Physicochemical Properties and Stability in the Acidic Solution of a New Macrolide Antibiotic, Clarithromycin, in Comparison with Erythromycin[1)]

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Clarithromycin (6-*O*-methylerythromycin), a new 14-membered macrolide antibiotic, has been studied to clarify its physicochemical properties and stability in acidic solution, as compared with erythromycin (EM).

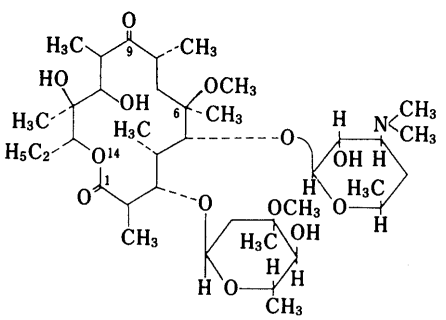
The solubility of clarithromycin (CAM) in distilled water was lower than that of EM and decreased with increasing temperature. The solubilities of CAM and EM in the phosphate buffer solution at 37 °C decreased with an increasing pH and kept constant above pH 9. From pH-solubility profiles, the dissociation constants of CAM and EM were determined to be 8.76 and 8.36, respectively. The partition coefficient of CAM took a higher value than that of EM and increased with an increasing pH.

In the acidic solution, the decomposition of CAM and EM obeyed the pseudo-first order kinetics. From the decomposition rate constants, the half life ($T_{1/2}$) of CAM and EM were determined. In pH 1.39, CAM degraded with a $T_{1/2}$ of 17 min while EM kinetics corresponded to a $T_{1/2}$ of 3 s. Therefore, CAM was 340-fold more stable in pH 1.39 and markedly more stable in the acidic solution than EM.

Keywords clarithromycin; erythromycin; physicochemical property; acid stability; pH-solubility profile; dissociation constant; partition coefficient

Erythromycin (EM) is widely used in the treatment of gram-positive infection and is used clinically because of its wide spectrum of antimicrobial activity.[2)] However, EM is rapidly inactivated by gastric acid and its absorption is reduced when the drug is given with meals.[3)] For these reasons, an enteric coated formulation and a number of derivertives have been developed. As for the enteric coated formulation, Watanabe *et al.*[4)] examined the bioavailability of the commercial dosage form in humans and suggested that it had very variable absorption because of its different disintegration times and dissolution rates in various pH solutions.

Clarithromycin (CAM) is synthesized in order to compensate for this disadvantage of EM and a new 14-membered macrolide antibiotic in which the hydroxy group of EM is methylated at the C6 position of the ring lactone as its chemical structure is shown in Fig. 1. Morimoto *et al.*[5)] examined the determination for the chemical structure of the acidic decomposition products of CAM and reported that the decomposition passway of CAM in the acidic solution was different from that of EM. However, their stabilities data were estimated under the condition of pH 2.0 at 33 °C alone and there was no discussion on the comparison of stability between CAM and EM under other acidic conditions. Suwa *et al.*[6)] reported that after the drugs were orally or intravenously administered to rats and beagle dogs, the serum concentration of CAM was significantly higher level than EM, and CAM was distributed more to the lungs than EM. Moreover, it has been confirmed that CAM had an excellent clinical efficacy in comparison with EM.[7)]



molecular formula : $C_{38}H_{69}NO_{13}$
molecular weight : 747.96

Fig. 1. Chemical Structure of Clarithromycin

In the present work, in order to elucidate the excellent absorption and efficacy of CAM from the aspects of the physicochemical properties, the solubilities in water, pH-solubility profiles, and partition coefficients (oil/water) of CAM and EM were investigated. In addition, the acidic stability between CAM and EM under several pH conditions at 37°C were compared and the simulation of acidic stabilities were carried out considering the physiological conditions.

Experimental

Materials Clarithromycin was synthesized at Taisho Pharmaceutical Co., Ltd., and used in every experiment.

Erythromycin USP grade was used. (Abbott Laboratory Co., Ltd.). All other chemicals were reagent grade and were used without further purification.

Solubility in Water Excess amounts of CAM and EM were added to distilled water in a flask, respectively. The suspensions were incubated at 20, 37, or 50 °C for 24 h and were filtered through a membrane filter (pore size 0.45 μm), then the concentration of CAM and EM were determined according to the high performance liquid chromatography (HPLC) method. The operating conditions of HPLC were as follows; detector, ultraviolet (UV) absorption spectra; detector wavelength, 210 nm; column temperature, 50 °C; flow rate, 1 ml/min; mobile phase, 1/15 M potassium dihydrogen phosphate, acetonitrile CAM (13:7), EM (12:8).

pH-Solubility Profile The solubilities of CAM in phosphate buffer solutions of several pH were determined at 37 °C in a similar manner as solubility in water. The concentration of EM in the filtrate was measured by a procedure similar to the one described by Ford *et al.*[8)] Namely, after some of the solution was diluted with 27 N sulfuric acid, the absorbance at 485 nm was measured and the concentration of EM was determined.

From the results of pH-solubility profiles, the relationship between the solubilities of CAM and EM and pH were derived and the pK_a of CAM, and EM were determined by the non-linear least squares method, respectively.

Partition Coefficient (*n*-Octanol–Phosphate Buffer) Partition coefficients of CAM and EM between *n*-octanol and phosphate buffer solutions were obtained by measuring the concentration of CAM or EM remaining

in the phosphate buffer solution at room temperature.

Stability in the Acidic Solution About 1 g of CAM and EM were dissolved in 100 ml of acetonitrile, respectively. After 10 ml of these solutions were added to 890 ml of hydrochloride solutions of several pH at 37 °C, some of the solution was withdrawn at regular time intervals and were neutralized with 1/10 M sodium phosphate and the residual concentration of CAM and EM were quantified by the HPLC method. The operating conditions of HPLC were in the similar manner as the solubility in water.

Results and Discussion

Solubilities of CAM and EM in Water The solubilities of CAM and EM in water at several temperatures are shown in Fig. 2. The solubilities of CAM and EM decreased with increasing temperature. These phenomena were observed for another macrolide, 9,3″-diacetylmidecamycin,[9] and it seems that the macrolide structure has similar properties in water. Fukumori *et al.*[10] and Shinoda *et al.*[11] attributed that the hydrophobic compounds, such as benzene and toluene were dissolved due to the hydrophobic hydration in the water. In the case of CAM and EM, it seems that water molecules were ordered regularly around the hydrophobic parts of CAM and EM at low temperatures. On the other hand, as the temperature increased, the hydrophobic hydration structure was destroyed and resulted in decreased solubilities of CAM and EM.

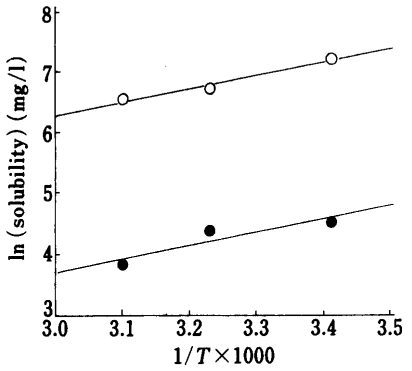


Fig. 2. Solubility of CAM and EM in Water at Several Temperatures
●, CAM; ○, EM.

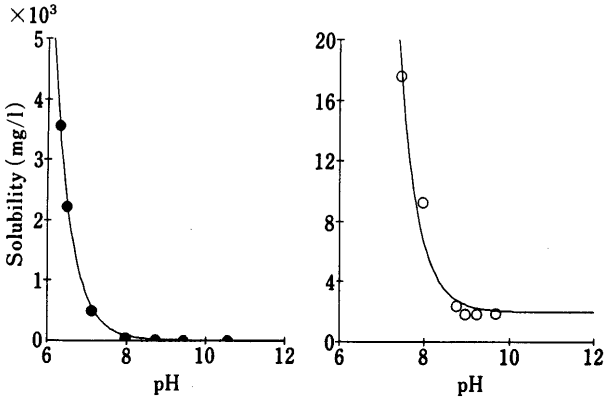


Fig. 3. pH-Solubility Profiles of CAM and EM at 37 °C
●, CAM; ○, EM.

pH-Solubility Profiles and Determination for pK_a Figure 3 illustrates the plots for the pH-solubilities of CAM and EM at 37 °C, respectively. Each solubility decreased with an increasing pH and kept constant above pH 9. Above pH 9, CAM and EM formed an undissociated structure.

The relationship between pH and solubility of the weak base compound can be explained by the following equation, the Henderson–Hasselbalch's equation.

$$pH = pK_a + \log(C_b/C_i) \quad (1)$$

where pK_a is the dissociation constant, C_i is the solubility of the dissociated molecule, and C_b is the solubility of the undissociated molecule. When Eq. 1 is transformed,

$$C_i = C_b 10^{(pK_a - pH)} \quad (2)$$

At any pH, the total concentration (S) of a compound is the sum of the solubility between the dissociated molecule and the undissociated molecule as shown in Eq. 3.

$$S = C_i + C_b \quad (3)$$

Substitution for C_i in Eq. 3 from Eq. 2

$$S = C_b + C_b 10^{(pK_a - pH)} = C_b(1 + 10^{(pK_a - pH)}) \quad (4)$$

Thus, the total concentration of a weak base compound is expressed as a function of pH. The solubility data of CAM and EM at any pH were substituted in Eq. 4 and the dissociation constant, pK_a and the concentration of the undissociated form, C_b were calculated by the non-linear least squares method, respectively. As a result, the pK_a and C_b of CAM were determined to be 8.76, 0.012 mg/l and those of EM were determined to be 8.36, 1.967 mg/l. The analyses of variance and the multiple correlation coefficients were monitored for determination of the best fit to Eq. 4 and were summarized in Table I. The multiple correlation coefficients (R) were calculated using the following equation.

$$R = (1 - (S.S._R/S.S._O))^{1/2} \quad (5)$$

where $S.S._R$ is the sum of squared residuals and $S.S._O$ is the sum of squared observations. As can be seen from Table I, the pH-solubilities data of CAM and EM fit with the Eq. 4, respectively. Moreover, the calculated curves were almost in agreement with the observed data, as shown in Fig. 3.

Partition Coefficient Table II shows the partition coefficients (*n*-octanol–phosphate buffer) for several pH levels. At every pH level, the partition coefficients of CAM were higher than that of EM. Since CAM differs structually

Table I. Analysis of Variance for Curve Fitting

Sample	Source	S.S.	D.F.	M.S.	F	S.L.	R	S.D.
CAM	Regression	17.766	2	8.8830	2400.8	3.55×10^{-8}	0.9995	0.0610
	Residual	0.0186	5	0.0037				
EM	Regression	403.56	2	201.78	121.83	2.61×10^{-5}	0.9919	1.2869
	Residual	6.6246	4	1.6562				

S.S., sum of square; D.F., degree of freedom; M.S., mean square; F, F value; S.L., significant level; R, multiple correlation coefficient; S.D., standard deviation.

from EM by a methyl substituted hydroxy group at position 6 in the aglycone ring as shown in Fig. 1, CAM had a more lipophylic property due to the methylation.

Hansch *et al.*[12] reported that the lipophilic character as defined by oil/water partition coefficients played an important role in the biological activity and the partition coefficients were correlated to the absorption, and the biological action. Suwa *et al.*[6] examined the absorption of CAM and EM in rats and beagle dogs by oral or intravenous administration and reported that the serum concentration of CAM was significantly higher than that of EM in each animal and CAM was distributed more to the lungs.

TABLE II. Partition Coefficients (*n*-Octanol/Buffer) of CAM and EM

pH	Partition Coefficient	
	CAM	EM
4.0	4.89	0.13
6.0	7.19	1.61
6.5	17.5	3.80
8.0	48.0	15.7

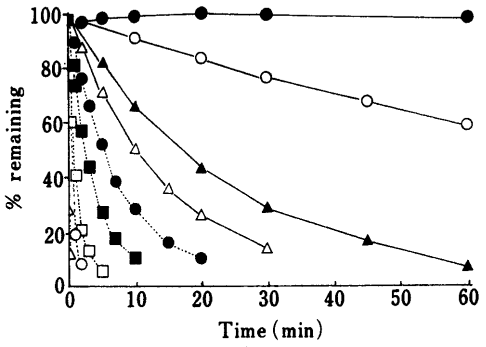


Fig. 4. Stability of CAM and EM in Acidic Solution at 37 °C

—●—, CAM (pH 3.0); ---●---, EM (pH 3.0); —○—, CAM (pH 2.0); ---■---, EM (pH 2.7); —▲—, CAM (pH 1.39); ---□---, EM (pH 2.3); —△—, CAM (pH 1.22); ---○---, EM (pH 2.0); ---△---, EM (pH 1.39).

Moreover, according to the results of clinical studies on CAM and EM, it was confirmed that CAM was distributed more to the lung in humans and had more efficacy to the repirotory pathogen than EM.[7]

Therefore, it is thought that the high lipophilic property of CAM is related to the above difference of the biological activity.

Stability in the Acidic Solution Figure 4 shows the results of the stability for CAM and EM in the acidic solution. The data indicated that EM was immediately degraded below pH 2.3 while CAM was slowly degraded in comparison with EM and was particularly stable in pH 3.0. From these data, on the basis of pseudo-first order decomposition, the decomposition rate constant (K_d) and the half life of decomposition ($T_{1/2}$) were calculated and are summarized in Table III. Considering the ratio of the decomposition rate constants in pH 1.39 between CAM and EM, the stability of CAM was found to increase 340-fold more than that of EM.

Atokins *et al.*[13] reported on the mechanism of the decomposition of EM in the acidic solution and that EM degraded to erythromicin 6,9-hemiketal, and anhydroerythromycin in the acidic solution as shown in Fig. 5A.

TABLE III. The Decomposition Rate Constants and Half Lives for Decomposition of CAM and EM in Acidic Solutions

pH	CAM		EM		*K*
	K_d (min^{-1})	$T_{1/2}$ (min)	K_d (min^{-1})	$T_{1/2}$ (sec)	
1.22	6.69×10^{-2}	10.4	—	—	—
1.39	4.08×10^{-2}	17.0	14.0	3.0	340
2.00	8.66×10^{-3}	80.0	1.54	27.0	178
2.30	—	—	0.838	49.6	—
2.70	—	—	0.262	156.0	—
3.00	1.51×10^{-4}	4590.0	0.127	330.0	835

K, ratio of the decomposition rate constants of CAM to that of EM.

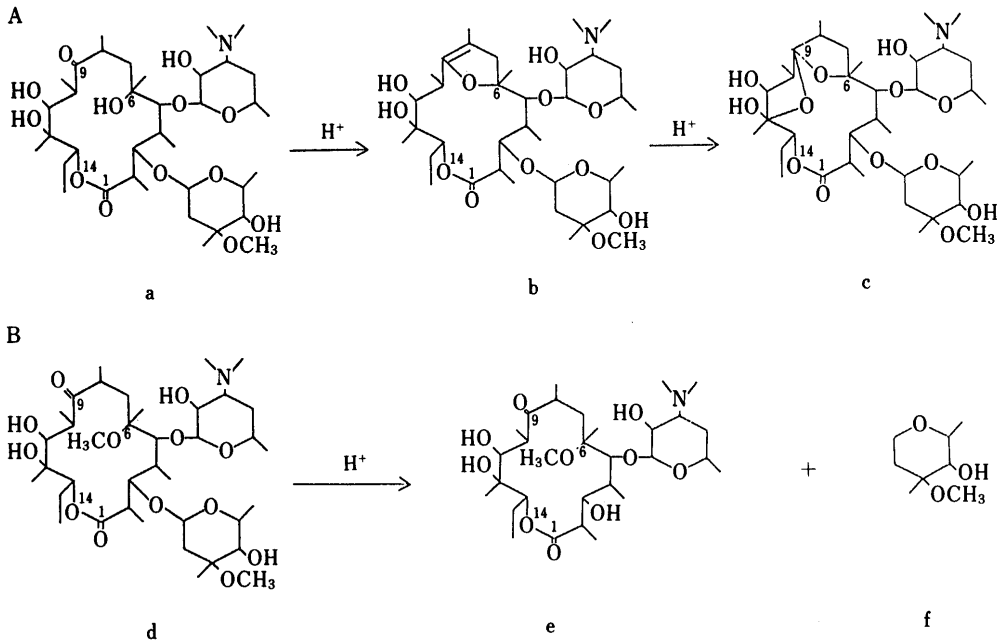


Fig. 5. Scheme of the Mechanism on CAM and EM in the Acidic Solution

a, EM; b, erythromycin 6,9-hemiketal; c, anhydroerythromycin; d, CAM; e, 5-*O*-desosaminyl-6-*O*-methylerythronolide; f, cladinose.

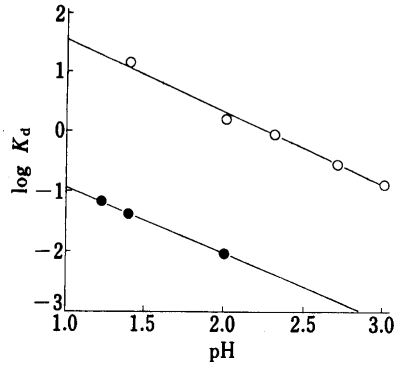


Fig. 6. The Relationship between pH and log K_d
●, CAM, ○, EM.

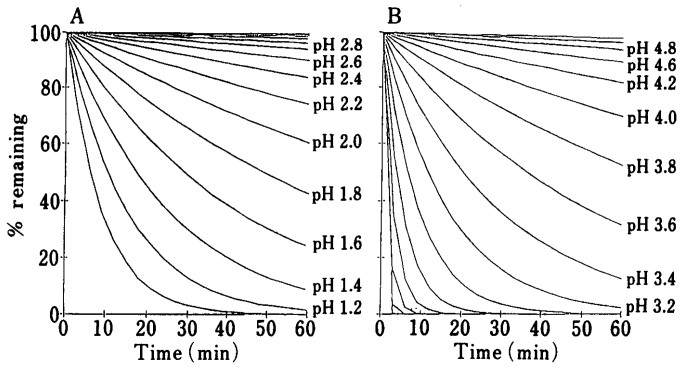


Fig. 7. Simulated Decomposition Patterns of CAM and EM in Several Acidic Solutions
A, CAM; B, EM.

On the other hand, Morimoto *et al.*[5] reported on the determination for the structure of the decomposition products and the decomposition pathway of CAM in the acidic solution. According to their report, in the acidic solution, CAM was degraded *via* cleavage of the neutral cladinose sugar and 6,9-hemiketal was not formed, as was the first step of the acidic decomposition of EM as shown in Fig. 5B. Thus, it was considered that the reason why CAM was markedly more stable than EM in the acidic solution is due to the inhibition of the formation of 6,9-hemiketal.

The logarithm of K_d of CAM and EM *versus* pH are plotted in Fig. 6. The relationship between log K_d and pH was linear, and the following equations were obtained.

$$\text{CAM}: \log K_d = -1.13\ \text{pH} + 0.186 \quad (r = 0.999) \tag{6}$$

$$\text{EM}: \log K_d = -1.26\ \text{pH} + 2.82 \quad (r = 0.995) \tag{7}$$

where r denotes the correlation coefficient. Moreover, on the basis of the above equations, the K_d for several pH were predicted and the decomposition rate of CAM and EM in several pH were simulated by using these values. The simulation results are shown in Fig. 7. Maekawa *et al.*[14] examined the gastric emptying rate in man using the X-ray method when the barium sulphate was orally administered and reported that most of the barium sulphate moved from the stomach to the small intestine within 1 h under fasting conditions. From the result shown in Fig. 7, if the gastric pH was over 3 and CAM remained in the stomach for 1 h, CAM was not degraded. On the other hand, EM was degraded more than 20% in pH 4 after 1 h and was stabilized above pH 5. From these results, it seems that EM is unstable owing to the acidic decomposition when it is orally administered, while CAM is more stable in the stomach than EM.

Conclusion

The lipophilic property of CAM and its stability in the acidic solution were enhanced in comparison with EM. It was also suggested that the results of these physicochemical properties were correlated to the difference of the absorption or the distribution between CAM and EM in the body.

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References and Notes

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