by the following results. (1) The hydrolysis rate of PGI_2Me was significantly influenced by the solvent polarity, as shown in Fig. 5. (2) In the case of PGI_2Me, a large negative contribution of ΔS^* was observed in 30 v/v% EtOH solution, as shown in Table III, indicating that some additional hydration and/or solvation may be necessary to form the cationic transition species in less polar solutions.

The different deceleration effects of CyDs on the hydrolysis of PGI_2 and PGI_2Me were also apparent in their thermodynamic activation parameters. In the presence of CyDs or EtOH, the rate changes of PGI_2 are largely controlled by ΔS^*, indicating that the PGI_2 molecule is located in a hydrophobic environment that prevents its hydrolysis. On the other hand, the contribution of E_a to ΔG^* is large in PGI_2Me–CyD systems, with a relatively small contribution of ΔS^*. Thus, it is reasonable to assume that the rate decelerations in PGI_2Me–CyD systems, particularly in the PGI_2Me–α-CyD system (see also k_c/k_o value in Table I), result from the blocking of solvation of the positive charge in the transition state[20] through inclusion of the active site of the PGI_2Me molecule, although the exact nature of this interaction was not determined. In the case of PGI_2–CyD systems, portions of the PGI_2 molecule other than the active site can be included within the cavity of CyD, and this may also result in the deceleration of PGI_2 hydrolysis by localizing the positive charge in the transition state.

Appendix

As shown in Chart 4, the hydrolysis of vinyl ethers[20] occurs through a rate-determining electrophilic addition of H^+ to a carbon-carbon double bond to form an alkoxycarbonium ion which is stabilized by resonance with an oxonium ion. This cationic intermediate is known to react rapidly with water to give a hemiketal intermediate, which subsequently decomposes to carbonyl and alcohol groups.

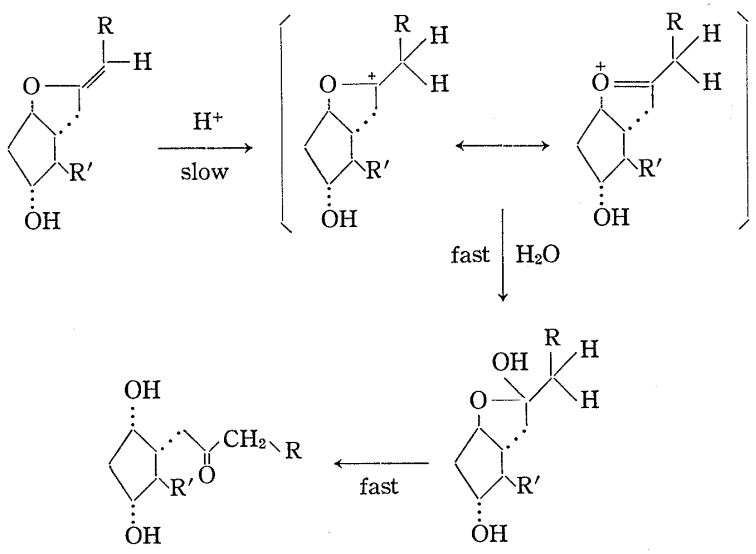


Chart 4

Acknowledgement This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged. The authors wish to express their gratitude to Kaken Chemical Co., Ltd. for the supply of PGI_2 sodium salt and Ono Pharmaceutical Co., Ltd. for the supply of PGI_2Me.

References and Notes

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