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Studies on the Kinetics and Mechanism of Drug Degradation. I. Kinetics and Mechanism of Degradation of Chlorphenesin Carbamate in Strongly Alkaline Aqueous Solutions[1)]

MATSUJI HARA,* HIDEHUMI HAYASHI, TSUGUCHIKA YOSHIDA, and HIROSHI MURAYAMA

Research Laboratory, Taisho Pharmaceutical Co., Ltd., 1–403 Yoshino-cho, Omiya, Saitama 330, Japan

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The kinetics and mechanism of the degradation of chlorphenesin carbamate (α-CPC) in strongly alkaline aqueous solutions were studied at 0 to 20 °C. The degradation did not obey pseudo-first order kinetics, but was shown to involve a complex reaction accompanied by consecutive, parallel and reverse reactions. The rate law of degradation could be approximated in terms of specific base-catalyzed hydrolysis, that is $k = k_{OH} a_{OH}$, from the rate constant–pH profile. The Arrhenius plots of the degradation were approximately linear. Chlorphenesin-2-carbamate (β-CPC) and chlorphenesin (CP) were detected as alkaline degradation products of α-CPC.

The mechanism proposed for the degradation of α-CPC involves migration of the carbamoyl group to the adjacent OH group and removal of the carbamoyl group in the presence of hydroxide ions.

Keywords——chlorphenesin carbamate; skeletal muscle relaxant; kinetics; stability; isomerization; hydrolysis; strongly alkaline aqueous solution

3-(*p*-Chlorophenoxy)-1,2-propanediol-1-carbamate (α-CPC) (Fig. 1) is an orally active agent effective in the treatment of skeletal muscle trauma and inflammation.[2,3)] The pharmacology,[4)] metabolism,[5)] pharmacokinetics[6)] and analytical characterization[7–10)] of this drug have already been reported, but no details of its degradation kinetics or mechanism have yet appeared. The elucidation of the degradation kinetics and mechanism is important in relation to toxicological studies and the planning of formulation. Therefore, in this study, the degradation kinetics and mechanism in a strongly alkaline environment were investigated.

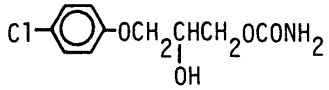

Fig. 1. Chemical Structure of α-CPC

Experimental

Materials——α-CPC, β-CPC (the isomer of α-CPC) and chlorphenesin (CP) (the degradation product of α-CPC) were kindly supplied by the manufacturer (Upjohn Company, U.S.A.) and used without further purification. All other chemicals used were of reagent grade.

Alkaline Solution——Alkaline solutions of 0.01, 0.02 and 0.1 N sodium hydroxide were prepared. These solutions were standardized with sulfamic acid (standard reagent) according to JP IX before use.

Kinetics of the Degradation of α-CPC in Strongly Alkaline Solutions——About 0.25 g of α-CPC was dissolved in 500 ml of water and this solution was used as the stock solution. A 100 ml aliquot of this stock solution was added accurately to 100 ml of alkaline solution pre-equilibrated to the desired reaction temperature (0 °C for studying the effect of alkali concentration and 10 and 20 °C for the measurement of activation energies). Final concentration of α-CPC was 1 mM.

The residual amount of α-CPC and the amounts of the degradation products formed in each reaction solution were determined periodically by high-performance liquid chromatography (HPLC). For the purpose of understand-

ing the degradation mechanism of α-CPC, the degradation kinetics of β-CPC were simultaneously investigated at a concentration of 0.5 mM (because of its low solubility in water).

Determination of α-CPC and Its Degradation Products——α-CPC and its degradation products in each reaction solution were determined by the HPLC method. At suitable intervals, 5-ml aliquots of the reaction solution were accurately withdrawn and placed in a test tube with a stopper, to which 1 ml of 5 N hydrochloric acid had been added beforehand. About 2 g of sodium chloride was then added in order to facilitate the extraction of related compounds, followed by 2 ml of a mixture of methylene chloride and *n*-butanol (1 : 1), and the whole was shaken for 5 min. The solution was then centrifuged at 3000 rpm for 10 min to separate it into two layers and the upper organic layer was transferred to a 5-ml volumetric flask. The lower layer was extracted twice with 2 ml and 1 ml of a mixture of methylene chloride and *n*-butanol (1 : 1). The extracted organic layers were combined and a mixture of methylene chloride and *n*-butanol (1 : 1) was added to make up the volume. Then 10 μl of the solution was injected into a Hitachi 633 high-performance liquid chromatograph equipped with a variable-wavelength photometer (233 nm). Separation of α-CPC and its degradation products was carried out in a stainless steel column (500 × 4 mm i.d.) packed with Lichrosorb SI 100, 5 μm (Merck Co., Ltd.) using a mixture of water-saturated methylene chloride, methylene chloride, *n*-butanol, *n*-hexane and methanol (38.5 : 38.5 : 10 : 10 : 3) as a mobile phase. The flow rate was 1.3 ml/min, and the sensitivity was 0.2 AUFS.

Results and Discussion

Separation of α-CPC and Its Degradation Products

Figure 2A shows the chromatogram of CP (1), α-CPC (2) and β-CPC (3) in a standard solution. Good separation was achieved. Figure 2B shows the chromatogram of the degraded solution in 1.04×10^{-2} N NaOH at 20 °C for 24 h. The degradation of α-CPC yielded two degradation products in strongly alkaline aqueous solution. The retention times of the degradation products were identical with those of standard CP (1) and β-CPC (3). Furthermore, the degradation products were confirmed to be CP and β-CPC by analysis of their nuclear magnetic resonance (NMR) spectra.

Degradation of α-CPC in Strongly Alkaline Aqueous Solution

Figure 3 shows the time courses of the decrease of α-CPC at various concentrations of sodium hydroxide. The degradation did not obey pseudo-first order kinetics in any of the

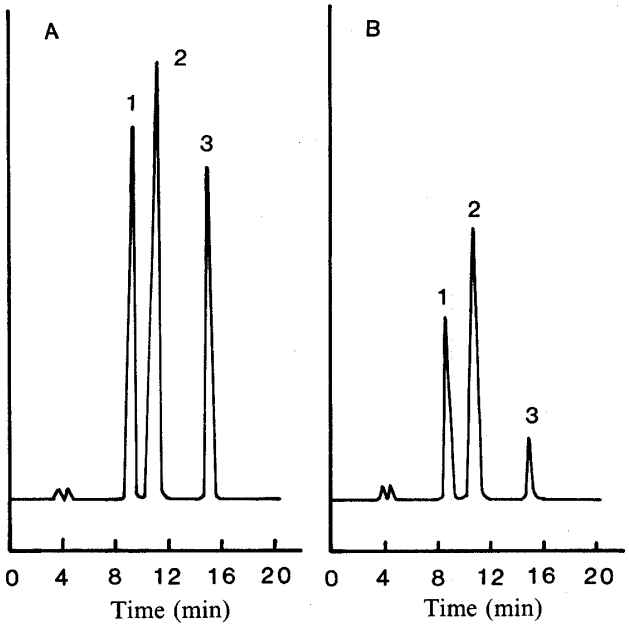

Fig. 2. Chromatograms of α-CPC and Its Degradation Products

A, standard solution; B, reaction solution of α-CPC in 1.04×10^{-2} N NaOH at 20 °C for 24 h; 1, CP; 2, α-CPC; 3, β-CPC.

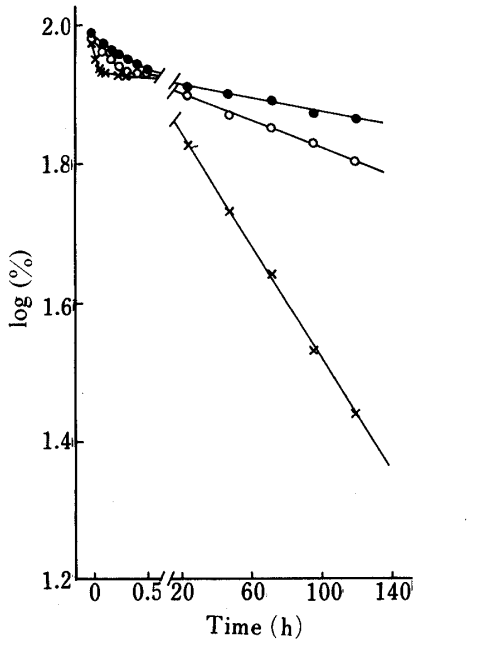

Fig. 3. Time Courses of the Degradation of α-CPC at 0 °C

●, 5.18×10^{-3} N NaOH; ○, 1.04×10^{-2} N NaOH; ×, 5.18×10^{-2} N NaOH.

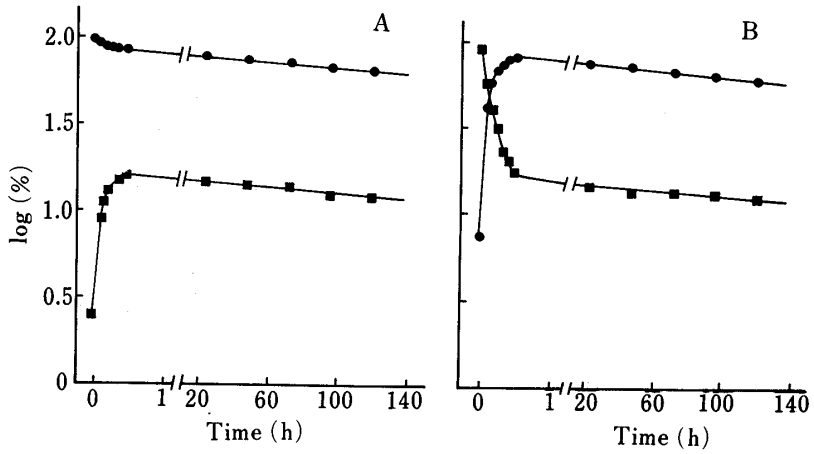

Fig. 4. Time Courses of the Degradation of α-CPC and β-CPC in 1.04×10^{-2} N NaOH at 0 °C

A, degradation of α-CPC; B, degradation of β-CPC; ●, α-CPC; ■, β-CPC.

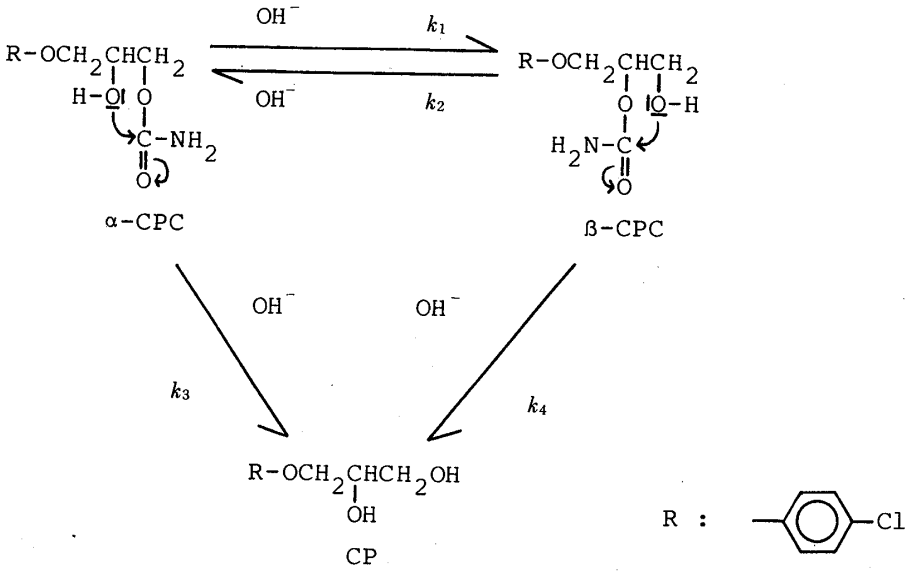

Chart 1. Mechanism of the Isomerization and Degradation of α-CPC

cases, but a double-exponential decrease was observed.

Figure 4A shows a gradual decrease of α-CPC accompanied by the formation of β-CPC, which is the inversion compound resulting from migration of the carbamoyl group ($CONH_2$) at the 1-OH position to the 2-OH position. Figure 4B shows the degradation of β-CPC under the same conditions as α-CPC. As can be seen from Figs. 4A and 4B, the slopes at the initial stage and the subsequent stage of the degradation were different and the attenuation curves of both compounds were observed to be approximately parallel at the later stage of the degradation. The concentration of α-CPC was very much larger than that of the isomer at equilibrium.

From these results, it was concluded that the degradation of α-CPC could be represented by Chart 1. Solving the differential equations for this model, we can derive Eqs. 1, 2 and 3, which give the amounts of α-CPC, β-CPC and CP as a function of time, respectively.

$$[\alpha\text{-CPC}]_t = \frac{k_2[\beta\text{-CPC}]_0 - (k_1 + k_4 + M_2)[\alpha\text{-CPC}]_0}{M_1 - M_2} e^{M_1 t} + \frac{(k_1 + k_4 + M_1)[\alpha\text{-CPC}]_0 - k_2[\beta\text{-CPC}]_0}{M_1 - M_2} e^{M_2 t} \quad \cdots (1)$$

TABLE I. Rate Constants of the Isomerization and Degradation of α-CPC and β-CPC under Various Conditions

Compound	Temp. (°C)	NaOH conc. (N)	Rate constant (h^{-1})			
			k_1	k_2	k_3	k_4
α-CPC	0	5.18×10^{-3}	5.93×10^{-1}	2.66	1.23×10^{-3}	1.20×10^{-3}
	0	1.04×10^{-2}	8.83×10^{-1}	4.68	2.32×10^{-3}	2.35×10^{-3}
	0	5.18×10^{-2}	4.81	27.3	8.78×10^{-3}	8.85×10^{-3}
	10	1.04×10^{-2}	2.23	10.5	6.63×10^{-3}	6.63×10^{-3}
	20	1.04×10^{-2}	4.87	22.5	1.44×10^{-2}	1.46×10^{-2}
β-CPC	0	1.04×10^{-2}	1.17	5.72	2.26×10^{-3}	2.33×10^{-3}

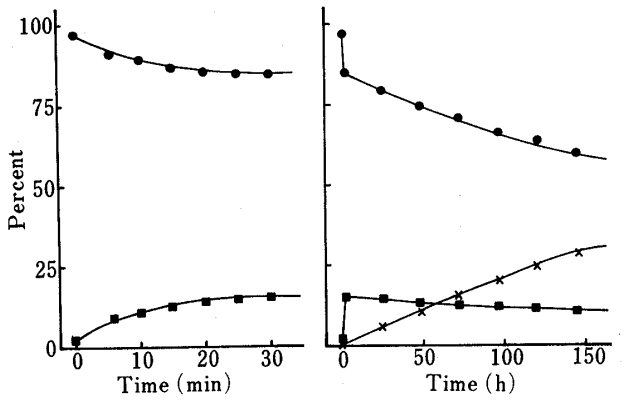

Fig. 5. Time Course of the Degradation of α-CPC in 1.04×10^{-2} N NaOH at 0 °C

●, α-CPC; ■, β-CPC; ×, CP; ●, ■, ×, experimental value; solid line, line calculated by using Eqs. 1, 2 and 3; $[\alpha\text{-CPC}]_0$, 97.47%; $[\beta\text{-CPC}]_0$, 2.53%.

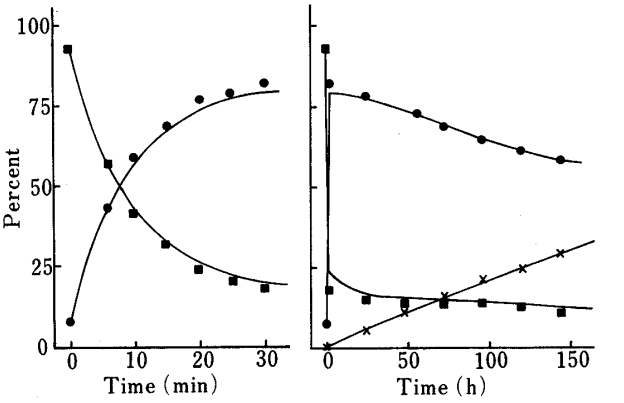

Fig. 6. Time Course of the Degradation of β-CPC in 1.04×10^{-2} N NaOH at 0 °C

●, α-CPC; ■, β-CPC; ×, CP; ●, ■, ×, experimental value; solid line, line calculated by using Eqs. 1, 2 and 3; $[\alpha\text{-CPC}]_0$, 7.59%; $[\beta\text{-CPC}]_0$, 92.41%.

$$[\beta\text{-CPC}]_t=\frac{(k_1+k_4+M_1)[\beta\text{-CPC}]_0+k_1[\alpha\text{-CPC}]_0}{M_1-M_2}e^{M_1t}-\frac{(k_1+k_4+M_2)[\beta\text{-CPC}]_0+k_1[\alpha\text{-CPC}]_0}{M_1-M_2}e^{M_2t} \quad (2)$$

$$[\mathrm{CP}]_t=[\alpha\text{-CPC}]_0+[\beta\text{-CPC}]_0-\frac{1}{M_2-M_1}[\{(M_2+k_4)[\alpha\text{-CPC}]_0+(M_2+k_3)[\beta\text{-CPC}]_0\}e^{M_1t}-\{(M_1+k_4)[\alpha\text{-CPC}]_0+(M_1+k_3)[\beta\text{-CPC}]_0\}e^{M_2t}] \quad (3)$$

where $|M_1|>|M_2|$, $M_1+M_2=-(k_1+k_2+k_3+k_4)$, $M_1*M_2=k_1k_3+k_2k_4+k_3k_4$, k_1 is the apparent rate constant of isomerization of α-CPC to β-CPC, k_2 is the apparent rate constant of the degradation of β-CPC to α-CPC, k_3 and k_4 are the pseudo-first order degradation constants of β-CPC and α-CPC to CP, respectively, and $[\alpha\text{-CPC}]_0$ and $[\beta\text{-CPC}]_0$ are the initial concentrations of α-CPC and β-CPC.

Using Eqs. 1 and 2, we can obtain the rate constants k_1, k_2, k_3 and k_4 by calculation using the residual method at the preequilibrium phase and an attenuation graph at the post-equilibrium phase. All of the parameters, k_1, k_2, k_3 and k_4, could be obtained by the above procedures. The results are summarized in Table I.

As shown in Table I, the rate constants, k_1, k_2, k_3 and k_4 obtained from α-CPC and from β-CPC as the initial substance corresponded well with each other. The rate constant k_2 was

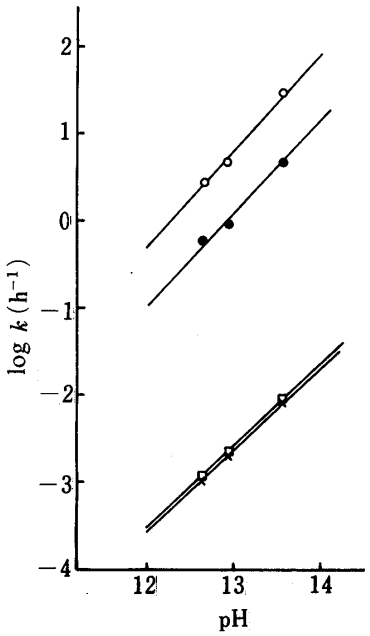

Fig. 7. Rate–pH Profile of the Degradation Rate Constants at 0 °C

●, k_1; ○, k_2; □, k_3; ×, k_4.

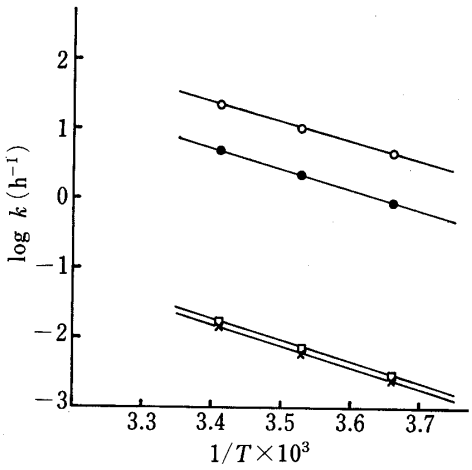

Fig. 8. Arrhenius Plots of the Rate Constants of the Isomerization and Degradation in 1.04×10^{-2} N NaOH

●, k_1; ○, k_2; □, k_3; ×, k_4.

about 5 times larger than k_1. Therefore, α-CPC was considered to be a more stable form than β-CPC. The isomerization rate constants between α-CPC and β-CPC were very much larger than the degradation rate constants of α-CPC and β-CPC to CP. Using these results (Table I) and Eqs. 1, 2 and 3, the calculated lines shown in Figs. 5 and 6 were generated. A fairly good fit between the experimental values and the calculated line was obtained. These results indicate that the hypothesis regarding the degradation pathways is reasonable.

Effect of Alkali Concentration on the Degradation of α-CPC

In the case of concentrated alkaline solutions, the mean activity coefficient must be taken into account because of the inaccuracy in pH determination. Therefore, the relationship between pH and sodium hydroxide concentration is defined by Eq. 4.

$$pH = p^{K_w} + \log(\gamma_{NaOH}\,[NaOH]) \tag{4}$$

where p^{K_w} is the ionization constant of water and γ_{NaOH} is the mean activity coefficient of sodium hydroxide. Using Eq. 4 and the literature value[11] of p^{K_w} and γ_{NaOH}, the pH of each concentrated alkaline solution was calculated. The results of the rate–pH profile are shown in Fig. 7. All of the degradation rate constants increased with increasing pH, and the plots of the logarithms of rate constants *versus* pH became linear with slopes of unity. This suggests that the isomerization and the degradation of α-CPC are dominated by OH^-.

From these results (Figs. 5, 6 and 7), α-CPC is considered to be in equilibrium with β-CPC. Furthermore, these two compounds degrade to CP. All these reactions are catalyzed by OH^- as judged from the rate– pH profile. Therefore, it is considered that these results support the degradation mechanism proposed for α-CPC in Chart 1. The isomerization of another propanediol ester derivative in alkaline solution has been reported by Schmid and Voak.[12] In that report it was considered that the isomerization occurred as a result of nucleophilic attack by the intramolecular OH group on the carbamoyl carbon in the presence of alkali. Furthermore, isomerization of the glycerin ester of monopalmitic acid in an aqueous perchloric acid solution has been reported to occur.[13] In the case of α-CPC, nucleophilic

attack on the carbonyl carbon of the carbamoyl ester by the electron pair of the oxygen in the adjacent OH group might well occur in the presence of alkali by means of the same mechanism as assumed by Schmid and Voak.[12)]

Effect of Temperature

The effect of temperature on the reaction can be expressed by using the Arrhenius equation. Figure 8 shows the Arrhenius plots of the isomerization rate constants and the degradation rate constants in a solution of 1.04×10^{-2} N NaOH. All the rate constants of the isomerization and degradation of α-CPC increased with increasing temperature, and the plots of the logarithms of rate constants *versus* the reciprocal of temperature were linear. The linearity of the Arrhenius plots indicates that the mechanism of degradation and isomerization is identical at all the temperatures employed.

From the slopes, the apparent activation energies for k_1, k_2, k_3 and k_4 were calculated to be 13.3, 12.2, 14.2 and 14.2 kcal mol^{-1}, respectively. Furthermore, from the intercepts, the apparent entropies at 10 °C for k_1, k_2, k_3 and k_4 were calculated to be −28.1, −29.1, −36.6 and −36.6 cal deg^{-1} mol^{-1}, respectively.

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