Erythromycin VI: Kinetics of Acid-Catalyzed Hydrolysis of Erythromycin Oxime and Erythromycylamine

TOMISLAV LAZAREVSKI ˣ, GORJANA RADOBOLJA, and SLOBODAN DJOKIĆ

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Abstract □ Kinetic data were obtained, by qualitative and quantitative analysis, of the hydrolytic degradation of erythromycin oxime and erythromycylamine separated by TLC. The pseudo-first-order rate constants were determined at three temperatures (17, 26, and 36°), and the temperature dependency of the reaction was studied. The activation energy for the hydrolysis and methanolysis of the compounds was calculated. The factors contributing to the differences in the reaction rate are discussed.

Keyphrases □ Erythromycin oxime and amine—kinetics of acid-catalyzed hydrolysis □ Kinetics—acid-catalyzed hydrolysis of erythromycin oxime and amine □ Hydrolysis, acid catalyzed—erythromycin oxime and amine, kinetics □ Antibacterials—erythromycin oxime and amine, kinetics of acid-catalyzed hydrolysis

Erythromycin (I*a*) in acidic media rapidly loses its biological activity because of extensive acid-catalyzed transformations (1). These transformations involve the formation of an internal enol ether (I*b*) by addition of the C-6 hydroxyl to the carbonyl at C-9 followed by dehydration. This step is followed by the participation of the C-12 hydroxyl in an irreversible addition to the double bond, giving a spiroketal (II). In a further reaction, the glycosidic linkage at the C-3 position is broken and the sugar cladinose (3-*O*-methylmycarose) (III) and erythralosamine (IV) are obtained. The degradation pathway is presented in Scheme I.

Erythromycin oxime (V) and erythromycylamine (VI) are prepared from erythromycin (2), and their structures differ only at the C-9 position. No information was available regarding the stability of these compounds. The purposes of this investigation were to establish the rates of hydrolysis and methanolysis of V and VI at various temperatures and to compare them with those of erythromycin (3).

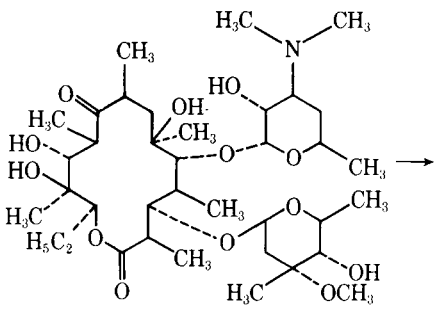
EXPERIMENTAL

Apparatus—The light absorption measurements were made on a densitometer[1] using a blue filter of 465-nm wavelength. Temperature control was carried out with thermostats maintained at 17, 26, and 36°. Constant-temperature baths with a mean variation of about 0.1° were used.

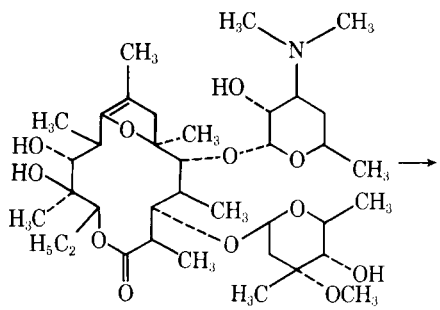
Materials—The standards cladinose (III) and methylcladinoside (VII) were prepared by a reported method (4), and their purity was verified by TLC. Other chemicals were analytical grade and were used without further purification.

Kinetic Measurements—Compounds V and VI were hydrolyzed in 0.23% (0.063 *N*) methanolic or aqueous hydrochloric acid at 17, 26, and 36° for 48 hr. The concentration of the investigated substances in acid solutions was 2%. Samples were withdrawn at various times and neutralized immediately with sodium carbonate. The solvent was removed *in vacuo*, and the residue was extracted with chloroform. The extract was dried, concentrated *in vacuo* to a solid, and redissolved in chloroform (10 mg/ml) for TLC analysis.

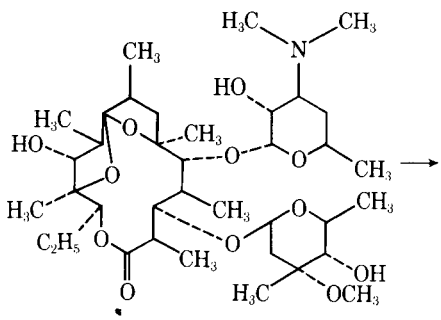
The chloroform solutions were spotted (10 μl) on 250-μm silica gel F_{254} plates. Components in the hydrolysates were separated on TLC plates by development in chloroform–benzene (19:1) saturated with ammonia vapor and were then visualized as dark-brown spots by spraying with



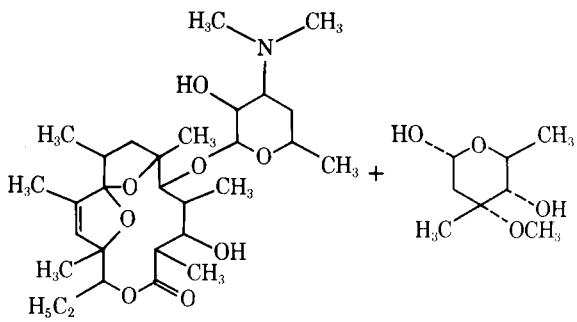
Ia



Ib



II



IV III

Scheme I

[1] Model 520A, Photovolt Corp., New York, N.Y.

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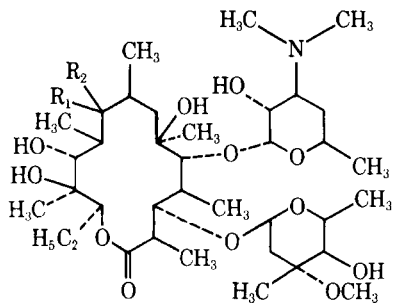
Table I—Rate Constants and Energy of Activation for the Acid Solvolysis of Erythromycin Oxime and Erythromycylamine

Compound	Temperature	Rate Constants for Hydrolysis, k, min^{-1}	Rate Constants for Methanolysis, k, min^{-1}	E_a for Hydrolysis, kcal/mole	E_a for Methanolysis, kcal/mole
V	17°	5.23×10^{-4}	2.01×10^{-3}	19.4	24.8
	26°	1.42×10^{-3}	7.50×10^{-3}		
	36°	4.12×10^{-3}	2.94×10^{-2}		
VI	17°	3.69×10^{-4}	1.59×10^{-3}	20.7	22.9
	26°	1.13×10^{-3}	5.11×10^{-3}		
	36°	3.48×10^{-3}	1.77×10^{-2}		



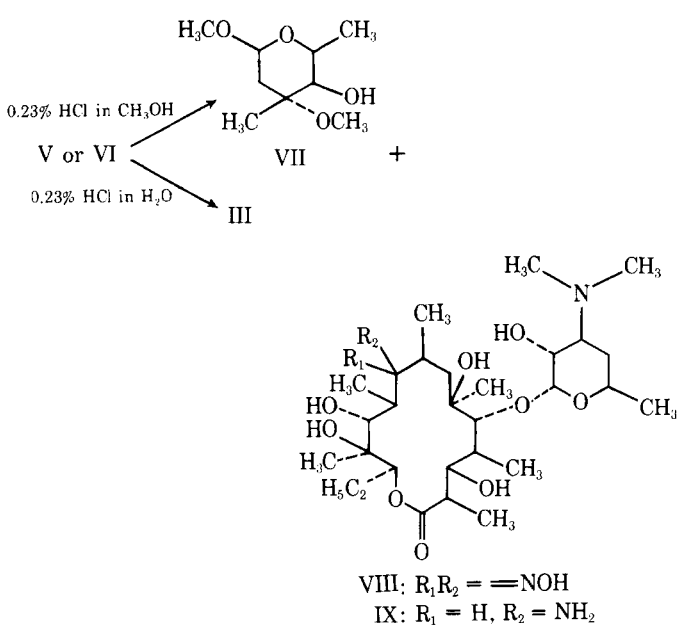
V: R_1R_2 = =NOH
VI: R_1 = H, R_2 = NH_2

phenol–sulfuric acid (5). The assay was based on the densitometric measurements of these spots.

RESULTS AND DISCUSSION

Solvolysis of V and VI with 0.23% HCl in water or methanol at 17, 26, and 36° was monitored by TLC; each degradation product was identified by the corresponding standard. The neutral sugar cladinose was removed by solvolysis. Cladinose (III) and methylcladinoside (VII), formed during the aging of V and VI in acid media (Scheme II), were determined quantitatively by densitometry of TLC plates; the results were used for calculating the velocity constants.

The amounts of sugar liberated during hydrolysis were determined by measuring the light absorbance. Linear calibration curves were obtained by the least-squares method for each sugar, and their equations were used to convert densitometric peak areas into amounts of sugar in the hydrolysate (5). From the amount of sugar, the amount of starting material present at any time was obtained indirectly.



VIII: R_1R_2 = =NOH
IX: R_1 = H, R_2 = NH_2

Scheme II

By plotting the hydrolysis time *versus* the degree of sample conversion, a linear relationship was obtained (Fig. 1). The straight line is a valid proof that the reaction follows first-order kinetics. The equation employed is:

$$k = \frac{2.303}{t} \log \frac{a}{a - x} \qquad \text{(Eq. 1)}$$

where a is the initial amount of sample and x is the amount of hydrolyzed sample at time t.

The values of the velocity constant at three different temperatures are given in Table I. The increased temperature accelerated the degradation rates of V and VI. No difference in the number or identity of the compounds formed was seen at the temperatures studied. The logarithms of the k values were plotted *versus* $1/T$. These Arrhenius plots produced straight lines, allowing the calculation of the activation energy (Table I).

The difference between the reaction rates for the solvolysis of V and VI suggests the influence of the oximino and amino groups at C-9, respectively. Erythromycylamine was slightly more stable than erythromycin oxime. Compared with the parent antibiotic erythromycin, the rate of acid-catalyzed hydrolysis of V and VI is believed to be mainly influenced by different substituents at C-9. Oximino and amino groups do not permit the formation of spiroketal (II), causing a decrease of the reaction rate and an increase of the stability in an acidic medium.

The velocity constants for methanolysis were higher than for hydrolysis because of the different polarity of the solvents. These results are consistent with the mechanism of glycoside hydrolysis (6). Few literature data are available for comparison of the activation energy values. However, the activation energy for the hydrolysis of V and VI agrees well with that calculated from the reported rate constants for inactivation of erythromycin (3).

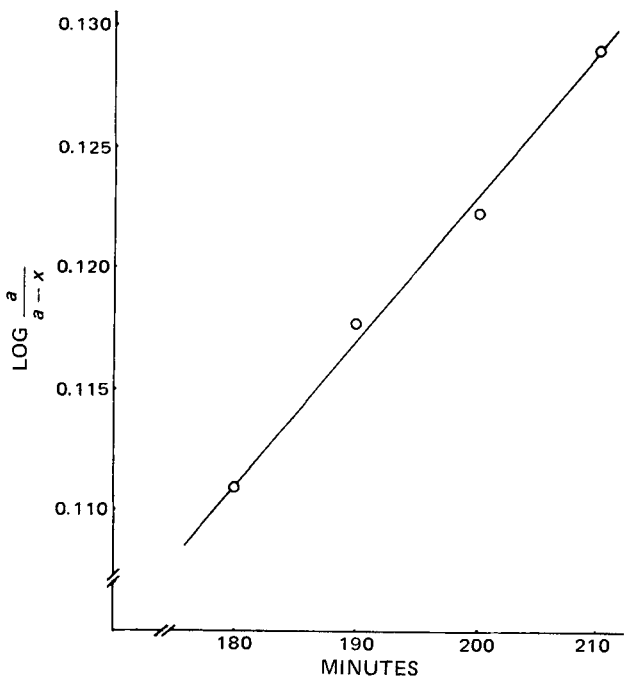


Figure 1—*First-order plot for the hydrolysis of erythromycin oxime in 0.23% aqueous hydrochloric acid at 26°.*

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Rapid Determination of Atenolol in Human Plasma and Urine by High-Pressure Liquid Chromatography

ORVILLE H. WEDDLE, EDWIN N. AMICK, and WILLIAM D. MASON ˣ

Received March 28, 1977, from the *Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri–Kansas City, Kansas City, MO 64108.* Accepted for publication November 2, 1977.

Abstract □ A rapid, specific, high-pressure liquid chromatographic determination of atenolol in plasma and urine was developed. This method employs the high sensitivity of fluorescence detection together with selective extraction and reversed-phase chromatography to measure concentrations as low as 20 ng of drug/ml of plasma with a coefficient of variation of 3.91%. The assay is specific enough to be valid in the presence of plasma and urine substances. The detection limit (*i.e.*, three times baseline noise) is 3 ng/ml.

Keyphrases □ Atenolol—high-pressure liquid chromatographic determination in biological fluids □ High-pressure liquid chromatography—analysis, atenolol in biological fluids □ Antiadrenergic agents—atenolol, high-pressure liquid chromatographic determination in biological fluids

Atenolol[1], 4-(2-hydroxy-3-isopropylaminopropoxy)-phenylacetamide (I), is a new β-adrenergic blocking agent. Studies in animals and humans suggest that this drug causes preferential blockade of cardiac β-adrenoreceptors, is free of intrinsic sympathomimetic activity, and is devoid of significant membrane-stabilizing activity (1–5). Initial clinical experience indicates that atenolol may be of value in the treatment of hypertension (6–9).

Previous procedures for the determination of atenolol in plasma and urine employed GLC with electron-capture detection (10, 11) or spectrophotofluorometry (12). The spectrophotofluorometric method is relatively simple but is of questionable specificity, and the detection limit is 50 ng/ml. Although the GLC methods are highly selective and have a detection limit of 20 ng/ml, they are relatively complex and rely on a demanding prechromatography derivatization.

The procedure reported here combines a selective two-step extraction with the separative capability of high-pressure liquid chromatography (HPLC) and the sensitivity of fluorescence detection. The method is rapid and specific, employs an internal standard, and is of sufficient sensitivity (3 ng/ml detection limit) for pharmacokinetic studies.

[1] Burdick and Jackson Laboratories, Muskegon, Mich.

EXPERIMENTAL

Materials—UV grade methanol[1], 1-butanol[1], and hexane[1] were used as obtained, as were reagent grade triethanolamine[2] and 1-heptane sulfonic acid in acetic acid[3]. Water was passed through an ion-exchange bed and then distilled. Atenolol[4] and procainamide[5] were used as obtained.

Apparatus—HPLC was performed in a system consisting of a universal injector[6], a high pressure pump[7], a bonded-phase column[8], and a fluorescence detector[9]. The fluorescence of atenolol was excited at 222 nm. A combination of a 7-54 and a UV 30 filter was used between the flowcell and the photomultiplier tube.

Chromatographic Parameters—The mobile phase was prepared by mixing 2.0 ml of 1-heptane sulfonic acid in acetic acid[3] with 100 ml of 0.1 *M* triethanolamine in distilled water and 1900 ml of methanol. The mobile phase was pumped at 2 ml/min and 20° through a stainless steel column (30.5 cm × 4 mm i.d.) packed with a high efficiency bonded-phase packing. Aliquots of 100–250 μl of the reextraction solution were injected directly on-column through the injector.

Analytical Procedure—*Plasma*—Two 1.0-ml aliquots of each plasma sample were placed in 15-ml screw-topped centrifuge tubes. To each tube were added 0.1 ml of 2.5 *N* NaOH and 5.0 ml of 10% 1-butanol in *n*-hexane. The tubes were vortexed for 30 sec and then centrifuged for 10 min at 2000 rpm. After centrifugation, the organic phase was removed and discarded.

Four milliliters of 50% 1-butanol in *n*-hexane was then added to each tube. This solution contained 50.0 ng of procainamide/ml as an internal standard. The tubes were again vortexed for 30 sec and centrifuged for 10 min at 2000 rpm. This second organic phase was transferred to a second centrifuge tube containing 300 μl of 0.1% acetic acid. After 30 sec of vortexing and 5 min of centrifugation at 2000 rpm, 100–250 μl of the lower (aqueous) phase was withdrawn with a syringe and injected into the injector loop.

The output of the detector was recorded on a chart recorder, and the areas of the atenolol and procainamide peaks were measured. The ratios of the peak areas were converted into plasma atenolol concentrations by comparison with a standard curve.

Urine—Urine, 100 μl, was added to 4.90 ml of 1.52 *M* phosphate buffer (pH 12.3) and mixed thoroughly. Then 1-ml aliquots of this diluted sample were placed in 15-ml centrifuge tubes and assayed by the same

[2] Fisher Scientific Co., St. Louis, Mo.
[3] B7 reagent, Waters Associates, Milford, Mass.
[4] Stuart Pharmaceutical Division of ICI United States, Wilmington, Del.
[5] E. R. Squibb & Sons, Princeton, N.J.
[6] U6K, Waters Associates, Milford, Mass.
[7] Model 6000A, Waters Associates, Milford, Mass.
[8] μBondapak CN, Waters Associates, Milford, Mass.
[9] Schoeffel, FS-970, Westwood, N.J.

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