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A new mechanism for the decomposition of erythromycin A in acidic aqueous solutions

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Summary

A new degradation mechanism for erythromycin A and erythromycin A enol ether in acidic aqueous solutions is proposed. It consists of an equilibrium between erythromycin A and erythromycin A enol ether coupled to a direct conversion reaction from erythromycin A to anhydroerythromycin A. The modelling of the experimentally obtained concentration profiles of erythromycin A, erythromycin A enol ether and anhydroerythromycin A is carried out using the BMDP statistical package containing a multiparameter fitting program based on the DUD algorithm. The values for the rate constants deduced from the erythromycin A and erythromycin A enol ether decays are in good agreement yielding support for the proposed mechanism.

Introduction

Erythromycin is the most important member of the macrolide antibiotics and was introduced into clinical use more than 35 years ago (McGuire et al., 1952). Although erythromycin is known to decompose rapidly in acidic conditions, only a few reports deal with the kinetics of erythromycin decomposition (e.g. Connors et al., 1986). The first systematic study of the kinetics of the decomposition of erythromycin in acidic and neutral buffers has been reported recently (Atkins et al., 1986). In their study a spectrophotometric method was used to generate most data. Although it is well established that erythromycin A decomposes in acidic solutions to anhydroerythromycin A with the loss of one molecule of water (Kurath et al., 1971; Connors et al., 1986), the detailed reaction mechanism is still open for discussion. In the study of Atkins et al. (1986), a mechanism was proposed involving two consecutive steps:

erythromycin A (EA)

$\xrightarrow{k_a}$ erythromycin A enol ether (EAEN)

followed by

EAEN $\xrightarrow{k_b}$ anhydroerythromycin A (AEA)

According to this mechanism EAEN (sometimes erroneously called erythromycin A 6–9-hemiketal) is an intermediate in the overall conversion process of EA to AEA. The rate constant, k_a, was

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determined in a variety of experimental conditions such as pH, buffer concentration and temperature using the 'initial rate' method (Connors, 1981) on the first-order decay of EA. In the same study (Atkins et al., 1986) values for k_b were determined from the decay of EAEN over a much broader concentration range up to 98% conversion. From the combination of both experiments, reliable rate coefficients can be obtained if the proposed two-step reaction mechanism is well established.

However, from the straightforward observation in our laboratory that EA is also formed as a degradation product of EAEN, we had to conclude that the foregoing mechanism was at least incomplete and that it was worthwhile to reinvestigate the kinetics of the degradation of both EA and EAEN in acidic aqueous solutions. In order to verify the reaction mechanism, the concentrations of EA, EAEN and AEA were simultaneously followed by HPLC as a function of the reaction time. It will be shown that contrary to the mechanism shown above, EA directly decomposes to AEA without having to pass through the EAEN intermediate. Moreover the direct reaction from EAEN towards AEA seems to be unimportant. Instead, the conversion must pass through EA itself.

We now propose another mechanism consisting of an equilibrium between EA and EAEN:

$$\mathrm{EA} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{EAEN}$$

with a simultaneous degradation path:

$$\mathrm{EA} \xrightarrow{k_3} \mathrm{AEA}$$

This mechanism is validated by comparing the experimentally obtained concentrations of the 3 components with the calculated profiles using a multiparameter fitting computer program available in the BMDP statistical package.

Materials and Methods

The preparation of the different compounds and the analytical techniques used in this study are described in detail in the companion paper (Cachet et al., 1989).

Results and Discussion

Experimental results

In order to examine the reaction mechanism, two experiments were carried out at a temperature of 22°C in a 0.2 M potassium phosphate buffer pH 3.86. In the first instance, the decay of EA was monitored as a function of the reaction time for about 6 h. Simultaneously with the decay of EA, the concentrations of both EAEN and AEA were measured. The concentration–time evolution is shown in Fig. 1. One clearly sees that AEA is formed at the expense of EA itself with EAEN being an intermediate always present in relatively low concentration. This observation would still confirm the mechanism presented by Atkins et al. (1986) where a consecutive reaction lies at the basis of the EA conversion to the ultimate product AEA.

In a second experiment under the same experimental conditions the decay of EAEN was followed as shown in Fig. 2. Here also it appears that EAEN is consumed to a large extent to form AEA. However, simultaneously a significant quantity of EA is also formed. EA is not at all expected to occur when a consecutive decomposition mechanism prevails. Additionally, the shape of the AEA concentration profile shows an induction period not observed for EA. This led us to believe that EA is now an intermediate in the conversion processes of EAEN.

The kinetic mechanism can be mathematically represented by a set of 3 differential equations describing the net chemical reaction rate of each component during the time of the experiment. Since the analytical solution of this set of differential equations is very complex and in many cases even impossible, the numerical solution for modelling the various concentration profiles is obtained by a non-linear least-squares multiparameter fitting program. A comparison between the calculated and the experimental concentrations serves as the validation criterion of the overall reaction mechanism.

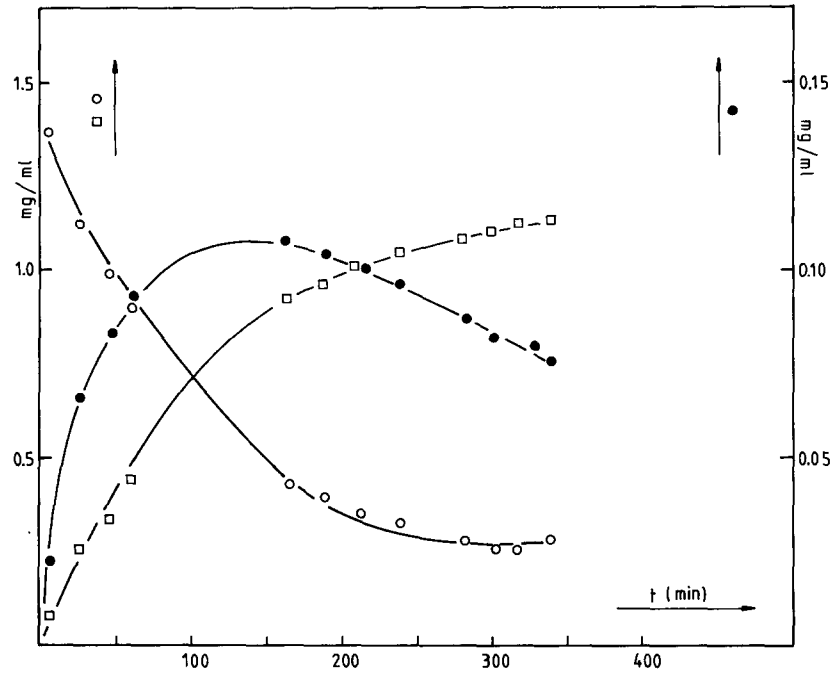

Fig. 1. Decay of erythromycin A (○) and formation of erythromycin A enol ether (●) and anhydroerythromycin A (□). Concentrations are expressed in mg/ml. The experimental conditions are: $T = 22°$ C, pH = 3.86, solvent: 0.2 M potassium phosphate buffer.

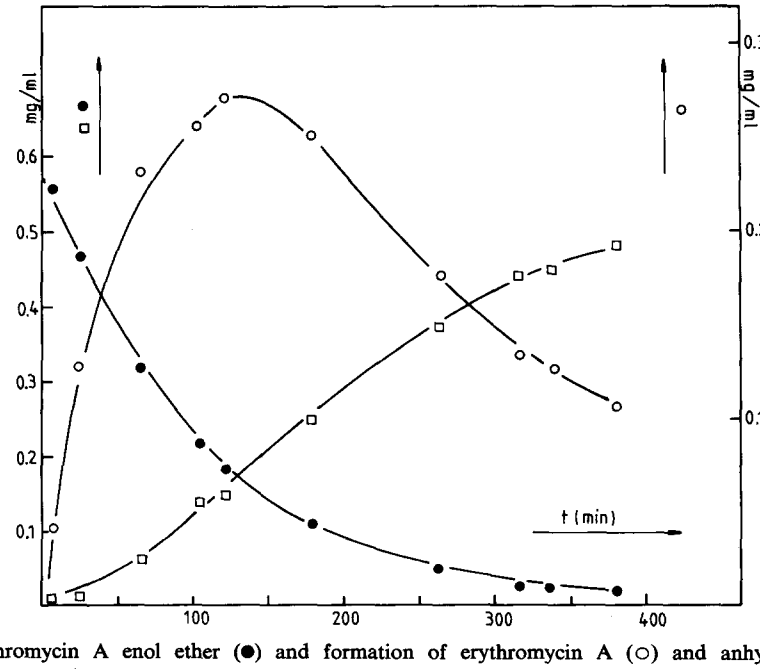

Fig. 2. Decay of erythromycin A enol ether (●) and formation of erythromycin A (○) and anhydroerythromycin A (□). Concentrations are expressed in mg/ml. Experimental conditions are the same as in Fig. 1.

Procedure

In order to calculate the concentration–time evolution of EA, EAEN and AEA, the statistical package BMDP (Ralston, 1983) is used. The basic principles and its possible application to kinetic systems have already been described by Copeland (1984). The non-linear regression program is based on the DUD algorithm (does not use derivatives) developed by Ralston and Jennrich (1978). While most regression programs require the analytical expression of the partial derivative of the regression function to each of the parameters, the DUD procedure starts the parameter search without the use of these derivative functions. Another advantage of this statistical package is that it contains a subprogram DEFUN which allows one to carry out numerical integrations for the evaluation of the regression functions when the latter are given as a set of differential equations. The only functions required for the calculations are dEA/dt, dEAEN/dt and dAEA/dt, each depending on the kinetic mechanism proposed.

The determination of the appropriate rate constants, k_i, treated in this program as parameters to be optimised, is carried out with a least-squares method for non-linear parameter estimation based on DUD. While in most kinetic studies the optimisation procedure is only applied to the concentration profile of one component, we use the experimental profiles of EAEN, EA and AEA simultaneously. In this way the derived parameters, and thus the reaction rate constants are obtained from a minimisation of the function Q which is the weighted sum of squares of the residuals between the experimental and calculated concentration of the 3 components EA, EAEN and AEA participating in the reaction process.

Model calculations

Decay of EAEN. Starting from EAEN in Fig. 2, concentration profiles of EAEN, EA and AEA are calculated as a function of the reaction time, t, using different reaction mechanisms compiled in Table 1. Mechanism I is the one adhered to by Atkins et al. (1986) while mechanisms II–V are alternative reaction sequences which may be proposed to explain the kinetic behaviour of EA, EAEN and AEA. The results of the calculations

TABLE 1

Reaction mechanisms for erythromycin conversion

Mechanism		Symbol *
I	$EA \xrightarrow{k_a} EAEN \xrightarrow{k_b} AEA$	■
II	$EAEN \underset{k_1}{\overset{k_2}{\rightleftharpoons}} EA \xrightarrow{k_3} AEA$	□
III	$EAEN \xrightarrow{k_2} EA \xrightarrow{k_3} AEA$	▲
IV	$EAEN \xrightarrow{k_4} AEA$; $\searrow k_2$ EA	●
V	$EAEN \xrightarrow{k_4} AEA$; $k_1 \nwarrow \searrow k_2$ EA	○

* The symbols refer to the mechanism used for the calculation of the various concentration profiles.

are shown in Figs. 3–5. In Fig. 3 it is shown that the decay of EAEN is fairly well predicted with all mechanisms except mechanism V, which shows a large deviation at long reaction times. Of course we have omitted mechanism I in these calculations since it does not allow for the formation of EA from EAEN, which is contradicted by the experimental findings as shown in Fig. 2. It is also clear that by only following the initial decay of EAEN no conclusive arguments can be drawn on the validity of mechanisms II–IV. The profiles of EA, however, are much more sensitive to the proposed reaction mechanisms since one observes that now both mechanisms IV and V result in bad fits (Fig. 4), while mechanisms II and III yield a good agreement between the calculated and experimental concentration profiles. These findings are confirmed in Fig. 5 where the build-up of AEA is followed. The experiment shows an induction period which mechanisms IV and V fail to include. This means that there is no direct path to form AEA from EAEN but that instead the latter compound must first be converted to EA which then directly degrades to AEA. From the foregoing calculations it is not yet possible to distinguish between mechanisms II and III; i.e. whether EAEN

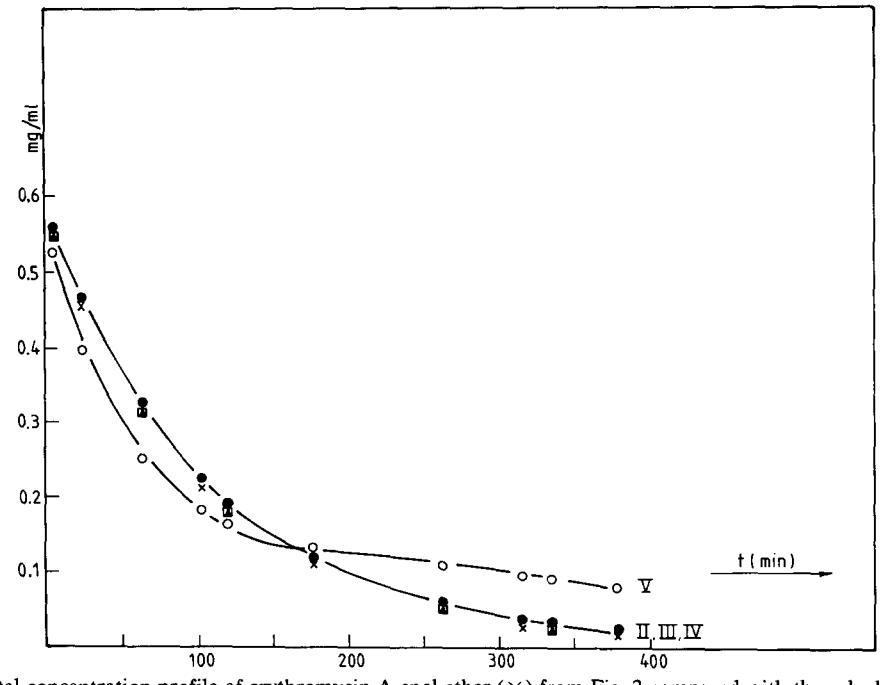

Fig. 3. Experimental concentration profile of erythromycin A enol ether (×) from Fig. 2 compared with the calculated concentration profiles according to the mechanisms II (□) and III (▲) which are superposed and represented as ▲, mechanisms IV (●) and V (○) from Table 1.

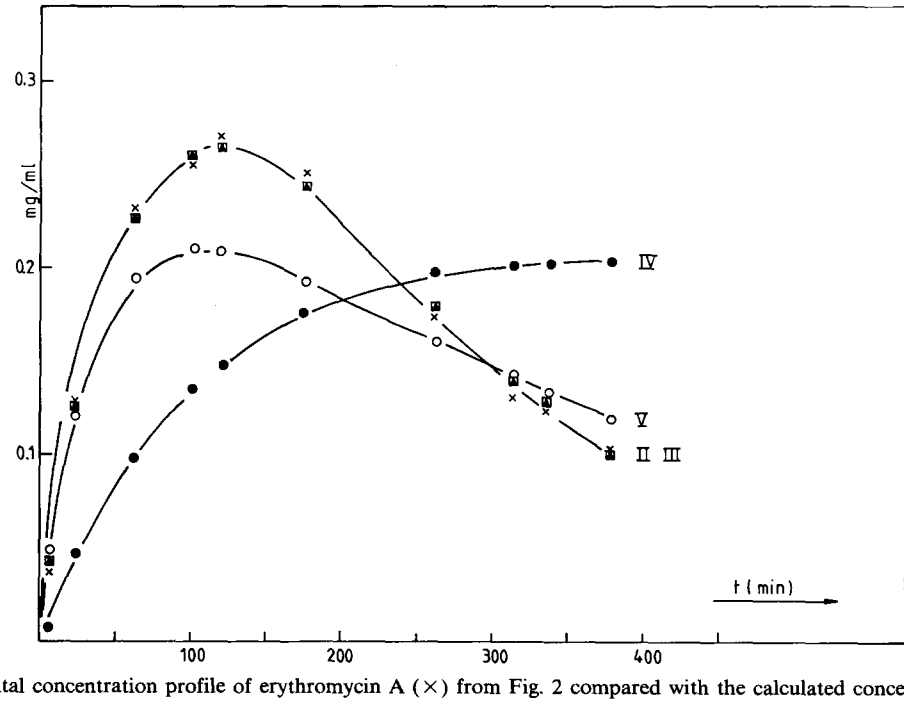

Fig. 4. Experimental concentration profile of erythromycin A (×) from Fig. 2 compared with the calculated concentration profiles according to various reaction mechanisms of Table 1. The symbols have the same meanings as in Fig. 3.

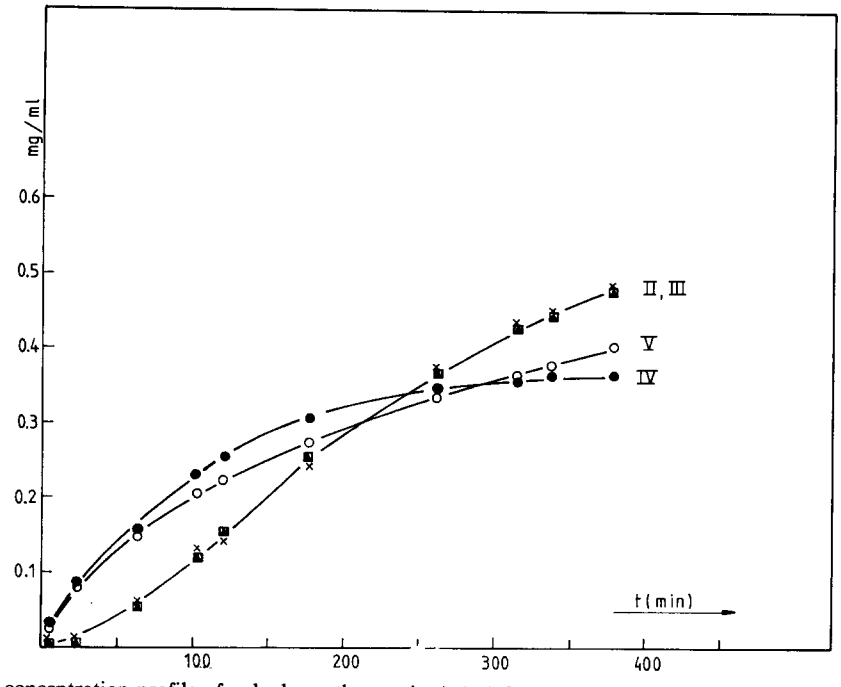

Fig. 5. Experimental concentration profile of anhydroerythromycin A (×) from Fig. 2 compared with the calculated concentration profiles according to various reaction mechanism of Table 1. The symbols have the same meanings as in Fig. 3.

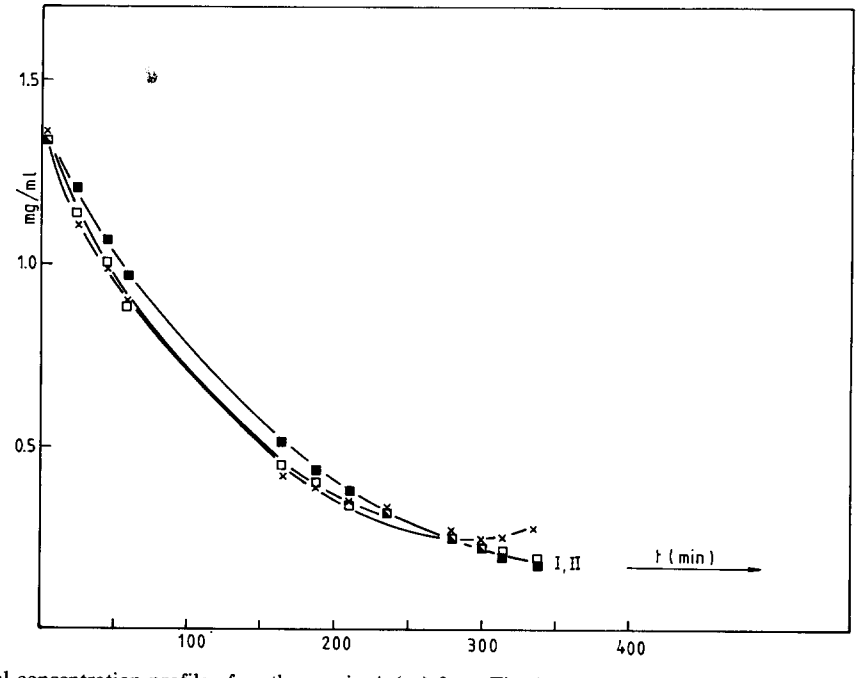

Fig. 6. Experimental concentration profile of erythromycin A (×) from Fig. 1 compared with the calculated concentration profiles according to mechanisms I (■) and II (□) from Table 1.

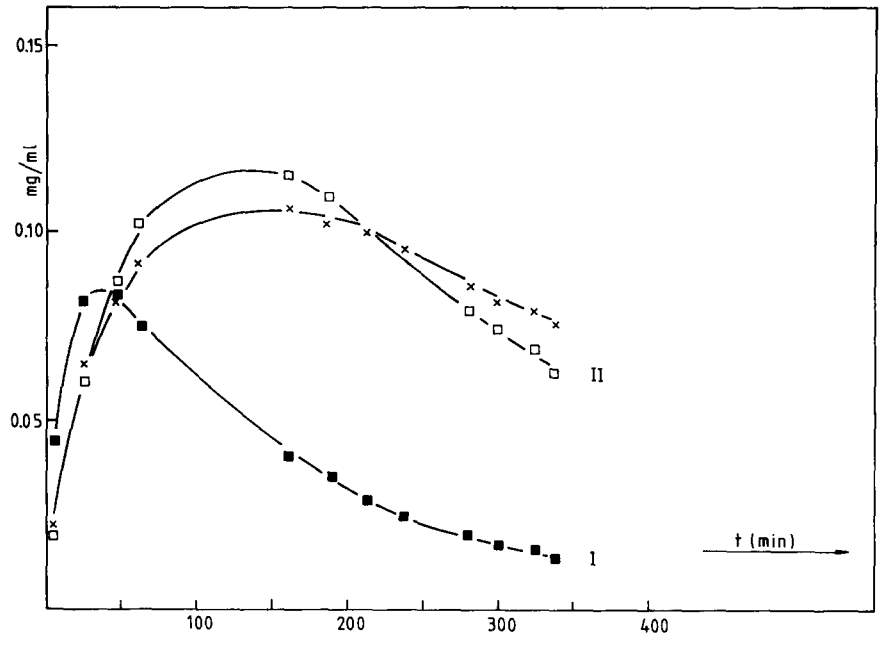

Fig. 7. Experimental concentration profile of erythromycin A enol ether (×) from Fig. 1 compared with the calculated profiles according to mechanisms I and II from Table 1. The symbols have the same meanings as in Fig. 6.

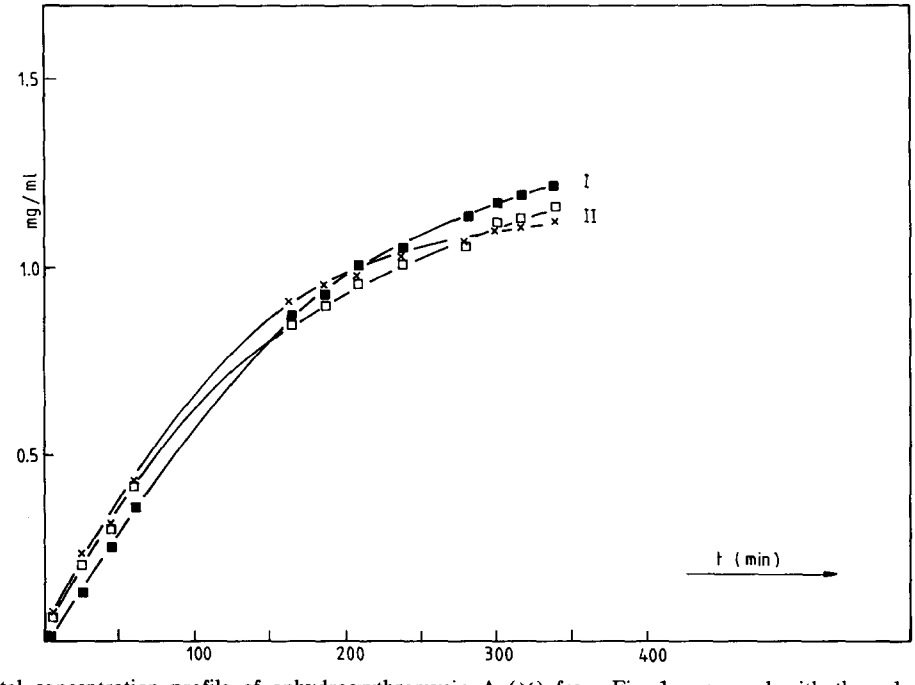

Fig. 8. Experimental concentration profile of anhydroerythromycin A (×) from Fig. 1 compared with the calculated profiles according to mechanisms I and II from Table 1. The symbols have the same meanings as in Fig. 6.

goes to an equilibrium with EA or not cannot be derived from this type of experiment. However, we know that the degradation of EA (Fig. 1) also yields EAEN, so that the reverse reaction EA → EAEN must be considered as is only the case in mechanism II.

Decay of EA. In order to check the validity of mechanism II, the calculations are now performed on the results of Fig. 1 where the decay of EA was followed. Although we already know that mechanism I cannot explain our results of the degradation of EAEN, it is interesting to examine the sensitivity of this mechanism to the results obtained from the degradation of EA. Its concentration–time behaviour is fairly well predicted by both mechanisms I and II, although at short reaction times mechanism II yields a better agreement (Fig. 6). However, when the concentration of EAEN is calculated according to mechanism I, it is too low by more than a factor of two demonstrating that this consecutive reaction sequence is unacceptable (Fig. 7). Finally one can observe that the concentration of AEA is also better modelled by mechanism II (Fig. 8). The foregoing leads us to the final conclusion that only mechanism II is capable of predicting the observed concentration–time evolution of all 3 components: EA, EAEN and AEA. This means that the conversion of EA goes directly to AEA without the need of going through the EAEN stadium. Vice versa this implies that the degradation of EAEN does not directly yield AEA but that it has to be first transformed into EA.

Derivation of the rate constants k_1, k_2 and k_3. Besides the quality of the profile fits, another important criterion for the validation of a reaction mechanism is the value of the derived parameters k_1, k_2 and k_3 as they are shown in Table 2. Data shown in Fig. 1 yield rate constants k_1 and k_2 with a standard deviation of about 20% while the uncertainty on k_3 is only of the order of 2%. This can be explained by the interdependence of both k_1 and k_2 since in mechanism II EA and EAEN evolve to an equilibrium so that both rate constants are correlated as shown by the values in the correlation matrix. In Table 2, a correlation factor close to unity means that the rate constants are so strongly coupled (positively or negatively) that it becomes impossible to derive them independently as is the case when chemical equilibrium is fully established. A correlation factor of zero indicates that the parameters are completely independent from each other and thus do not influence each other's accuracy. From Table 2 one can conclude that k_3 is nearly independent from k_1 and k_2 which explains the low error on k_3, while k_1 and k_2 are rather strongly coupled and can only be determined within 20% standard deviation. The calculations on the profiles of Fig. 2 allowed one to derive k_2 and k_3 fairly well in good agreement with the previous values. However, the profiles do not seem to be sensitive to the rate constant k_1 in view of the extremely large calculated error on k_1. This can be explained as follows: EA is in this experiment an intermediate nearly always present in minor concentrations. Additionally the value of

TABLE 2

Rate constants (min^{-1}) and correlation matrices for k_1, k_2 and k_3 calculated for the erythromycin conversion according to mechanism II at pH = 3.86

Experiment			
	k_1	k_2	k_3
Erythromycin A (Fig. 1)	$2.1 \pm 0.39 \times 10^{-3}$	$1.0 \pm 0.22 \times 10^{-2}$	$6.2 \pm 0.11 \times 10^{-3}$

Correlation matrix	k_1	k_2	k_3
k_1	1.0	0.88	0.35
k_2	0.88	1.0	0.17
k_3	0.35	0.17	1.0

	k_1	k_2	k_3
Erythromycin A enol ether (Fig. 2)	$1.6 \pm 1.4 \times 10^{-4}$	$1.0 \pm 0.01 \times 10^{-2}$	$6.7 \pm 0.06 \times 10^{-3}$

Correlation matrix	k_1	k_2	k_3
k_1	1.0	0.80	−0.10
k_2	0.80	1.0	−0.27
k_3	−0.10	−0.27	1.0

	k_2	k_3
k_1 fixed at 2.1×10^{-3}	$1.1 \pm 0.02 \times 10^{-2}$	$6.6 \pm 0.1 \times 10^{-3}$

Correlation matrix	k_2	k_3
k_2	1.0	−0.35

k_1 as calculated from the previous experiment is a factor of 5 lower than k_2 so that the product $k_1 \cdot |EA|$ will always be too small in comparison with $k_2 \cdot |EAEN|$ in order to calculate k_1 accurately in this system. This brings us to the point of selecting the suitable experimental conditions for the derivation of the kinetic coefficients. In view of their sensitivity to the individual concentration profiles, it is only possible to calculate k_1, k_2 and k_3 from experiments with EA as starting reactant and not from EAEN. Therefore the technique of modelling is not only required for the exact derivation of the rate constants but also for the determination of the experimental conditions most favourable for accurate parameter calculations. So we were obliged to keep the value of k_1 fixed at 2.1×10^{-3} min^{-1} as obtained from the first calculation. As shown in Table 2, we then found values for k_2 and k_3 in satisfactory agreement with those obtained from the EA decay (Fig. 1).

A number of experiments have also been carried out in a 0.2 M potassium phosphate buffer of pH 1.97. The temperature was kept at 22°C. The results are summarized in Table 3. Again it is demonstrated that k_1, k_2 and k_3 can be reasonably well determined with EA as a starting compound, while with EAEN the value of k_1 had to be fixed at 0.2 min^{-1}. Under these circumstances the values for k_2 or k_3 derived from the two experiments are within each others' error range. The experiments at pH 1.97, where the individual values of k_1, k_2 and k_3 are almost two orders of magnitude larger than at pH 3.86, nicely confirm our proposed reaction mechanism II for the decay of erythromycin compounds in acidic aqueous solutions.

It should be finally mentioned that the other reaction mechanisms also allowed one to generate values for the rate constants k_1, k_2 and k_3. But as already demonstrated, these sets did not reproduce well the concentrations of all components involved. For these mechanisms the sum of the residual-squares Q values were a factor of 4–10 higher than for mechanism II.

It may be concluded that a simple consecutive reaction mechanism cannot explain the overall erythromycin conversion processes. A more complicated mechanism involving an equilibrium between EA and EAEN has to be included. Also a direct reaction path from EA to AEA must be considered. This system is quite a good illustration of the dangers of using the 'initial rate' method for deriving kinetic coefficients, especially when the reaction mechanism is not well established. When the experimental concentration profiles of all components involved are available, the BMDP statistical package is a very powerful tool for the validation of the kinetic mechanism and for the derivation of the appropriate rate constants.

TABLE 3

Rate constants (min^{-1}) and correlation matrices for k_1, k_2 and k_3 calculated for the erythromycin conversion according to mechanism II at pH 1.97.

Experiment			
	k_1	k_2	k_3
Erythromycin A	0.20 ± 0.04	0.73 ± 0.19	0.91 ± 0.02

Correlation matrix	k_1	k_2	k_3
k_1	1.0	0.92	0.50
k_2	0.92	1.0	0.38
k_3	0.50	0.38	1.0

	k_2	k_3
Erythromycin A enol ether		
k_1 fixed at 0.2	0.90 ± 0.05	0.88 ± 0.06

Correlation matrix	k_2	k_3
k_2	1.0	−0.41

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