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Decomposition kinetics of erythromycin A in acidic aqueous solutions

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Summary

The decomposition of the antibiotic, erythromycin A, in acidic aqueous solutions has been investigated by means of a high-performance liquid chromatographic assay with ultraviolet detection at 215 nm. During decomposition erythromycin A is in equilibrium with erythromycin A enol ether and simultaneously erythromycin A is directly converted to anhydroerythromycin A. The calculations of the observed rate coefficients of the partial reactions (k_1, k_2 and k_3) were performed using a multiparameter fitting program present in the BMDP statistical software. The decomposition kinetics have been studied as a function of the type and concentration of the buffer, ionic strength, pH and temperature.

Introduction

In a companion paper a new mechanism for the decomposition of erythromycin is proposed (Vinckier et al., 1989). The experimental data to support the new mechanism were obtained by high-performance liquid chromatography (HPLC). This HPLC method will be briefly discussed here. More kinetic data for the decomposition of erythromycin in acidic conditions are presented and factors affecting the degradation reaction (pH, ionic strength, buffer and temperature) are discussed. Both this report and the companion paper have to be considered as an updating of knowledge of the decomposition mechanism of erythromycin.

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Materials and Methods

Preparation of the solutions

Pure erythromycin A (EA) was obtained by 5 consecutive crystallisations from acetone/water (1 : 1). Erythromycin A enol ether (EAEN) and anhydroerythromycin A (AEA) were prepared according to known procedures described in the literature (Kurath et al., 1971; Wiley et al., 1957). Pseudoerythromycin A enol ether (psEAEN) was prepared as reported previously (Kibwage et al., 1987). The chemical structures of the erythromycin derivatives involved are shown in Figs. 1 and 2. Chemicals used for the preparation of buffers were of analytical grade (Merck, Darmstadt, F.R.G.) and bidistilled water was used throughout. For most kinetic studies, potassium phosphate buffers were used in the pH range from 2 to 6. To examine the influence of the buffer type, separate experiments were carried out at pH 4 using 0.2 M

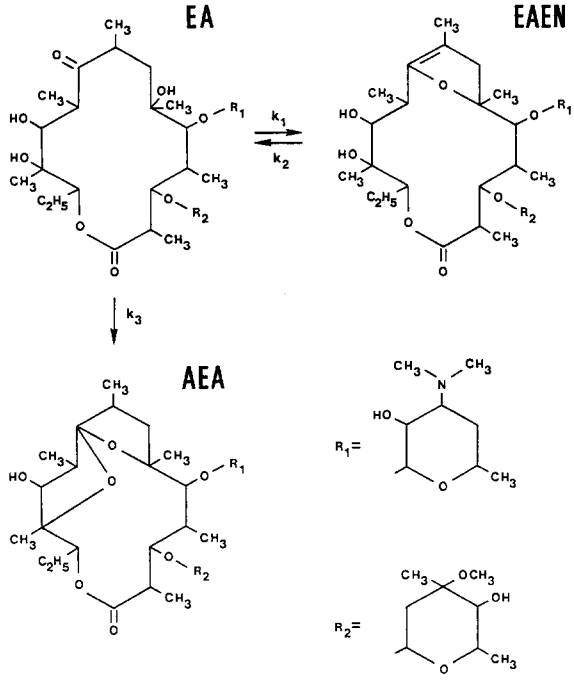

Fig. 1. Decomposition scheme of erythromycin A (EA) in acidic aqueous solutions. EAEN = erythromycin A enol ether; AEA = anhydroerythromycin A.

buffer solutions of sodium phosphate, ammonium phosphate, sodium citrate or sodium acetate. The ionic strength (μ) of the buffers was adjusted with potassium chloride. Reference buffers for calibration in pH measurements were prepared according to instructions of the European Pharmacopoeia (V.6.3, 1980).

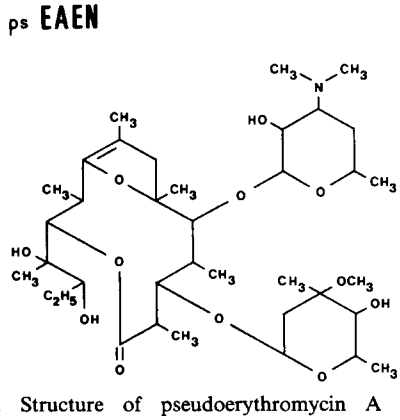

Fig. 2. Structure of pseudoerythromycin A enol ether (psEAEN).

In order to follow the decomposition reactions at pH values equal to or above 4, solutions were prepared by dissolving in the buffer solution an appropriate amount of EA (about 1.5 mg/ml) or EAEN (about 0.5 mg/ml). In the more acidic range of pH, below 4, where the decomposition reaction is fast, solutions were prepared by diluting 5.0 ml of a 1% w/v solution of EAEN or a 3% w/v solution of EA in methanol to 100 ml with the buffer solution.

HPLC method

For kinetic measurements, samples were withdrawn from the reaction vessel at appropriate reaction times. The analysis of the samples was carried out by HPLC. The chromatographic system consisted of a Waters M45 solvent delivery system (Milford, MA, U.S.A.), a Valco Model CV-6-UHPa N60 injection valve (Houston, TX, U.S.A.) equipped with a 100 μl loop, a Waters Model 440 UV Detector equipped with a Waters Extended Wavelength Module for 215 nm and a HP 3390 Integrator (Hewlett Packard, Avondale, PA, U.S.A.). The 25.0 × 0.46 cm i.d. column was home-packed with 10 μm Partisil ODS3 (Whatman, Clifton, NJ, U.S.A.). The mobile phase was acetonitrile/0.2 M ammonium phosphate buffer pH 4.0/0.2 M tetramethylammonium phosphate (TMA) pH 4.0/water (35 : 5 : 20 : 40). Acetonitrile HPLC grade S was obtained from Rathburn (Walkerburn, U.K.). The 0.2 M TMA solution was prepared from a 20% w/v solution of tetramethylammonium hydroxide (Janssen Chimica, Beerse, Belgium) in methanol. The pH was adjusted to 4.0 with 85% phosphoric acid pro analysi (Merck) before the solution was brought to the final volume. The mobile phase was degassed by ultrasonication. The flow rate was 1.5 ml/min. The column was maintained at 35 °C by means of a waterjacket.

The HPLC method was derived from a method described previously (Cachet et al., 1987). A buffer of pH 4.0, instead of that described at pH 6.5, was used in the mobile phase. This allowed faster chromatography with sufficient selectivity for EA, EAEN, AEA and psEAEN, a compound which is formed in neutral solutions. A typical chromatogram is shown in Fig. 3. No degradation of the

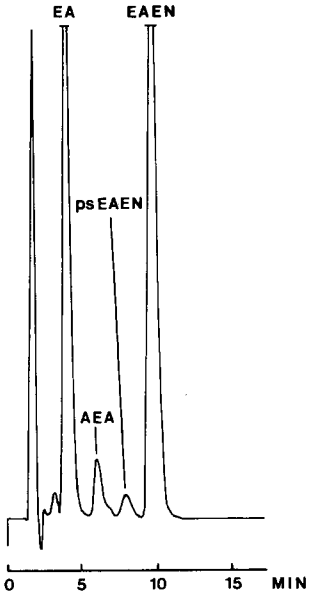

Fig. 3. HPLC chromatogram of a decomposition mixture of erythromycin A in 0.2 M potassium phosphate pH 5.83 after 48 h at 22°C. EA = erythromycin A; AEA = anhydroerythromycin; psEAEN = pseudoerythromycin A enol ether; and EAEN = erythromycin A enol ether.

acid-labile EA occurred on the column as evidenced by the good peak symmetry and the absence of related substances formed by acid degradation. Even with a mobile phase at pH 3, no degradation of EA was observed during the analysis. This can partly be explained by the short residence time of EA on the column. Additionally it is reasonable to accept that EA is only liable to degradation when present in solution. During a large portion of the chromatographic run, EA is adsorbed on the column.

Quantitation of EA and the decomposition products, EAEN and AEA, was based on peak area measurements. Calibration curves for the laboratory standards for EA, EAEN and AEA were obtained using solutions dissolved in a 0.2 M potassium phosphate buffer pH 7.45. The relationships between area y and the amount (μg) of product injected x are given in Table 1, where r = correlation coefficient, $S_{y,x}$ = standard error of estimate and CR = range of injected mass (μg) examined.

TABLE 1

Calibration curves for EA, EAEN and AEA

	Intercept	Slope	r	$S_{y,x}$	CR (μg)
EA	2157	10.6×10^4	0.9999	996	20–170
AEA	−419	35.3×10^3	0.9999	409	20–190
EAEN	129	27.8×10^5	0.9999	4689	1– 75

The detection limits were 0.6 μg for EA, 1.2 μg for AEA and 0.04 μg for EAEN. The reproducibility of the method was determined by analysing a sample of EA 42 times over a period of 5 days. The relative standard deviation was 0.5%.

Mechanism and model calculations

The calculations of the kinetic parameters based on a new degradation mechanism were performed using a multiparameter fitting program present in the BMDP statistical software (Ralston, 1983). More details about the use of this program are given in a companion paper (Vinckier et al., 1989). The essential elements of the EA decomposition scheme are given in Fig. 1. It consists of an equilibrium between EA and EAEN coupled to a direct conversion reaction from EA to AEA. This mechanism is in disagreement with literature results (Atkins et al., 1986), stating that the degradation of EA to AEA passes through EAEN. Evidence in favour of the new mechanism is extensively given in the companion paper (Vinckier et al., 1989). AEA shows no decomposition in the conditions used and can be considered to be the endproduct of EA decomposition in acidic medium. In neutral media (pH 6–8) small amounts of psEAEN are formed by translactonisation of erythromycin (Kibwage et al., 1987), resulting in a ring contraction of the aglycone (Fig. 2). This translactonisation is more important in alkaline medium above pH 8, where the formation of EAEN and AEA is less important. The mechanism of decomposition presented in the companion paper and used here as the basis for the calculations of the various rate constants is thus restricted to the decomposition of erythromycin below pH 6. The decomposition of EA in neutral and alkaline medium is not discussed in this paper.

The mass balance being better than 95% indicates that in our experiments all important decomposition products were measured by the HPLC method. The non-linear least-squares curve-fitting computer program was used to calculate the individual rate constants (k_1, k_2 and k_3). The calculations were based on the concentration–time profile of all products involved in the decomposition, thus not solely on the profile obtained for EA. Standard deviations given for each kinetic parameter were directly calculated by the BMDP program and reflect a statistical uncertainty. Linear regression was carried out by means of the SAS statistical package using the weighted values of the kinetic parameters to derive slopes and intercepts. The quoted error limits are the standard deviations.

TABLE 2

Observed rate constants (min^{-1}) for the degradation of EA as a function of the type of the 0.2 M buffer at pH = 3.97 with an ionic strength of 0.6 and at 22 °C

Buffer	$k_1 \times 10^{-2}$	$k_2 \times 10^{-2}$	$k_3 \times 10^{-2}$
Potassium phosphate	0.121 ± 0.008	0.652 ± 0.060	0.371 ± 0.002
Ammonium phosphate	0.141 ± 0.009	0.720 ± 0.006	0.431 ± 0.002
Sodium phosphate	0.153 ± 0.018	0.790 ± 0.117	0.380 ± 0.004
Sodium acetate	0.354 ± 0.083	1.61 ± 0.49	1.09 ± 0.03
Sodium citrate	0.215 ± 0.015	0.942 ± 0.093	0.512 ± 0.004

Results and Discussion

The various factors influencing the degradation rate of EA and EAEN such as the buffer type and concentration, the pH, the ionic strength and the temperature are now discussed in more detail. As already pointed out, AEA is stable in the experimental conditions used here.

Influence of the buffer type and concentration

The decomposition of EA is affected by the type of buffer used. This appears from experiments reported in Table 2 where other experimental conditions i.e. concentration of the buffer (0.2 M), pH (3.97), ionic strength (0.6) and temperature (22 °C), were constant. The cation of the buffer exerts some effect on the stability of EA. The reaction rates are somewhat larger for the sodium and ammonium phosphate buffers as compared to those obtained with the potassium phosphate buffer. The effect of the anion was also investigated. Erythromycin is most labile in the sodium acetate buffer and most stable in the phosphate buffer while intermediate decomposition rates are observed for citrate. The effect of the buffer concentration was examined with the

TABLE 3

Observed rate constants (min^{-1}) for the degradation of EA as a function of the concentration of the potassium phosphate buffer at pH = 3.86 with an ionic strength of 0.6 and at 22 °C

Buffer concentration (M)	$k_1 \times 10^{-2}$	$k_2 \times 10^{-2}$	$k_3 \times 10^{-2}$
0.075	0.141 ± 0.004	0.742 ± 0.027	0.440 ± 0.001
	0.124 ± 0.011	0.670 ± 0.087	0.438 ± 0.003
0.10	0.190 ± 0.002	0.894 ± 0.012	0.494 ± 0.005
0.15	0.206 ± 0.005	1.06 ± 0.04	0.546 ± 0.001
	0.197 ± 0.002	1.00 ± 0.02	0.543 ± 0.006
0.20	0.232 ± 0.017	1.05 ± 0.13	0.623 ± 0.004
0.30	0.296 ± 0.004	1.44 ± 0.03	0.756 ± 0.001
0.40	0.338 ± 0.005	1.67 ± 0.03	0.887 ± 0.001
Intercept (k_0)	(0.12 ± 0.01) × 10^{-2}	(0.60 ± 0.03) × 10^{-2}	(0.350 ± 0.007) × 10^{-2}
Slope	0.0055 ± 0.0006	0.027 ± 0.001	0.0130 ± 0.0003

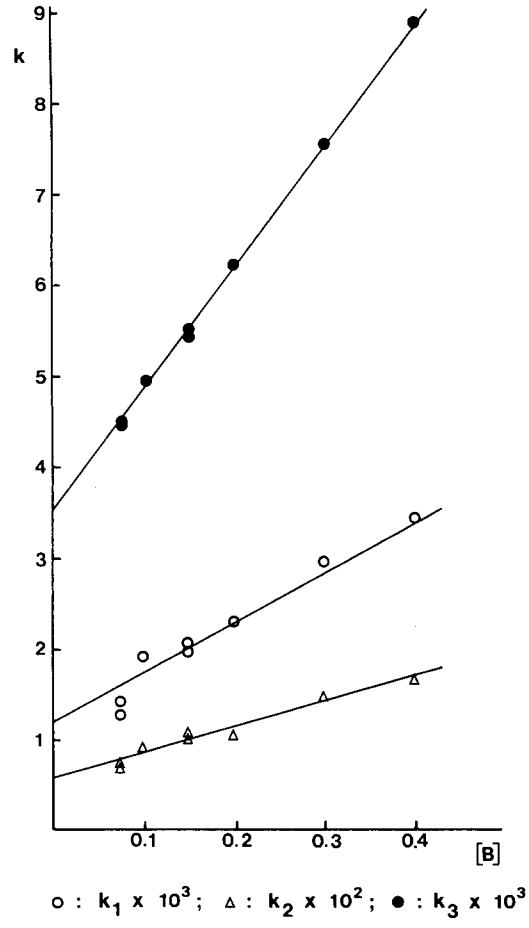

Fig. 4. Plot of k_{obs} against the concentration of potassium phosphate buffer pH 3.86 with ionic strength of 0.6 and at 22°C.

TABLE 4

Observed rate constants (min^{-1}) for the degradation of EA as a function of the ionic strength of the 0.2 M potassium phosphate buffer at pH 3.86 and at 22°C

Ionic strength (μ)	$k_1 \times 10^{-2}$	$k_2 \times 10^{-2}$	$k_3 \times 10^{-2}$
0.27	0.224 ± 0.009	1.05 ± 0.06	0.626 ± 0.002
0.33	0.237 ± 0.009	1.15 ± 0.06	0.625 ± 0.002
0.40	0.224 ± 0.007	1.11 ± 0.05	0.629 ± 0.002
0.47	0.229 ± 0.007	1.11 ± 0.04	0.629 ± 0.002
0.60	0.232 ± 0.017	1.05 ± 0.13	0.623 ± 0.004

potassium phosphate buffer at pH 3.86 (Table 3). The relationships between the observed rate constants, k_1, k_2 and k_3, and the buffer concentration are linear (Fig. 4).

Influence of ionic strength

To investigate the influence of ionic strength (μ) on the decomposition of EA various amounts of potassium chloride were added to a solution in 0.2 M potassium phosphate buffer at pH 3.86. From Table 4 it can be concluded that there is a negligible effect of ionic strength on the rate constants, k_1, k_2 and k_3, in the range examined, i.e. $\mu = 0.27$–0.60.

Influence of pH

The rate constants obtained from experiments at different pH values are given in Table 5. The

TABLE 5

Observed rate constants (min^{-1}) for the degradation of EA as a function of pH, in 0.2 M potassium phosphate buffer with an ionic strength of 0.6 and at 22°C

pH	k_1	k_2	k_3
1.97	0.205 ± 0.043	0.733 ± 0.197	0.913 ± 0.018
3.00	(0.219 ± 0.011) × 10^{-1}	(0.841 ± 0.107) × 10^{-1}	(0.917 ± 0.004) × 10^{-1}
3.86	(0.210 ± 0.039) × 10^{-2}	(1.04 ± 0.22) × 10^{-2}	(0.620 ± 0.011) × 10^{-2}
4.79	(0.385 ± 0.031) × 10^{-3}	(1.59 ± 0.25) × 10^{-3}	(1.14 ± 0.016) × 10^{-3}
5.83	(0.76 ± 0.11) × 10^{-4}	(6.23 ± 1.44) × 10^{-4}	(1.20 ± 0.02) × 10^{-4}
Slope	−0.98 ± 0.03 *	−0.96 ± 0.02 *	−1.09 ± 0.08 *
	−0.93 ± 0.04	−0.85 ± 0.08	−1.05 ± 0.05
Intercept	1.3 ± 0.1 *	1.8 ± 0.1 *	2.2 ± 0.2 *
	1.1 ± 0.2	1.4 ± 0.3	2.1 ± 0.2

* Rates obtained at pH 5.83 were not included in the calculation.

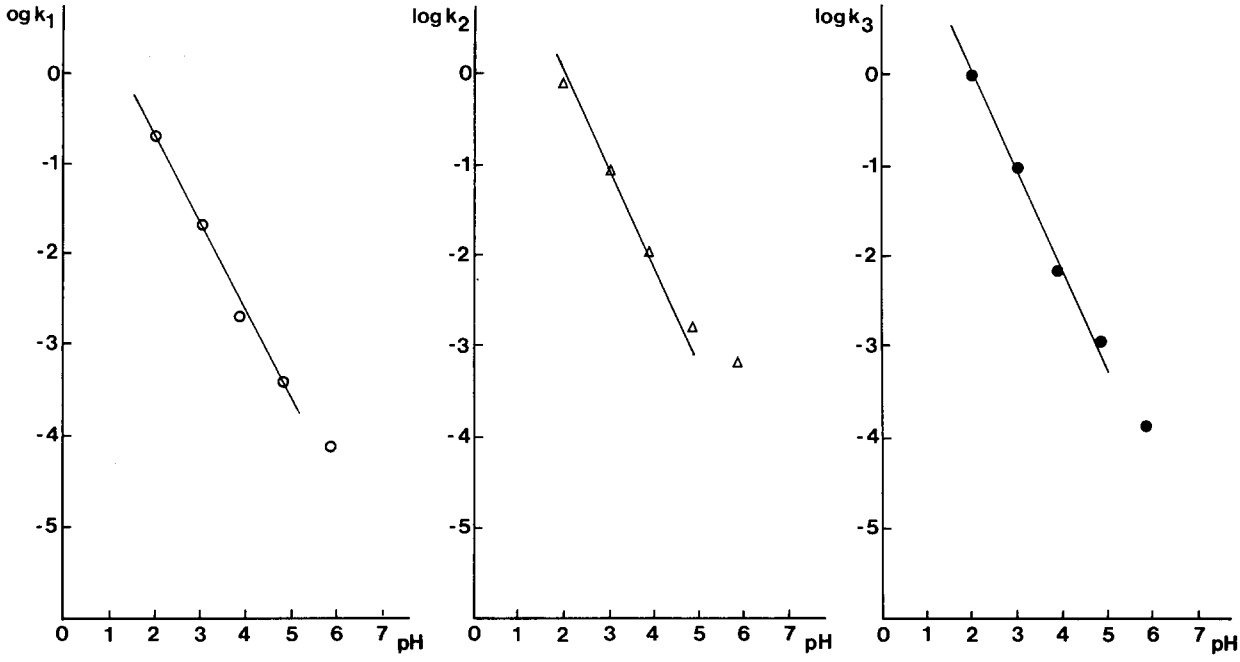

Fig. 5. Log k–pH profile for the degradation of erythromycin A in 0.2 M potassium phosphate buffer with ionic strength of 0.6 and at 22° C.

other experimental conditions, i.e. buffer type and concentration (0.2 M potassium phosphate), ionic strength (0.6) and temperature (22° C) were constant. The log k versus pH graphs of the various rate constants are depicted in Fig. 5. The linearity of the profiles in the pH range 1.97–4.79 indicates the occurrence of specific acid catalysis in the decomposition reaction. This is further confirmed by the slopes for k_1, k_2 and k_3 which are respectively -0.98 ± 0.03, -0.96 ± 0.02 and -1.09 ± 0.08. At higher pH, deviation from linearity starts to occur. Different explanations are possible. In the first place psEAEN is observed to be formed at a pH of about 6 and therefore the rate constants, calculated using the mechanism depicted in Fig. 1, are less valid. Secondly, since a specific base catalysis has been observed for the decomposition of erythromycin (Connors et al., 1986), one may expect some influence from it to occur already at neutral pH.

TABLE 6

Observed rate constants (min^{-1}) and Arrhenius parameters for the decomposition of EA as a function of temperature, in 0.2 M potassium phosphate buffer at pH 5.10 with an ionic strength of 0.6

Temp. (° C)	$k_1 \times 10^{-4}$	$k_2 \times 10^{-4}$	$k_3 \times 10^{-4}$
4	0.65 ± 0.06	4.81 ± 0.97	1.23 ± 0.02
22	2.06 ± 0.11	8.19 ± 1.12	7.58 ± 0.03
37	7.12 ± 0.41	17.78 ± 3.61	25.71 ± 0.13
50	22.52 ± 1.16	47.33 ± 6.40	85.43 ± 0.52
Slope ($-E_{obs}/R$)	−7268 ±646	−4743 ±897	−8098 ±312
E_{obs} (kJ/mol)	60.4 ± 5.4	39.4 ± 7.5	67.3 ± 2.6

Influence of temperature

The influence of temperature was investigated in the range 4–50 °C using a solution of EA in 0.2 M potassium phosphate buffer at pH 5.10. When the natural logarithms of the rate constants are plotted versus $1/T$, straight lines are obtained. From the slopes the apparent activation energies can be calculated as shown in Table 6.

Conclusion

The BMDP statistical software was also used in this paper to give evidence for a new mechanism for the decomposition of EA in acidic conditions. This mechanism, deduced in the companion paper, is confirmed in this work. The rate of decomposition of EA is largely dependent on the pH through acid catalysis and the effect of the buffer is significant. The effect of the temperature can be described by Arrhenius plots and the apparent activation energies of the reactions can be calculated.

Acknowledgements

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