Effects of Cyclodextrins on Degradations of Emetine and Cephaeline in Aqueous Solution

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The stabilizing effects of β-, γ- and 2,6-dimethyl-β-cyclodextrins (β-, γ- and DM-β-CyDs) against photodegradation and thermal degradation of emetine (EM) and cephaeline (CP) in aqueous solution were examined. The degradation rates of EM and CP increased with decreasing pH below about 3 and with increasing pH above about 5, showing V-shaped pH-profiles, and the photodegradation of EM and CP was significantly faster than the thermal degradation. The photostability of EM and CP was improved by the complexation with γ- and DM-β-CyDs, while it was reduced slightly by β-CyD. The emetic syrup was prepared with γ-CyD or DM-β-CyD in pH 4 phosphate buffer solution, and its photostability was investigated. The results suggested that γ- and DM-β-CyD complexations are useful for the stabilization of EM and CP in the syrup preparation.

Keywords emetine; cephaeline; syrup preparation; degradation; cyclodextrin; inclusion complex; stabilization

Introduction

Emetine (EM) and cephaeline (CP) are the main alkaloids contained in ipecac. In Europe and the U.S.A., ipecac syrup is often used as a first-aid medicine when children of less than five years old have ingested harmful substances. In some hospitals in Japan, ipecac syrup is also used for the treatment of accidental poisonings. However, EM and CP are known to decompose thermally and photochemically to various products such as emetamine, *O*-methylpsychotrine and psychotrine (Fig. 1), which may significantly alter the pharmacological effect of ipecac.[1,2]

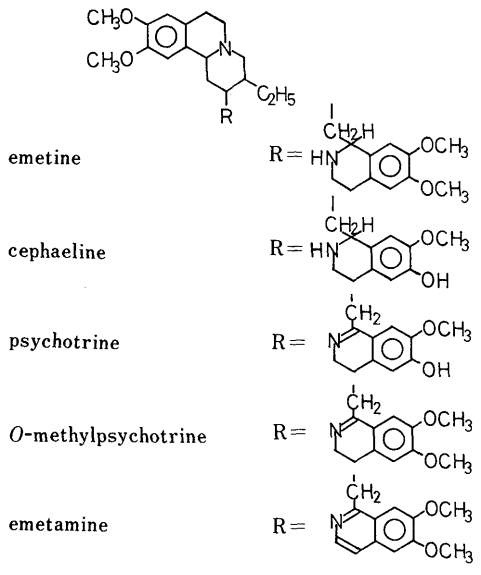

Fig. 1. Emetine and Related Alkaloids from Ipecac

Cyclodextrins (CyDs) have been utilized extensively to improve various physico- and bio-pharmaceutical properties such as chemical instability, poor dissolution characteristics, and low bioavailability of drugs, through inclusion complexation.[3] In this study, therefore, the effects of CyDs on the degradation rates of EM and CP were kinetically investigated in the hope of improving the chemical stability of the alkaloids; little work has yet been done on the stabilizing effect of CyDs on large molecules.

Experimental

Materials EM was extracted from its hydrochloride salt (Sigma Chemical Co.) with ether under alkaline conditions (10% NH_4OH). CP was obtained according to the method reported previously.[4] β-CyD, γ-CyD and heptakis (2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) were purchased from Nihon Shokuhin Kako Co. and recrystallized from water. All other materials and solvents were of analytical reagent grade.

Kinetic Studies The degradation study of EM and CP in the dark was carried out in phosphate buffer (pH 2—9) at 65±0.5 °C. The reaction was initiated by addition of a stock solution of EM or CP in ethanol to the buffer solution, where the final concentrations of the alkaloids and ethanol were 4.6×10^{-4} M and 9.1% (v/v), respectively. The photodegradation of the alkaloids was conducted at 25±2 °C, by irradiating sample solutions from a distance of 15 cm using a UV-B (Toshiba EL20S, E-30; $\lambda=305$ nm) lamp (2.2 mW/cm^2). Other conditions for the photodegradation were the same as those in the dark. At appropriate intervals, 0.2 ml of the sample was withdrawn, then 50 μl of an internal standard (chloroquine 6 mg/ml) and 50 μl of hydrochloric acid solution (5×10^{-3} M) were added immediately, and the mixture was subjected to high-performance liquid chromatography (HPLC) for determination of EM and CP. Emetic and ipecac syrups were also irradiated under the above conditions, and 0.2 ml of the sample solution was withdrawn and analyzed according to the method described in a previous report.[5] The HPLC conditions were as follows: a Shimadzu liquid chromatograph (model LC-3A) equipped with a variable-wavelength Shimadzu UV spectrometer (model SPD-2A) was used; column, prepacked TSK gel ODS-80TM (5 μm, 15 cm×4.6 mm, Tosoh); injection volume, 20 μl; mobile phase, 10 mM sodium 1-heptanesulfonate solution adjusted to pH 4 with glacial acetic acid–methanol (46:54); flow rate, 1 ml/min; detection, 285 nm. Components were quantitated by measuring peak height and comparing it with that of a known amount of the internal standard. The pH of the sample solution was confirmed to be identical before and after the reaction. First-order plots for the degradation of EM and CP were linear within the half-life, and the degradation rate constants (k) were calculated from them.

Preparation of the Emetic Syrup EM hydrochloride salt (50 mg) or CP hydrochloride salt (125 mg) (equivalent to the amount in the U.S.P. ipecac syrup) was dissolved in a 30 ml of phosphate buffer solution (pH 4) containing γ- or DM-β-CyD (3.6×10^{-2} M), and then the same volume of glycerin and syrup simplex was added, according to the preparative method in the U.S.P.[6] (Fig. 7).

Results and Discussion

Effects of pH Figure 2 shows the log k–pH profiles for the degradations of EM and CP in the dark at 65 °C. The degradation rates of EM and CP increased with decreasing pH in the low pH region and with increasing pH in the high pH region, showing V-shaped pH-profiles.

Slopes of the profiles were in the ranges of −0.8—−1.0 and 0.3—0.5 below and above pHs 3 and 5, respectively, suggesting the participation of specific acid–base and buffer

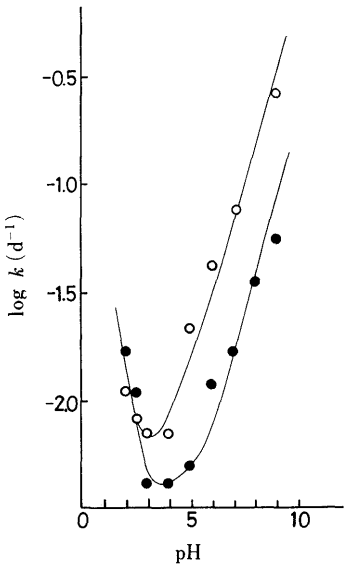

Fig. 2. pH-Profiles for Thermal Degradation Rates of EM and CP at 65°C in the Dark

●, EM; ○, CP.

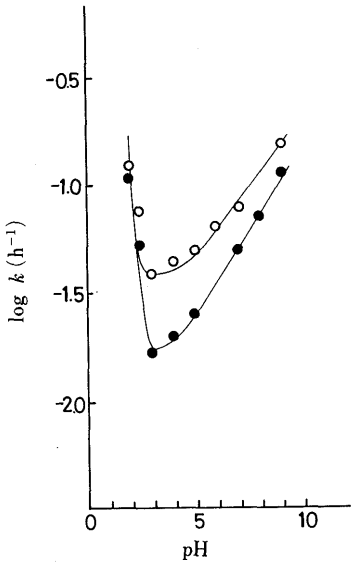

Fig. 3. pH-Profiles for Photodegradation Rates of EM and CP at 25°C

●, EM; ○, CP.

catalyses in the degradation. The rate constants were not extrapolated to zero buffer concentration because of the slowness of the reaction.

Thus, the slope of less than unity in the neutral and alkaline regions may be ascribable to buffer catalysis, since the reaction was catalyzed by the buffer components. CP was more susceptible to the base-catalyzed degradation than EM, whereas the acid-catalyzed degradation rate was almost the same for EM and CP. CP has a phenolic hydroxyl group on the isoquinoline moiety, whereas this hydroxyl group is methylated in the EM molecule (Fig. 1). Thus, the high susceptibility of CP to the base-catalyzed degradation may be ascribable to the presence of the phenolic group in the molecule,[7] although the degradation mechanism was not fully elucidated. Both alkaloids showed greater stability at pH 3—4.

TABLE I. Photodegradation Rate Constants ($\times 10\,h^{-1}$) of EM and CP in the Absence and Presence of CyDs in Phosphate Buffer ($\mu=0.2$) at 25 °C

Compd.	pH	In the absence of CyDs	In the presence of CyDs[a]		
			β-CyD	γ-CyD	DM-β-CyD
EM	3	0.165	0.270	0.138	0.090
	5	0.253	0.380	0.186	0.149
	7	0.513	0.575	0.367	0.300
	9	1.104	1.225	0.980	0.855
CP	3	0.380	0.416	0.312	0.257
	5	0.505	0.527	0.402	0.300
	7	0.652	0.721	0.543	0.480
	9	1.560	1.590	1.470	1.250

a) CyD concentration was 1.5×10^{-2} M.

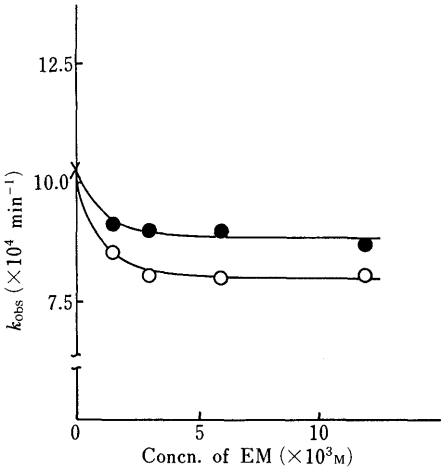

Fig. 4. Observed Rate Constants for the Degradation of EM as a Function of CyD Concentration in Phosphate Buffer (pH 7.5, $\mu=0.2$) at 25°C

●, γ-CyD complex; ○, DM-β-CyD complex.

Figure 3 shows the log k–pH profiles for the photodegradations of EM and CP at 25 °C, where the thermal degradation of the alkaloids was negligible. The rates of photodegradation of the alkaloids were significantly faster than those of thermal degradation (Fig. 2), and the pH dependence of the photodegradation above pH 4 was smaller than that of the thermal degradation. These results indicate that the photodegradation rather than the thermal degradation of the alkaloids is more critical in quality assurance from a practical standpoint. In the following study, therefore, the effect of CyDs on photodegradations of EM and CP was investigated in detail.

Table I shows the photodegradation rate constants of EM and CP in the absence and presence of CyDs in pH 3—9. The degradation rates of EM and CP were decreased by the addition of γ- and DM-β-CyDs in pH 3—9, while they were accelerated slightly by β-CyD. The stabilizing effect of DM-β-CyD was greater than that of γ-CyD.

Effects of CyD Concentration Figure 4 shows the effects of γ- and DM-β-CyD concentrations on the observed photodegradation rate constant (k_{obs}) of EM in phosphate buffer (pH 7.5). The reaction rates decreased hyperbolically with increasing CyD concentration, showing typical saturation kinetics. Similar results were obtained for the CP complexes with γ- and DM-β-CyDs. The dependency of

$$\begin{array}{ccc} \text{EM} + \text{CyD} & \overset{K_c}{\rightleftharpoons} & \text{EM–CyD} \\ \downarrow k_o & & \downarrow k_c \\ \text{other ipecac alkaloids} & & \text{other ipecac} + \text{CyD alkaloids} \end{array}$$

Chart 1

TABLE II. Rate Constants and Stability Constants of EM– and CP–CyD Complexes at pH 7.5 and 25 °C

System	k_o ($\times 10^4$ min^{-1})	k_c ($\times 10^4$ min^{-1})	k_c/k_o	K_c (M^{-1})
EM alone	10.30	—	—	—
EM–γ-CyD	—	8.62	0.837	830
EM–DM-β-CyD	—	8.08	0.784	1560
CP alone	11.80	—	—	—
CP–γ-CyD	—	10.53	0.892	1110
CP–DM-β-CyD	—	10.46	0.886	1550

k_{obs} on the CyD concentration was quantitatively treated by Eq. 1[8] to obtain the apparent stability constant (K_c) and the rate constant (k_c) of the complex, assuming the 1 : 1 complexation scheme (Chart 1), where k_o and $(CyD)_t$ are the rate constant in the absence of CyDs and the total concentration of CyDs, respectively. The plots according to Eq. 1 gave a good straight line with a correlation coefficient (r) of over 0.99, and the results for k_o, k_c, k_c/k_o and K_c are summarized in Table II. The K_c values of the DM-β-CyD complexes were larger than those of the γ-CyD complexes, indicating a higher affinity of the alkaloids to DM-β-CyD. On the other hand, the difference between the K_c values of EM– and CP–CyD complexes is insignificant.

$$\frac{(CyD)_t}{k_o - k_{obs}} = \frac{1}{k_o - k_c}(CyD)_t + \frac{1}{K_c(k_o - k_c)} \quad (1)$$

Effects of Temperature Figure 5 shows Arrhenius plots of the thermodegradation rates of CP in the absence and presence of CyDs over a temperature range of 50—65 °C at pH 9. Similarly, the Arrhenius relationship held well for EM systems over the temperature range employed. The thermodynamic activation parameters[9] calculated from the linear plots are listed in Table III. It is apparent that the low reactivity of EM compared with CP is due to the smaller activation entropy (ΔS^*), together with the greater activation enthalpy (ΔH^*). On the other hand, the decelerating effect of CyDs resulted from the increase in ΔH^*, which was partly cancelled out by the increase in ΔS^*. This effect was greater in the DM-β-CyD complex than in the γ-CyD complex. Figure 6 shows the relationships between ΔH^* and ΔS^* for the degradation of EM and CP.

The plots of ΔH^* *versus* ΔS^* were linear ($r = 0.99$), from which the compensation temperatures were calculated to be 366 and 357 K for EM and CP, respectively. The linear relationship between ΔH^* and ΔS^* suggests that the degradation pathway of EM and CP is not changed by the binding to CyDs, and the hydration of the transition species plays an important role in the degradation.[10]

Thus, the alkaloids may degrade through an ionic transition state, which is destabilized energetically and is desolvated in the hydrophobic environment of the CyD cavity, leading to the larger positive ΔH^* and ΔS^* values in the CyD systems.

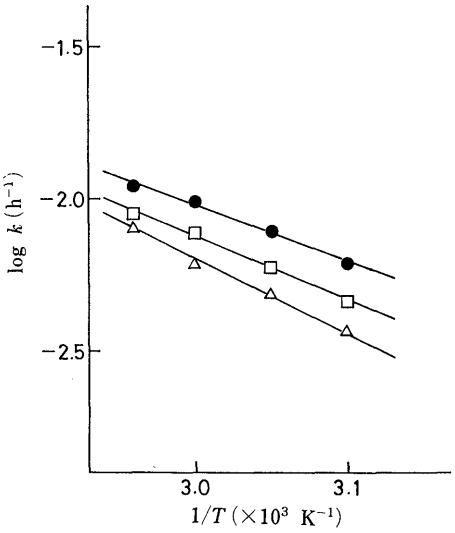

Fig. 5. Arrhenius Plots for the Degradation of CP[a] in the Absence and Presence of CyDs[b] in Phosphate Buffer (pH 9.0, $\mu = 0.2$)

a) CP concentration was 1.9×10^{-4} M. *b*) CyD concentration was 1.5×10^{-2} M. ●, CP alone; □, γ-CyD complex; △, DM-β-CyD complex.

TABLE III. Thermodynamic Activation Parameters for the Degradation of EM and CP in the Absence and Presence of CyDs[a] in Phosphate Buffer (pH 9.0, $\mu = 0.2$)

System	ΔG^*_{338} (kcal/mol)	ΔH^* (kcal/mol)	ΔS^*_{338} (e.u.)
EM alone	30.4	8.04	−66.1
EM–γ-CyD	30.6	8.66	−64.9
EM–DM-β-CyD	0.8	13.20	−52.0
CP alone	28.4	7.67	−61.3
CP–γ-CyD	28.5	9.37	−56.5
CP–DM-β-CyD	8.6	11.77	−49.8

a) CyD concentration was 1.5×10^{-2} M.

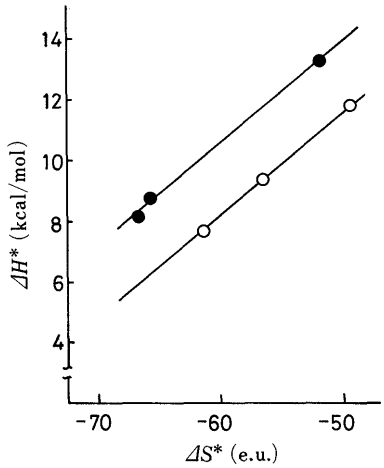

Fig. 6. Relationship between ΔH^* and ΔS^* for EM– and CP–CyD complexes at 65 °C

●, EM; ○, CP.

Photostability of EM and CP in Syrup Preparation From a practical point of view, the stabilizing effects of γ- and DM-β-CyDs on the photodegradation of EM and CP in a syrup preparation were investigated. The preparative method of emetic syrup is shown in Fig. 7. EM and CP

preparation of emetic syrup		preparation of U.S.P. ipecac syrup (100 ml)	
EM·HCl and CP·HCl	(154—196 mg)[a]		
in CyDs/phosphate buffer (pH 4)	30 ml	extract[b]	7 ml
glycerin	10 ml	glycerin	10 ml
syrup simplex	80 ml	syrup simplex	83 ml

Fig. 7. Preparation of the Emetic Syrup and U.S.P. Ipecac Syrup

a) Equivalent amounts to the contents in U.S.P. *b*) Extracted from U.S.P. powdered ipecac with EtOH : H_2O (3 : 1).

TABLE IV. Photodegradation Rate Constants (h^{-1}) of EM and CP in U.S.P. Ipecac Syrup or in the Absence and Presence of CyDs in Emetic Syrup at 25 °C

Compd.	Ipecac syrup (U.S.P.)	In the absence of CyD	In the presence of CyD[a]	
			γ-CyD	DM-β-CyD
EM	0.204	0.079	0.064	0.057
CP	0.148	0.083	0.050	0.046

a) CyD concentration was 3.6×10^{-2} M.

were dissolved in phosphate buffer (1/30 M, pH 4) containing γ- or DM-β-CyD (3.6×10^{-2} M), where the alkaloids have higher stability (see Fig. 3). The amounts of EM·HCl and CP·HCl added to the syrup were equivalent to those contained in the U.S.P. ipecac syrup. The effect of CyDs on the photodegradation rates of EM and CP in the emetic syrup is shown in Table IV. The stabilizing ability of DM-β-CyD was slightly larger than that of γ-CyD. In comparison with the U.S.P. ipecac syrup, the degradation rates of EM and CP were decreased about 3.3 times by the addition of γ- and DM-β-CyDs, although pH-correction should be done (pH values of the emetic syrup and the U.S.P. ipecac syrup were about 4.0 and 3.0—4.0, respectively). The data presented here will provide a rational basis for formulation design and for stabilizing alkaloids in pharmaceutical dosage forms.

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References and Notes

1) G. Bayracter-Alpmen, *Eczacilik Bulteni.* Istanbul, **13**, 1 (1971).
2) C. Schuijt, G. M. J. B. von Henegouwen and K. W. Gerritsma, *Pharmaceutisch Weekblad Sci. Ed.*, **114**, 186 (1979).
3) J. L. Lach and T-F. Chin, *J. Pharm. Sci.*, **53**, 924 (1964); T-F. Chin, P-H. Chung and J. L. Lach, *ibid.*, **57**, 44 (1968); K. Koizumi and K. Fujimura, *Yakugaku Zasshi*, **92**, 32 (1972).
4) D. Teshima, T. Tsuchiya, T. Aoyama and M. Horioka, *Iyakuhin Kenkyu*, **15**, 63 (1984).
5) D. Teshima, K. Ikeda, K. Shimomura and T. Aoyama, *Chem. Pharm. Bull.*, **37**, 197 (1989).
6) United States Pharmacopoeia, XXI, 554 (1985).
7) L. Gattermann and H. Wieland, "Die Praxis Des Organischen Chemikers," Vol. 41, ed. by T. Wieland, Walter De Gruyter & Co., Berlin, 1962, pp. 214—221.
8) S. Tanaka, K. Uekama and K. Ikeda, *Chem. Pharm. Bull.*, **24**, 2825 (1976).
9) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Inc., New York, 1969.
10) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley & Sons Inc., New York, 1963.