International Journal of Pharmaceutics, 56 (1989) 159–168
Elsevier

IJP 01903

Erythromycin prodrugs: kinetics of hydrolysis of erythromycin and various erythromycin 2′-esters in aqueous solution and human plasma

Bente Steffansen and Hans Bundgaard

The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, Copenhagen (Denmark)

(Received 2 March 1989)
(Accepted 10 May 1989)

Key words: Erythromycin; Erythromycin 2′-ester; Prodrug; Stability; Enzymatic hydrolysis

Summary

The kinetics of hydrolysis of various alkyl, dicarboxylic acid, carbonate and aromatic 2′-esters of erythromycin was studied in aqueous solution (pH 4–11.5) and human plasma with the aim of developing enzymatically labile erythromycin prodrug esters. The stability of erythromycin was also determined over the pH range 4–10 at 37 °C. Whereas the esters possessed almost the same stability as parent erythromycin in acidic solutions, they were much more unstable at pH > 6. The facile hydrolysis of the esters to erythromycin in neutral and alkaline solutions was attributed to an intramolecular catalytic effect by the neighbouring dimethylamino group in the compounds. Human plasma inhibited the rate of hydrolysis of the more lipophilic esters whereas the rate of hydrolysis of ionized dicarboxylic acid esters and less lipophilic alkyl esters was accelerated or unaffected by plasma. Suggestions are made for the design of erythromycin esters that are more readily converted to the parent drug in vivo than those presently used clinically.

Introduction

Erythromycin (**I**) is a widely used macrolide antibiotic active against most Gram-positive bacteria and some Gram-negative organisms. Since its introduction more than 25 years ago, a number of formulations and chemical derivatives and salts have been developed in an effort to provide a product with optimal gastrointestinal absorption, reduced gastrointestinal side-effects and without the bitter taste possessed by the drug. Erythromycin is very easily degraded in acidic solution corresponding to the conditions prevailing in the stomach (Boggiano and Gleeson, 1976; Atkins et al., 1986) and, accordingly, various approaches have been used to protect the drug against gastric inactivation. Commonly used preparations include enteric-coated tablets, capsules or pellets of erythromycin base, tablets of the slightly water-soluble stearate salt of erythromycin and tablets, capsules or suspensions of 2′-esters of erythromycin, notably the ethylsuccinate and propionate ester, the latter in the form of a lauryl sulfate salt called erythromycin estolate. Recently, the 2′-acetate ester of erythromycin in the form of the stearate salt (erythromycin acistrate) has been introduced.

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

As reviewed by Fraser (1980) considerable differences in the bioavailability and tolerability of these various formulations have been reported. For pediatric use the preferred dosage forms seem to be aqueous suspensions of the tasteless erythromycin esters whereas enteric-coated preparations of erythromycin base are generally preferred as the solid oral dosage forms. The acetate ester has, however, recently been shown to involve fewer and milder gastrointestinal side-effects than enteric-coated erythromycin base tablets (Gordin et al., 1988a) and also to be better absorbed (in terms of total erythromycin and ester blood concentrations) than various commercial erythromycin preparations (Männistö et al., 1988; Tuominen et al., 1988).

2′-Esters of erythromycin are antimicrobially inactive or at least much less active than the parent drug (Murphy, 1953; Tardrew et al., 1969; Sinkula, 1974) and, accordingly, they should be regarded as prodrugs requiring hydrolytic conversion to the free erythromycin in vivo in order to exhibit antibacterial activity. An important but often overlooked drawback of the esters used today is that they are only partly converted to erythromycin in the body. Thus, the propionate and ethylsuccinate esters are largely excreted unchanged, with only 20–35% of an orally given dose being present in the blood as free erythromycin (Stephens et al., 1969; Bechtol et al., 1976, 1981; Welling et al., 1979; Frazer, 1980; Yakatan et al., 1980, 1985). Similarly, only about 25% of the acetate ester is converted to erythromycin following oral administration in humans (Männistö et al., 1988; Gordin et al., 1988b).

While the stability of erythromycin has been thoroughly characterized in aqueous solution at pH 3–7 (Atkins et al., 1986; Amer and Takla, 1978), only sparse information is available on the stability of erythromycin esters in aqueous solution or in the presence of plasma (Tardrew et al., 1969; Stephens et al., 1969; Tsuji and Goetz, 1978a; Tserng and Wagner, 1976; Taskinen and Ottoila, 1988). The present study was undertaken to obtain detailed knowledge of the chemical stability of erythromycin 2′-esters in aqueous solution as a function of pH and on the kinetics of hydrolysis in human plasma solutions. In addition, the degradation of erythromycin was studied over a wide pH range. Several 2′-esters encompassing both aliphatic, aromatic and ionic esters were included in the study since information on the influence of ester structure on the hydrolysis rates was considered useful in achieving an ultimate aim of the study: the design of an erythromycin prodrug that is completely converted to the parent active drug in vivo and hence being more bioavailable than the presently used ester prodrugs.

Materials and Methods

Apparatus

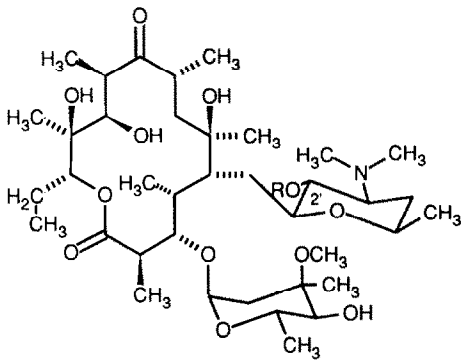
High-performance liquid chromatography (HPLC) was done with a system consisting of a Waters pump model 6000A, a variable wavelength UV-detector (Waters Type Lambda Max 480), a 20-μl loop injection valve (Rheodyne 7125) and a Chrompack column (100 × 3 mm) packed with CP Spher C18 (5-μm particles). In some cases, a deactivated reversed-phase Supelcosil C8 (3-μm particles) column (33 × 4.6 mm) was used. Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Microanalyses were performed at Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

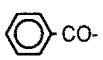
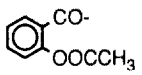
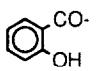
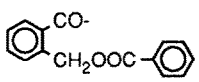
Erythromycin and erythromycin ethylsuccinate were purchased from Sigma Chemical Co., St. Louis. Chemicals and solvents used in the kinetic studies were of reagent grade.

Preparation of erythromycin 2′-esters

The acetate (**II**) and propionate (**III**) esters were prepared by reacting erythromycin with the appropriate acid anhydrides in acetone as described by Stephens and Conine (1959). The succinate ester (**V**) was obtained as described by Stephens (1959) and the glutarate ester (**VI**) as reported by Sinkula (1974). The methyl carbonate ester (**VII**)



R

I	H
II	CH_3CO-
III	CH_3CH_2CO-
IV	$C_2H_5OOCCH_2CH_2CO$-
V	$HOOCCH_2CH_2CO$-
VI	$HOOCCH_2CH_2CH_2CO$-
VII	CH_3OCO-
VIII	
IX	
X	
XI	

was prepared by reacting erythromycin with methyl chloroformate as described by Murphy (1954) whereas the benzoate (**VIII**), 2-acetoxybenzoate (**IX**) and salicylate (**X**) esters were obtained by using the general procedure described by Dall'Asta et al. (1988). The melting points for these already-described esters agreed with those reported in the literature (Clark and Warner, 1957; and the above-mentioned references).

The 2-benzoyloxymethylbenzoate ester **XI** has not been reported before. It was prepared as follows: a mixture of erythromycin (2.94 g, 4 mmol), sodium bicarbonate (1.7 g, 20 mmol) and 2-benzoyloxymethylbenzoyl chloride (1.1 g, 4 mmol) (purchased from Fluka, Switzerland) in acetone (25 ml) was stirred at room temperature for 5 h and filtered. A 2% aqueous sodium bicarbonate solution (about 10 ml) was added to the filtrate and the mixture kept at 4°C for 3 h. The precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol–acetone to give the ester **XI** in a yield of 60%. M.p. 126–128°C. *Anal.*: Calc. for $C_{52}H_{77}NO_{16}$: C, 64.24; H, 7.98; N, 1.44. Found: C, 64.25; H, 7.86; N, 1.41.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were acetate, phosphate, *N*-ethylmorpholine, borate and carbonate buffers. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The progress of the decomposition was followed by using reversed-phase HPLC methods. Mobile phase systems of 10% v/v methanol, 10% v/v 0.2 M ammonium acetate and 35–45% v/v acetonitrile–water mixtures with pH adjusted to 6.1 were generally used. The concentration of acetonitrile was adjusted for each compound to give an appropriate retention time (2–6 min). The column effluent was monitored at 233 nm in cases of the esters **VIII–XI** and at 215 nm for the remaining esters as well as erythromycin. Quantitation of the compounds was done by measuring the peak areas (Spectra-Physics SP-4290 integrator) in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 100–200 μl of a stock solution of the compounds in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 10^{-5} M (compounds **VIII–XI**) or 10^{-3} M (compounds **I–VII**). The solutions were kept in a water bath at 37°C and at appropriate intervals samples were taken and chromatographed. Pseudo-first-order rate constants for the degradation were determined

from the slopes of linear plots of the logarithm of residual derivative against time.

In case of the hydrolysis studies performed in human plasma diluted to 80% with 0.02 M phosphate buffer of pH 7.40, the reactions were initiated as described above. At appropriate intervals, samples of 250 µl were withdrawn and added either to 500 µl of acetonitrile or 500 µl of a 2% solution of zinc sulphate in 50% v/v methanol in order to deproteinize the plasma. After immediate mixing and centrifugation at 13,000 rpm for 3 min, 20 µl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

The kinetics of decomposition of various 2′-esters (**II–XI**) of erythromycin (**I**) was studied in aqueous solution at 37° C over a wide range of pHs (4–11.5). For the sake of comparison the kinetics of decomposition of erythromycin itself was also determined at the same conditions. At constant pH and temperature the disappearance of the esters or of erythromycin displayed strict first-order kinetics over several half-lives and all reactions proceeded to completion. Some examples demonstrating the first-order kinetics of degradation at constant pH are shown in Fig. 1. This finding strongly indicates that the HPLC procedures used to follow the progress of decomposition are capable of separating the esters (and erythromycin) from their various degradation products.

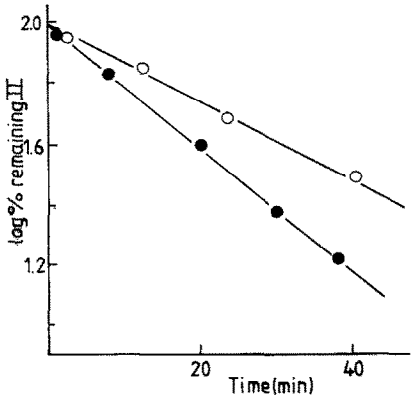


Fig. 1. First-order plots for the degradation of erythromycin 2′-acetate (**II**) in aqueous buffer solution of pH 4.00 (●) and pH 7.40 (○) at 37° C.

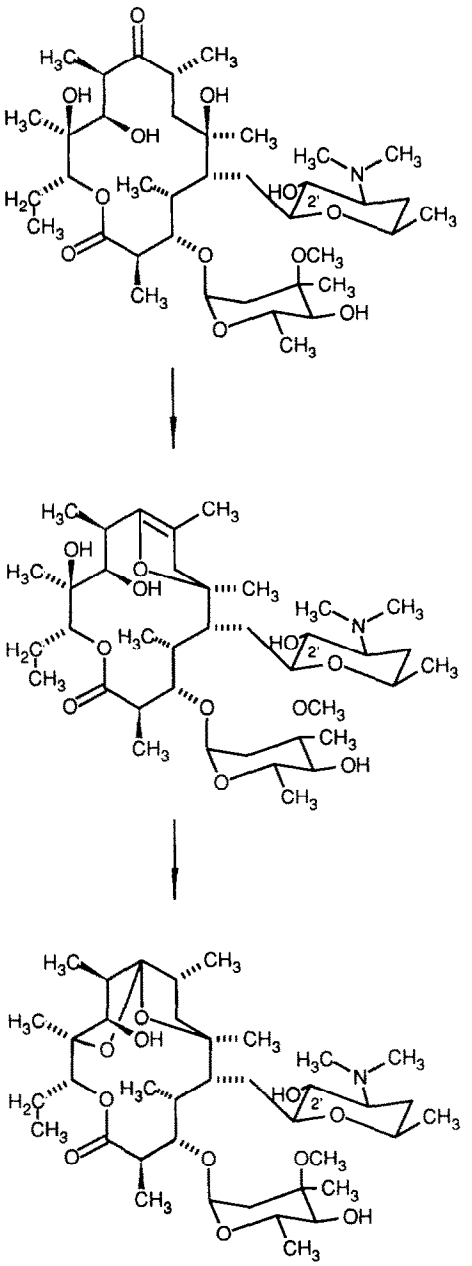


Fig. 2. Acid-catalyzed conversion of erythromycin to anhydroerythromycin via erythromycin 6,9-hemiketal. Similar conversions occur for erythromycin 2′-esters.

In acidic aqueous solution erythromycin is known to be degraded to anhydroerythromycin through dehydration of the initially formed 6,9-hemiketal erythromycin (Fig. 2) (Wiley et al., 1957; Stephens and Conine, 1959; Kurath et al., 1971; Atkins et al., 1986). In alkaline solutions "dihydroerythromycin" has been reported to be a major degradation product (Tsuji and Goetz, 1978b). Hydrolytic opening of the lactone moiety may also be an expected route of decomposition in basic solutions (Flynn et al., 1954). In cases of erythromycin esters the same types of dehydration reactions in acidic solutions as for erythromycin occur (Fig. 2) (Stephens and Conine, 1959; Tsuji and Goetz, 1978b). The latter authors also showed for erythromycin ethylsuccinate that at pH 6–8 the major product of degradation was erythromycin.

In agreement with these earlier reports we found that all the esters studied were quantitatively hydrolyzed to erythromycin in aqueous solution at pH 7–10 as well as in the presence of human plasma at pH 7.4 as evidenced by HPLC analysis of the reaction solutions. A typical plot illustrating the formation of erythromycin from an ester at pH 7.4 is shown in Fig. 3. Similarly, by following the reaction progress spectrophotometrically at 230 nm as described by Atkins et al. (1986) for erythromycin, the appearance and subsequent disappearance of the 6,9-hemiketal product from some esters (**II–IV**) at pH 4 was observed.

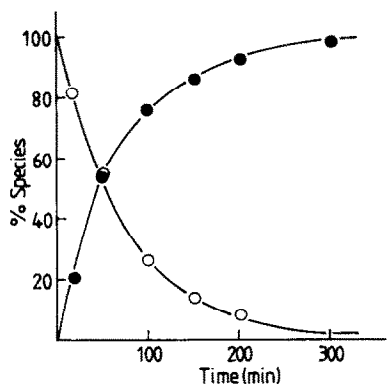


Fig. 3. Time-courses for erythromycin 2′-propionate (○) and erythromycin (●) in the degradation of the propionate ester at pH 7.4 (37 °C).

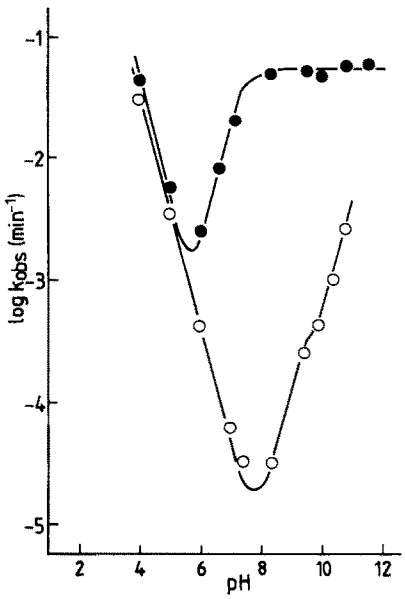


Fig. 4. The pH–rate profiles for the degradation of erythromycin (○) and erythromycin 2′-acetate (●) in aqueous solution ($\mu = 0.5$; 37 °C).

Kinetics of degradation of erythromycin

The influence of pH on the rates of degradation of erythromycin at 37 °C is shown in Fig. 4 in which the logarithm of the observed pseudo-first-order rate constants (k_{obs}) has been plotted against pH. The k_{obs} values were obtained from runs carried out in 0.02 M buffer solutions of fixed pH values. At this buffer concentration no significant catalysis of the buffer substances used was observed. Only at higher concentrations is a buffer catalytic effect of importance (cf. Atkins et al., 1986). As can be seen from Fig. 4 the maximal stability of erythromycin occurs at pH around 8. The shape of the pH–rate profile can be accounted for by the following rate expression:

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of

erythromycin in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated *N,N*-dimethylamino group in the compound; k_H is a specific acid-catalyzed rate constant, and k_{OH} and k'_{OH} are the second-order rate constants for the hydroxide ion-catalyzed degradation of the protonated and unprotonated species, respectively. The following rate and ionization constants were obtained:

$k_H = 350\ M^{-1} \cdot min^{-1}$

$k_{OH} = 5.3\ M^{-1} \cdot min^{-1}$

$k'_{OH} = 1.7\ M^{-1} \cdot min^{-1}$

$pK_a = 9.5$

The value for k_H is somewhat higher than that (105 $M^{-1} \cdot min^{-1}$) reported by Atkins et al. (1986) at a similar temperature and ionic strength. No pK_a value has apparently been reported previously for erythromycin in water. In 66% *N,N*-dimethylformamide a pK_a value of 8.6 (at 20 °C) has been reported (Stephens and Conine, 1959). Taking the different solvents and temperatures into consideration these values do not disagree.

Kinetics of degradation of erythromycin esters

The influence of pH on the rates of degradation of some esters is shown in Figs. 4 and 5. Similarly shaped pH–rate profiles were observed for the other esters of the study.

The data indicate that the degradation of erythromycin 2′-esters can be described in terms of specific acid- and base-catalyzed reactions of the protonated ester according to the following rate expression:

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} \qquad (2)$$

where k_H and k_{OH} are the specific rate constants for hydrogen and hydroxide ion-catalyzed degradation, respectively, of the protonated form of the esters and K_a is the apparent ionization constant for the protonated amino group in the compounds.

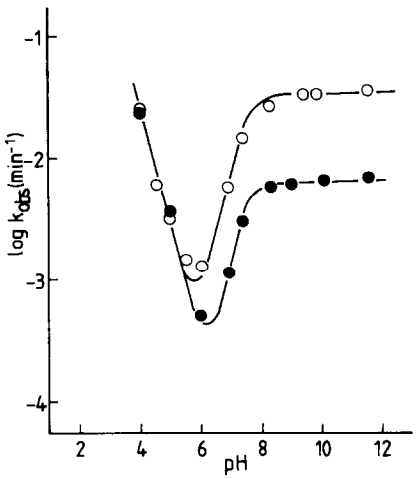


Fig. 5. The pH–rate profiles for the degradation of the 2′-propionate (○) and 2′-benzoate (●) esters of erythromycin in aqueous solution (μ = 0.5; 37 °C).

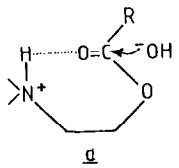
The various rate and ionization constants derived from the pH–rate profiles are listed in Table 1. The esters are considerably less basic than erythromycin (pK_a 7.7 vs 9.5) which can be ascribed to the greater electron-withdrawing effect of the ester moiety relative to a hydroxyl group. In 66% *N,N*-dimethylformamide pK_a values of 6.5–6.9 have been reported for various aliphatic erythromycin esters (Stephens and Conine, 1959).

TABLE 1

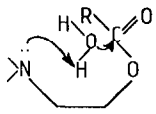
Ionization constants and rate data for the hydrolysis of various erythromycin 2′-esters (μ = 0.5; 37 °C)

Compound	k_H ($M^{-1} \cdot min^{-1}$)	k_{OH} ($M^{-1} \cdot min^{-1}$)	pK_a
II	5.0×10^2	6.6×10^4	7.7
III	2.5×10^2	3.6×10^4	7.8
IV	2.3×10^2	1.3×10^5	7.7
V	–	7.9×10^3	–
VI	–	1.1×10^4	–
VII	4.8×10^2	1.3×10^4	7.8
VIII	2.5×10^2	5.3×10^3	7.7
IX	2.3×10^2	1.6×10^4 [a]	7.7
X	–	8.5×10^3	7.7
XI	1.3×10^2	3.0×10^3	7.6

[a] The k_{OH} value for the ester **IX** is for the total degradation, i.e. hydrolysis to erythromycin as well as to the ester **X**.



a



b

Scheme 1.

A comparison of the rate data for erythromycin and its esters shows that the compounds possess similar stability in acidic solution (pH < 5). According to the dehydration reaction accounting for the degradation under these conditions (Fig. 2) this result is as expected since esterification of the 2′-OH group should not influence the dehydration leading to 6,9-hemiketal formation. At pH > 6 the esters are much more unstable than erythromycin because of their facile hydrolysis. Overall, these reactions imply that optimal stability of the esters occurs at pH 5.5–6.

The facile hydrolysis of the erythromycin 2′-esters in neutral solutions can be attributed to intramolecular catalysis by the *N*,*N*-dimethylamino group. One possible catalytic mechanism involves intramolecular general acid-catalyzed hydroxide ion attack on the ester moiety as depicted in (a) in Scheme 1. An alternative mechanism involves general base catalysis by the unprotonated amino group of water attack on the ester group (mechanism (b) in Scheme 1). These mechanisms are kinetically equivalent and cannot be distinguished from one another using the present kinetic data. The rate expression of Eqn. 2 has been formulated on the basis of mechanism (a). Erythromycin can be regarded as a β-aminoalcohol and the hydrolysis of esters of various other β-aminoalcohols is similarly known to be facilitated by the neighbouring amino group (Zaslowsky and Fisher, 1963; Hansen, 1963; Chu and Mautner, 1966; Bruice and Mautner, 1973; Bundgaard et al., 1988).

It is of interest to note that just as the neighbouring dimethylamino group enhances the rate of ester hydrolysis, it behaves as an intramolecular catalyst for the esterification of erythromycin. Erythromycin contains five OH-groups but the 2′-OH function is much more readily esterified than other OH-groups in the molecule (Jones et al., 1972; Banaszek et al., 1969).

Hydrolysis in human plasma

At initial concentrations of 10^{-5}–10^{-3} M the rate of degradation of the erythromycin esters in 80% human plasma displayed good first-order kinetics. Examples of first-order kinetic plots are shown in Fig. 6. All esters were quantitatively converted to erythromycin. In case of the acetylsalicylate ester **IX**, its degradation in plasma solutions proceeded with the formation of the corresponding salicylate ester **X** to an extent of more than 90% as determined by HPLC analysis. Once formed, the ester **X** then slowly degraded to yield erythromycin and salicylic acid. The analysis for salicylic acid and acetylsalicylic acid was performed by the HPLC method previously described (Nielsen and Bundgaard, 1989). The behaviour of the ester **IX** is similar to that of various alkyl esters of acetylsalicylic acid in that plasma-catalyzed deacetylation in such esters greatly predominates over hydrolysis of the other ester bond to yield free acetylsalicylic acid (Nielsen and Bundgaard, 1989).

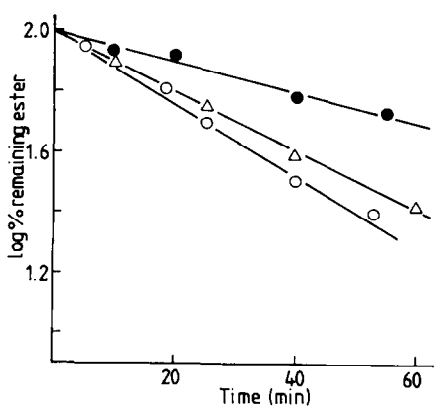


Fig. 6. Plots showing the first-order kinetics of hydrolysis of the 2′-acetate (○), 2′-ethylsuccinate (△) and 2′-methylcarbonate (●) esters of erythromycin in 80% human plasma (37 °C).

TABLE 2

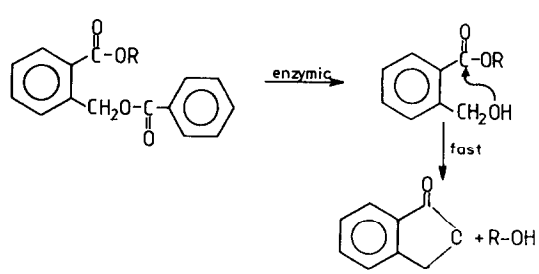
Rates of hydrolysis of erythromycin and various erythromycin esters in 0.02 M phosphate buffer (pH 7.4) and 80% human plasma (pH 7.4) at 37 °C

Compound	Half-life (min)	
	Buffer	Plasma
I	2.19×10^4 (365 h)	–
II	23	25
III	49	48
IV	19	31
V	147	60
VI	107	57
VII	86	61
VIII	235	1700
IX	77	53 [a]
X	147	905
XI	460	5900

[a] Half-life for the hydrolysis of ester **IX** to ester **X**.

The observed half-lives for the hydrolysis of the esters in 80% plasma solutions are listed in Table 2 along with the half-lives in buffer solutions of the same pH. As seen from the data the rate of hydrolysis of several esters is reduced in the presence of plasma. This rate-retarding effect which has been observed before for some erythromycin esters (Tardrew et al., 1969; Tsuji and Goetz, 1978a; Taskinen and Ottoila, 1988) may be due to binding of the esters to plasma proteins, the bound ester being protected against enzymatic and chemical hydrolysis. Serum albumin may be involved in the binding but as recently shown by Taskinen and Ottoila (1988) α_1-acid glycoprotein, which is known to bind several basic drugs (Müller and Stillbauer, 1983), may also be involved. It was found that the hydrolysis of the acetate ester of erythromycin was significantly inhibited in vitro in the presence of physiological concentrations of this protein. If binding of the esters to proteins is the cause of the rate-retarding effect by plasma, one should expect an increased rate retardation with increased hydrophobicity or lipophilicity of the ester. Support for this has been given by Taskinen and Ottoila (1988) who observed increased half-lives of hydrolysis in human plasma for lower aliphatic erythromycin esters in going from the acetate ester to the valerate ester. The data in Table 2 further support this view. The hydrolysis of the more lipophilic aromatic esters **VIII–XI** is strongly inhibited by plasma whereas the hydrolysis of the less lipophilic esters **II**, **III** and **VII** are only slightly affected by plasma. Furthermore, the succinate and glutarate esters **VI** and **VII** which are negatively charged at pH 7.4 and hence quite hydrophilic, hydrolyze even faster in the presence of plasma than in buffer solution. However, despite the apparent lack of plasma inhibition, these esters hydrolyze slower than the lower alkyl esters. Esters containing an ionized carboxylate group are generally poor substrates for hydrolytic enzymes (Nielsen and Bundgaard, 1987; and references cited therein) and this may be the reason why the hydrolysis of the esters **V** and **VI** is not more accelerated in the presence of plasma.

The 2-benzoyloxymethylbenzoate ester (**XI**) of erythromycin was prepared with the hope of obtaining an ester which was rapidly hydrolyzed by the action of esterases on the ester group not directly connected to the erythromycin molecule as outlined in Scheme 2. By hydrolysis of the terminal ester group compound **XI** should be converted to a 2-hydroxymethylbenzoate ester (Nielsen and Bundgaard, 1986) which is known (Fife and Benjamin, 1973, 1976) to undergo a rapid non-enzymatic cyclization in aqueous solution at pH 7.4 with formation of phthalide and concurrent release of the parent alcohol, i.e. erythromycin. The stability experiments showed, however, that the ester **XI** was extremely slowly hydrolyzed in plasma. Apparently, binding of this very lipophilic compound to plasma proteins not only protects the erythromycin ester linkage against enzymatic attack but also the benzyl benzoate ester moiety.



Scheme 2.

In conclusion, the results obtained show that erythromycin esters are much more unstable than erythromycin in neutral and basic solutions due to an intramolecularly facilitated hydrolysis. The results further indicate that in the development of an ester prodrug which is rapidly hydrolyzed in vivo to the parent active erythromycin and hence not eliminated from the body as inactive ester, more attention should be paid to the design of esters with a reduced lipophilicity and less propensity to be bound to plasma proteins. Studies are in progress to examine the behaviour of such esters (e.g. esters containing protonable amino functions) as well as other types of potential prodrug derivatives of erythromycin.

References

Amer, M.M. and Takla, K.F., Studies on the stability of some pharmaceutical formulations. V. Stability of erythromycin. *Bull. Fac. Pharm. Cairo Univ.*, 15 (1978) 325–339.

Atkins, P.J., Herbert, T.O. and Jones, N.B., Kinetic studies on the decomposition of erythromycin A in aqueous acidic and neutral buffers. *Int. J. Pharm.*, 30 (1986) 199–207.

Banaszek, A., Pyrek, J.St. and Zamojski, A., Esterification of erythromycin A. *Rocz. Chem.*, 43 (1969) 763–774.

Bechtol, L.D., DeSante, K.A., Foglesong, M.A., Spradlin, C.T. and Winely, C.L., The bio-availability of pediatric suspensions of two erythromycin esters. *Curr. Ther. Res.*, 29 (1981) 52–59.

Bechtol, L.D., Stephens, V.C., Pugh, C.T., Perkal, M.B. and Coletta, P.A., Erythromycin esters – comparative in-vivo hydrolysis and bioavailability. *Curr. Ther. Res.*, 20 (1976) 610–622.

Boggiano, B.G. and Gleeson, M., Gastric inactivation of erythromycin stearate in solid dosage forms. *J. Pharm. Sci.*, 65 (1976) 497–502.

Bruice, P.Y. and Mautner, H.G., Intramolecular catalysis and the involvement of tetrahedral intermediate partitioning in the hydrolysis of benzoylcholine, benzoylthionocholine and their dimethylamino analogs. *J. Am. Chem. Soc.*, 95 (1973) 1582–1586.

Bundgaard, H., Buur, A., Chang, S.-C. and Lee, V.H.L., Timolol prodrugs: synthesis, stability and lipophilicity of various alkyl, cycloalkyl and aromatic esters of timolol. *Int. J. Pharm.*, 46 (1988) 77–88.

Chu, S.-H. and Mautner, H.G., Analogs of neuroeffectors. V. Neighbouring-group effects in the reactions of esters, thiol-esters and selenoesters. The hydrolysis and aminolysis of benzoylcholine, benzoylthiolcholine, benzoylselenolcholine, and of their dimethylamino analogs. *J. Org. Chem.*, 31 (1966) 308–312.

Clark, R.K., Jr. and Warner, E.L., New esters of erythromycin. *Antibiot. Chemother.*, 7 (1957) 187–189.

Dall'Asta, L., Comini, A., Garegnani, E., Alberti, D., Coppi, G. and Quadro, G., Studies on the bioavailability of some new erythromycin esters. *J. Antibiot.*, 41 (1988) 139–141.

Fife, T.H. and Benjamin, B.M., General base catalyzed intramolecular transesterification. *J. Am. Chem. Soc.*, 95 (1973) 2059–2061.

Fife, T.H. and Benjamin, B.M., Intramolecular general base catalyzed transesterification. The cyclization of ethyl 2-hydroxymethylbenzoate and ethyl 2-hydroxymethyl-4-nitrobenzoate to phthalide and 5-nitrophthalide. *Bioorg. Chem.*, 5 (1976) 37–50.

Flynn, E.H., Sigal, M.V., Wiley, P.F. and Gerzon, K., Erythromycin. I. Properties and degradation studies. *J. Am. Chem. Soc.*, 76 (1954) 3121–3131.

Fraser, D.G., Selection of an oral erythromycin product. *Am. J. Hosp. Pharm.*, 37 (1980) 1199–1205.

Gordin, A., Kalima, S., Hulmi, S., Mäkelä, P. and Antikainen, R., Comparison of erythromycin acistrate and enterocoated erythromycin base in acute respiratory infections. *J. Antimicrob. Chemother.*, 21, Suppl. D (1988a) 85–92.

Gordin, A., Männistö, P.T., Antikainen, R., Savolainen, S., Ylikoski, J., Kokkonen, P. and Rauramaa, V., Concentrations of erythromycin, 2′-acetyl erythromycin, and their anhydro forms in plasma and tonsillar tissue after repeated dosage of erythromycin stearate and erythromycin acistrate. *Antimicrob. Agents Chemother.*, 32 (1988b) 1019–1024.

Hansen, B., Kinetics of the reactions of *O*-acetyl-ethanolamine. *Acta Chem. Scand.*, 17 (1963) 1307–1314.

Jones, P.H., Perun, T.J., Rowley, E.K. and Baker, E.J., Chemical modifications of erythromycin antibiotics. 3. Synthesis of 4″- and 11-esters of erythromycin A and B. *J. Med. Chem.*, 15 (1972) 631–634.

Kurath, P., Jones, P.H., Egan, R.S. and Perun, T.J., Acid degradation of erythromycin A and erythromycin B. *Experientia*, 27 (1971) 362.

Murphy, H.W., Esters of erythromycin. 1. Some water-insoluble, substantially tasteless esters. *Antibiot. Annu.*, (1953–1954) 500–513.

Müller, W.E. and Stillbauer, A.E., Characterization of a common binding site for basic drugs on human alpha$_1$-acid glycoprotein (orosomucoid). *Naunyn-Schmiedebergs Arch. Pharmacol.*, 322 (1983) 170–173.

Männistö, P.T., Taskinen, J., Ottoila, P., Solkinen, A., Vuorela, A. and Nykänen, S., Fate of single oral dosis of erythromycin acistrate, erythromycin stearate and pelleted erythromycin base analysed by mass-spectrometry in plasma of healthy human volunteers. *J. Antimicrob. Chemother.*, 21, Suppl. D (1988) 33–43.

Nielsen, N.M. and Bundgaard, H., Evaluation of glycolamide esters and various other esters of aspirin as true aspirin prodrugs. *J. Med. Chem.*, 32 (1989) 727–734.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. Part 42. 2-Hydroxymethylbenzamides and 2-acyloxymethylbenzamides as potential prodrug forms for amines. *Int. J. Pharm.*, 29 (1986) 9–18.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: a reference system for designing prodrug esters of carboxylic acid agents. *Int. J. Pharm.*, 39 (1987) 75–85.

Sinkula, A.A., Chemical modification of erythromycin: synthesis and preliminary bioactivity of selected amides and esters. *J. Pharm. Sci.*, 63 (1974) 842–848.

Stephens, V.C., Esters of erythromycin. II. Derivatives of cyclic anhydrides. *Antibiot. Annu.*, (1958–1959) 514–520.

Stephens, V.C. and Conine, J.W., Esters of erythromycin. III. Esters of low molecular weight aliphatic acids. *Antibiot. Annu.*, (1958–1959) 346–353.

Stephens, V.C., Pugh, C.T., Davis, N.E., Hoehn, M.M., Ralston, S., Sparks, M.C. and Thompkins, L., A study of the behaviour of propionyl erythromycin in blood by a new chromatographic method. *J. Antibiot.*, 22 (1969) 551–557.

Tardrew, P.L., Mao, J.C.H. and Kenney, D., Antibacterial activity of 2′-esters of erythromycin. *Appl. Microbiol.*, 18 (1969) 159–165.

Taskinen, J. and Ottoila, P., Hydrolysis of 2′-esters of erythromycin. *J. Antimicrob. Chemother.*, 21, Suppl. D (1988) 1–8.

Tserng, K.-Y. and Wagner, J.G., Fluorometric determination of erythromycin and erythromycin propionate in whole blood or plasma and correlation of results with microbiological assay. *Anal. Chem.*, 48 (1976) 348–353.

Tsuji, K. and Goetz, J.F., Elevated column temperature for the high-performance liquid chromatographic determination of erythromycin and erythromycin ethylsuccinate. *J. Chromatogr.*, 157 (1978a) 185–196.

Tsuji, K. and Goetz, J.F., HPLC determination of erythromycin. *J. Chromatogr.*, 147 (1978b) 359–367.

Tuominen, R.K., Männistö, P.T., Pohto, P., Solkinen, A. and Vuorela, A., Absorption of erythromycin acistrate and erythromycin base in the fasting and non-fasting state. *J. Antimicrob. Chemother.*, 21, Suppl. D (1988) 45–55.

Welling, P.G., Elliott, R.L., Pitterle, M.E., Corrick-West, H.P. and Lyons, L.L., Plasma levels following single and repeated doses of erythromycin estolate and erythromycin stearate. *J. Pharm. Sci.*, 68 (1979) 150–155.

Wiley, P.F., Gerzon, K., Flynn, E.H., Sigal, M.V., Jr., Weaver, O., Quarck, U.C., Chauvette, R.R. and Monahan, R., Erythromycin. X. Structure of erythromycin. *J. Am. Chem. Soc.*, 79 (1957) 6062–6070.

Yakatan, G.J., Poynor, W.J., Breeding, S.A., Lankford, C.E., Dighe, S.V., Martin, A.N. and Doluisio, J.T., Single and multiple-dose bioequivalence of erythromycin pharmaceutical alternatives. *J. Clin. Pharmacol.*, 20 (1980) 625–638.

Yakatan, G.J., Rasmussen, C.E., Feis, P.J. and Wallen, S., *J. Clin. Pharmacol.*, 25 (1985) 36–42.

Zaslowsky, J.A. and Fisher, E., The hydrolysis of β-diethylaminoethyl acetate. *J. Phys. Chem.*, 67 (1963) 959–961.