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Cyclodextrin Inclusion Catalysis in the Isomerization of Prostaglandin A_1

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Base-catalyzed isomerization of prostaglandin A_1 (PGA_1) to prostaglandin B_1 (PGB_1) was significantly accelerated by α- and β-cyclodextrins (α-CyD, β-CyD), where catalytic effect of β-CyD was larger than that of α-CyD. Stability constants and rate constants of PGA_1-cyclodextrin (CyD) complexes were kinetically determined on the base of 1:1 inclusion complex formation. In order to elucidate the catalytic mechanism of CyDs, effects of variables such as pH, ionic strength, cationic and anionic salts, and solvents on the isomerization rate were studied in the absence and in the presence of CyDs. Furthermore, activation parameters for CyD-catalyzed isomerization were determined. The results indicated that the catalytic effect of CyDs was mainly ascribable to conformational changes of PGA_1 molecule within the cavity of CyDs and hydrophobic interaction appeared to be predominant as a binding force.

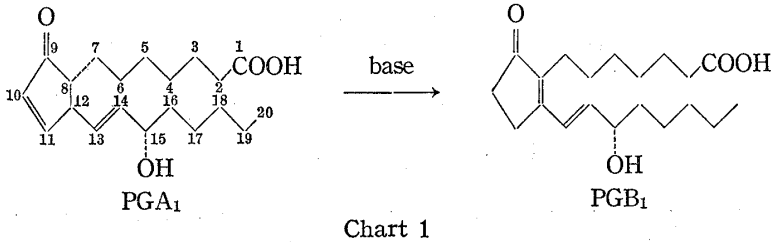
Keywords——prostaglandin A_1; prostaglandin B_1; α- and β-cyclodextrins; isomerization rate of prostaglandin A_1; cyclodextrin inclusion catalysis; stability constant; activation parameter; hydrophobic interaction; conformational change; acceleration mechanism

Prostaglandins are widely distributed in body and have a variety of physiological and pharmacological activities. Since subtle changes in structures of prostaglandins cause quite different activities, it has been suggested that stereospecific interaction between prostaglandins and hypothetical receptor sites is important for appearance of their activities.[2] From this standpoint, conformational analyses on stable conformers of prostaglandins have been investigated by several authors.[3,4] However, reactive conformers rather than stable forms should be considered in dynamic systems such as enzyme-catalyzed reactions. At present time little is known about reactive conformers of prostaglandins.

Cyclodextrins (CyDs) are known to form inclusion complexes with many kinds of molecules,[5,6] and utilized as enzyme models since CyD-catalyzed reactions exhibit many of kinetic features shown by enzyme reactions, *i.e.*, catalyst-substrate complex formation, competitive inhibition, saturation, and stereospecific catalysis.[7,8] We recently reported that some naturally occurring prostaglandins form inclusion complexes with α- and β-cyclodextrins (α-CyD and β-CyD) in aqueous solution.[9] In this paper we report that α-CyD and β-CyD, by virtue of their ability to fix prostaglandin A_1 (PGA_1) to reactive conformer,

1) Location: *5-1, Oe-honmachi, Kumamoto 862, Japan.*
2) S. Bergström, L.A. Carlson, and J.R. Weeks, *Pharmacol. Rev.*, **20**, 1 (1968).
3) I. Rabinowitz, P. Ramwell, and P. Davison, *Nature New Biol.*, **233**, 88 (1971).
4) A. Murakami and Y. Akahori, *Chem. Pharm. Bull.* (Tokyo), **22**, 1133 (1974); *idem, ibid.*, **25**, 2870 (1977).
5) J. Cohen and J.L. Lach, *J. Pharm. Sci.*, **52**, 132 (1963).
6) W. Saenger, "Environmental Effects on Molecular Structures and Properties," ed. by B. Pullman, D. Reidel Publishing Company, Dordrecht-Holland, 1976, p. 265.
7) D.W. Griffiths and M.L. Bender, *Advan. Catal.*, **23**, 209 (1973).
8) M.L. Bender and M. Komiyama, "Bioorganic Chemistry," Vol. 1, ed. by E.E. van Tamelen, Academic Press, New York, 1977, p. 19.
9) *a*) K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, *J. Pharm. Sci.*, **66**, 706 (1977); *b*) K. Uekama, F. Hirayama, S. Yamasaki, M. Otagiri, and K. Ikeda, *Chem. Lett.*, **1977**, 1389; *c*) K. Uekama, F. Hirayama, and M. Daiguji, *ibid.*, **1978**, 327; *d*) K. Uekama, F. Hirayama, and T. Irie, *ibid.*, **1978**, 661; *e*) K. Uekama and F. Hirayama, *Chem. Pharm. Bull.* (Tokyo), **26**, 1195 (1978).

enhanced the base-catalyzed isomerization of PGA_1 to give prostaglandin B_1 (PGB_1), which consequently elicites the loss of biological activity.[10,11] To gain insight into the acceleration mechanism, effects of pH, temperature, solvents, and salts on isomerization rate of PGA_1 in the absence and in the presence of CyDs were investigated.



PGA_1 PGB_1

Chart 1

Experimental

Materials——PGA_1 was donated by Ono Pharmaceutical Co., Ltd. α- and β-CyDs were the gift of Teijin Ltd. and recrystallized from water. All other materials and solvents were of analytical reagent grade.

Kinetics——The isomerization of PGA_1 in the absence and in the presence of CyDs was followed spectrophotometrically by measuring the appearance of PGB_1 at 284 nm, as reported previously.[9c] The reaction was initiated by addition of a stock solution of PGA_1 in EtOH to phosphate buffer (constant pH and ionic strength) at generally 60°, where the final concentrations of PGA_1 and EtOH were 3.8×10^{-5} M and 3.2 v/v %, respectively. In these experimental conditions, no appreciable side reactions[12] such as formations of 8-iso-PGA_1 and 15-epi-PGA_1 were observed.[13]

Results and Discussion

Effects of Cyclodextrin Concentrations

Treatment of PGA_1 with base results in internal rearrangement of the double bond ($\Delta^{10,11}$) to form thermodynamically stable PGB_1 containing a dienone chromophore (see Appendix). This isomerization reaction is known to exhibit a first-order dependency on PGA_1 concentration.[11] In the present study, effects of α- and β-CyDs having different cavity size on isomerization of PGA_1 was investigated in phosphate buffer (pH 11.9). High pH and temperature of 60° were chosen to accelerate the reaction rate because the measurement was kinetically convenient.

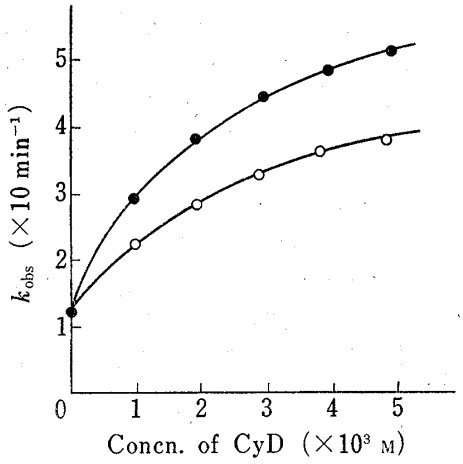


Fig. 1. Observed Rate Constants for the Isomerization of PGA_1 as a Function of CyD Concentration in Phosphate Buffer (pH 11.9, $\mu = 0.2$) at 60°

○: PGA_1—α-CyD system,
●: PGA_1—β-CyD system.

Figure 1 shows the effects of α- and β-CyDs on the observed rate constant (k_{obs}), where both CyDs significantly enhanced the rate of isomerization with non-linear fashion. Since PGA_1 forms 1:1 inclusion complexes with CyDs in aqueous solution,[9] the depen-

$$\begin{array}{ccc} PGA_1 + CyDs & \overset{K_c}{\rightleftarrows} & PGA_1\text{-}CyDs \\ \downarrow k_o & & \downarrow k_c \\ PGB_1 & & PGB_1 + CyDs \end{array}$$

Chart 2

10) N.H. Andersen, *J. Lipid Res.*, **10**, 320 (1969).
11) D.C. Monkhaus, L. VanCampen, and A.J. Aguiar, *J. Pharm. Sci.*, **62**, 576 (1973).
12) R.G. Stehle and T.O. Oesterling, *J. Pharm. Sci.*, **66**, 1590 (1977).
13) Liquid chromatography was employed to check the impurity and by-products in reaction solution, according to the method described previously [K. Uekama, F. Hirayama, Y. Yamada, K. Ikeda, and K. Inaba, *J. Pharm. Sci.*, in press].

dency of k_{obs} on CyD concentration was quantitatively treated by Eq. (1)[14,15] to yield stability constant (K_c) and rate constant (k_c) of the complex, based on Chart 2, where k_o and $(CyD)_t$ are rate constant in the absence of CyDs and total concentration of CyD, respectively. As

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

shown in Fig. 2, the plots according to Eq. (1) were linear, verifying 1:1 complexation (Chart 2). Table I summarizes the results on k_o, k_c, the ratio of k_c/k_o, and K_c. In the

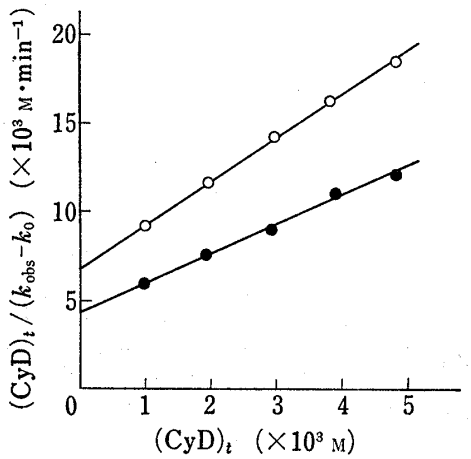


Fig. 2. Determination of K_c and k_c for PGA_1–CyD Complexes from Kinetic Data (Fig. 1) according to Eq. (1)

○: PGA_1—α-CyD system,
●: PGA_1—β-CyD system.

Table I. Rate Constants and Stability Constants[a] of PGA_1–CyD Systems

System	k_o (min^{-1})	k_c (min^{-1})	k_c/k_o	K_c (M^{-1})
PGA_1	0.124	—	—	—
PGA_1–α-CyD	—	0.567	4.57	320
PGA_1–β-CyD	—	0.770	6.21	360

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

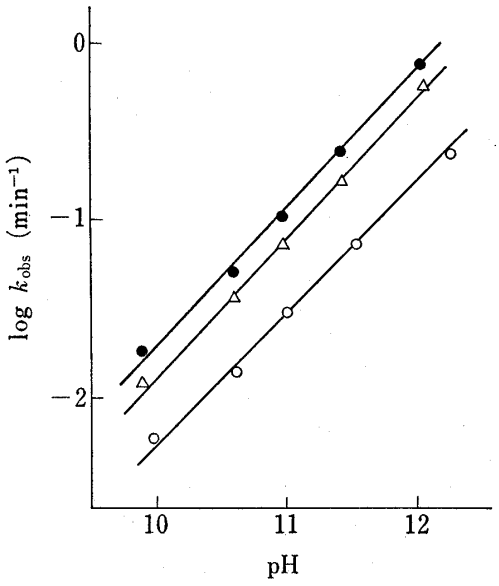


Fig. 3. pH Profiles for the Isomerization of PGA_1 in the Absence and in the Presence of CyDs (5×10^{-3} M) at 60°

○: PGA_1 alone, △: PGA_1+α-CyD,
●: PGA_1+β-CyD.

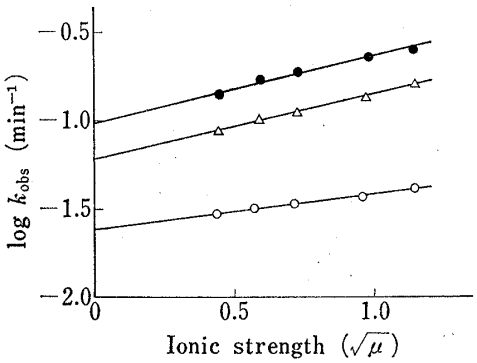


Fig. 4. Effect of Ionic Strength on Rate of Isomerization of PGA_1 in the Absence and in the Presence of CyDs (5×10^{-3} M) in Phosphate Buffer (pH 11.0) at 60°

○: PGA_1 alone, △: PGA_1+α-CyD,
●: PGA_1+β-CyD.

14) R.L. VanEtten, J.F. Sebastian, G.A. Clowes, and M.L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
15) S. Tanaka, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 2825 (1976).

comparison with α-CyD, PGA_1 complexed more strongly with β-CyD and the resultant complex is more labile to isomerization reaction. Carbon 13 nuclear magnetic resonance (NMR) study suggested[9e] that α-CyD interacts preferably with ω-side chain (C_{13}—C_{20}) of the prostaglandins because of the smaller cavity. On the other hand, larger β-CyD cavity is capable to include both α-(C_1—C_7) and ω-side chains, which may result in rigid complex formation with larger stability constant. Thus, this different mode of binding between α- and β-CyDs may affect greatly the conformation of PGA_1 side chains in reactant state, as mentioned later.

Effects of pH, Ionic Strength, and Temperature

Above results suggested that PGA_1 indeed forms inclusion complex with CyDs, which may consequently enhance the rate of isomerization. Then, to gain insight into the acceleration mechanism effects of pH, ionic strength, and temperature on isomerization rate were investigated. Figure 3 shows the pH–log rate profiles in the absence and in the presence of CyDs over the pH range of 10.0—12.2. It was found that slopes of the pH profiles for PGA_1 and its CyD complexes were identical (slope=0.75) even at high pH. This indicates that any alkoxide ion effect of CyDs[16] on this isomerization reaction was not operative. Figure 4 shows effects of ionic strength on k_{obs} in the absence and in the presence of CyDs at higher buffer concentration. In the absence of CyDs, only a small effect was noted presumably due to overriding phosphate buffer catalysis. In the presence of CyDs, however, slope in Fig. 4 became greater (slope: without CyDs=0.19, with α-CyD=0.31, and with β-CyD=0.32). This indicates that primary salt effect[17] upon the reaction between negative charge developed in PGA_1 and hydroxide ion was promoted by the formation of inclusion complexation.[18]

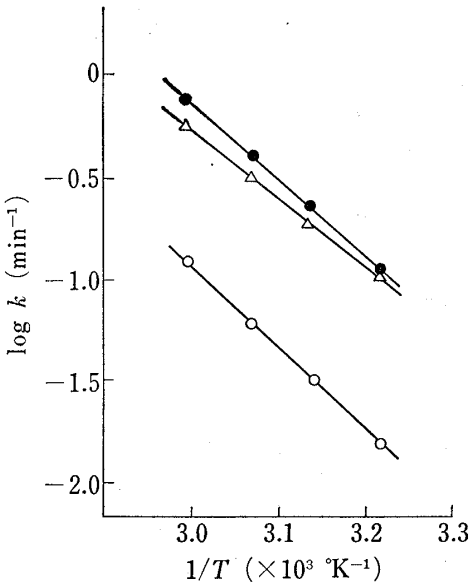


Fig. 5. Arrhenius Plots for k_o and k_c at pH 11.9 and μ=0.2

○: PGA_1 alone, △: PGA_1–α-CyD, ●: PGA_1–β-CyD.

Table II. Thermodynamic Activation Parameters[a] of PGA_1–CyD Systems

System	ΔG^*_{333} (kcal/mol)	E_a (kcal/mol)	ΔS^*_{333} (e.u.)
PGA_1	23.6	18.1	−18.7
PGA_1–α-CyD	22.6	15.3	−24.1
PGA_1–β-CyD	22.4	17.4	−17.1

a) Kinetic conditions were the same as in Fg. 4. Accuracy of ±3%.

Figure 5 shows Arrhenius plots[19] for k_o and k_c in the temperature range of 35°—60°. The thermodynamic activation parameters calculated from the linear plots are listed in Table II. It is noted that the rate accelerations induced by both α- and β-CyDs appeared to be somewhat different thermodynamically each other, which is reflected especially in entropy of activation (ΔS^*). Although the difference in entropy can be generally explained by orientation requirements of solvent molecules around the reaction center, conformational

16) pK_a for alkoxyl group of CyDs is reported to be 12.2 [T.-F. Chin, P.-H. Chung, and J.L. Lach, *J. Pharm. Sci.*, **57**, 44 (1968)].
17) A.A. Frost and R.G. Pearson, "Kinetics and Mechanism," 2nd ed., Wiley, New York, N.W., 1961, p. 150.
18) Y. Matsui and K. Mochida, *Bull. Chem. Soc. Jpn.*, **51**, 673 (1978).
19) No experiments have been conducted to extrapolate the isomerization rate to zero buffer concentration.

effects in PGA_1 molecule seem to be predominant in the present system. Prostaglandins are known to exist in many conformational isomers,[4] since they are very flexible systems. A most stable form of the five-membered ring is half-chair conformation puckered at C_8 or C_{12}, which allows a maximum dispersion forces of attraction between α- and ω-side chains.[3] Thus, complexation of PGA_1 with CyDs is expected to cause a great conformational changes. In other words, inclusion of PGA_1 side chains within the cavity of CyDs would provide favorable conformation to allow the introduction of negative charge in cyclopentanone ring. The larger negative value in ΔS^* obtained for α-CyD system may reflect a significant requirement in molecular orientation, because α-CyD is assumed to include only one of side chain in PGA_1.[9e] The larger acceleration effect of β-CyD may be derived from the favorable change in ΔS^*. Since interaction of whole PGA_1 molecule with β-CyD[9e] would cause the association of each side chain more proximately and the resultant complex may contain a lower absolute entropy in reactant state than PGA_1 itself. Similar explanation has been made to differentiate the reactivities between PGA_1 and PGA_2.[11]

Effects of Salts and Solvents

To determine the binding forces involving in complexation of PGA_1 with CyDs, effects of salts and solvents on isomerization rate were conducted. Table III summarizes the salts

Table III. Effects of Inorganic Salts on Rate of Isomerization of PGA_1 in the Absence and in the Presence of CyDs[a] at 60°

Ionic species	Rate constant ($\times 10^2$ min^{-1})		
	in the absence of CyDs	in the presence of CyDs	
		α-CyD	β-CyD
Cation[b]			
Li^+	1.28	4.51	7.06
Na^+	1.24	4.32	5.36
K^+	1.21	3.84	5.27
Rb^+	1.06	3.77	5.19
Cs^+	1.10	3.50	5.30
Anion[c]			
F^-	2.50	7.43	11.8
Cl^-	2.45	7.01	12.0
Br^-	2.33	6.87	11.7
I^-	2.20	4.94	9.86
ClO_3^-	2.18	5.53	9.89
BrO_3^-	2.20	5.86	9.91
IO_3^-	2.27	6.94	11.4
NO_3^-	2.23	6.88	11.5

a) Concentration of CyDs was 5×10^{-3} M.
b) Ionic strength and pH were adjusted to 0.5 and 10.4 with its chloride salt and 0.1 M NaOH–0.1 M HCl, respectively.
c) Ionic strength and pH were adjusted to 0.5 and 10.8 with its potassium salt and 0.1 M NaOH–0.1 M H_3PO_4, respectively.

effects on k_{obs} in the absence and in the presence of CyDs. The greater the water structure-forming tendency in both of cationic ($Li^+ > Na^+ > K^+ > Cs^+$) and anionic ($F^- > Cl^- > Br^- > I^-$) ions,[20] the larger the rate enhancement was generally noted. This indicates that hydrophobic interaction may play an important role in the association of PGA_1 with CyDs. In the case of bulky ion systems such as KI, $KClO_3$, and $KBrO_3$, however, isomerization rate

20) W.P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, Inc., 1969, p. 389.

was significantly inhibited, particularly for α-CyD system, indicating a competition between salts and guest molecule for CyD inclusion.[21]

Figure 6 shows the effect of ethanol concentration on isomerization rate. In the absence of CyDs, a linear negative slope was obtained, indicating a decreasing rate as the dielectric constant of the solvent decreases.[22] In the presence of CyDs, however, k_{obs} decreased rapidly with increasing ethanol concentration. Similar results were obtained when hydroxylic or aprotic solvents such as 2-propanol, acetonitrile, dimethyl sulfoxide, and N,N-dimethyl formamide were added to reaction solution. The rapid decrease of k_{obs} in CyD systems may be ascribable to competitive inclusion between solvent and guest molecule toward CyD cavity.[15,23]

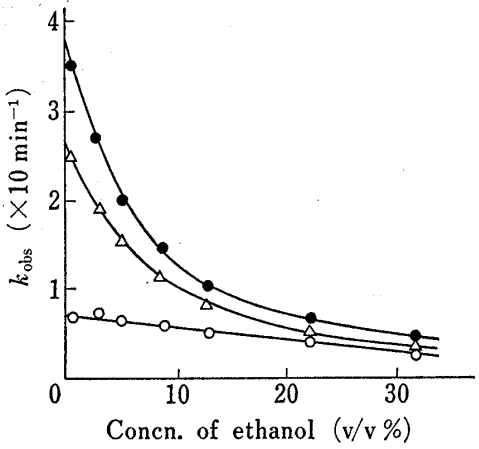


Fig. 6. Effect of Ethanol Concentration on Rate of Isomerization of PGA_1 in the Absence and in the Presence of CyDs (5×10^{-3} M) in Phosphate Buffer (pH 11.4, $\mu=0.2$) at 60°

○: PGA_1 alone, △: PGA_1+α-CyD, ●: PGA_1+β-CyD.

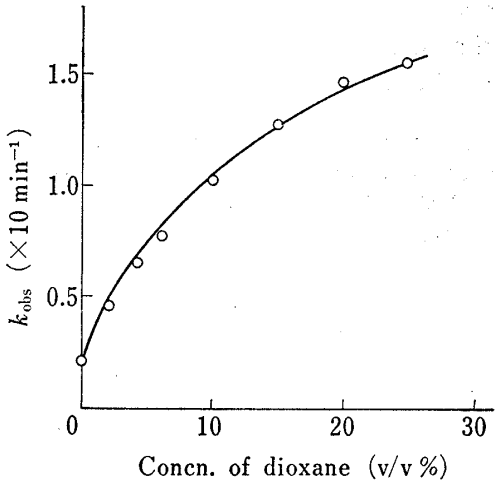


Fig. 7. Effect of Dioxane Concentration on Rate of Isomerization of PGA_1 in Phosphate Buffer (pH 10.8, $\mu=0.2$) at 60°

It is interesting to note that when dioxane was added to PGA_1 solution, significant rate acceleration was observed, showing a feature of CyD-catalyzed isomerization, as shown in Fig. 7. Bender *et al.*[14] reported that the ultraviolet (UV) spectrum of *p-t*-butylphenol bound to ether-like CyD cavity resembles closely to that dissolved in dioxane. We recently reported that nonionic micellar system of Brij-35 which also provides both hydrophobic and ether-like fields accelerates the isomerization of PGA_1.[24] Above results suggest that hydrophobic and/or ether-like environment of CyD cavity is responsible for conformational change of PGA_1 molecule to exert the rate acceleration.

Appendix

The isomerization reaction of PGA_1 to PGB_1 in alkaline pH region is assumed to proceed through prostaglandin C_1 (PGC_1)[25] rather than direct conversion, as shown in Chart 3. In this reaction pathway, the step 1, proton abstraction at C_{12}, appeared to be rate-determining

21) F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967); H. Schlenk and D.M. Sand, *ibid.*, **83**, 2312 (1961); E.A. Lewis and L.D. Hansen, *J. Chem. Soc. Perkin II*, **1973**, 2081.
22) G. Scatchard, *Chem. Rev.*, **10**, 229 (1932).
23) M. Otagiri, J.H. Perrin, K. Uekama, K. Ikeda, and K. Takeo, *Pharm. Acta Helv.*, **51**, 343 (1976).
24) K. Uekama, F. Hirayama, and S. Yamasaki, *Bull. Chem. Soc. Jpn.*, **51**, 1229 (1978).
25) H. Polet and L. Levine, *J. Biol. Chem.*, **250**, 351 (1975).

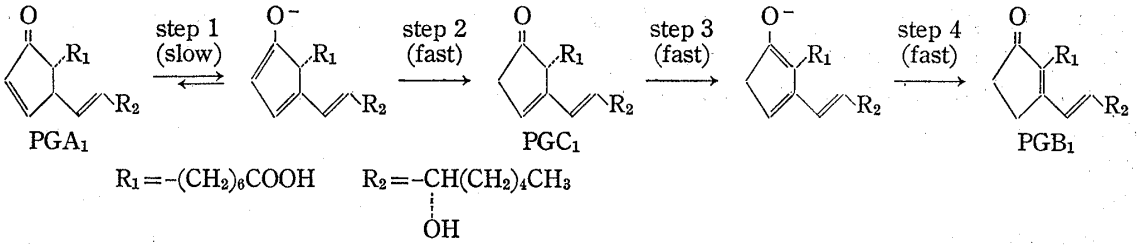


Chart 3

because of the following reasons: (1) No significant UV spectral change due to the appearance of intermediate PGC_1 (λ_{max}=235 nm) was detectable in the present kinetic runs, indicating that the steps 3 and 4 after PGC_1 formation may be rapid proceeding ones. (2) No appreciable solvent isotope effect[26] on the isomerization reaction was observable in the absence and in the presence of CyDs. This suggests that step 2, protonation of trienolate, is not rate-determining step.

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26) The solvent isotope effect was conducted at 60° (pH 10.6, ionic strength 0.5). The k_{H_2O}/k_{D_2O} value was found to be 1.1—1.2 in the absence and in the presence of CyDs (5.0×10^{-3} M). pD was estimated using the equation of pD=pH meter reading+0.4 [P.K. Glasoe and F.A. Long, *J. Phys. Chem.*, **64**, 188 (1960)].