Acid-Catalyzed Degradation of Clarithromycin and Erythromycin B: A Comparative Study Using NMR Spectroscopy

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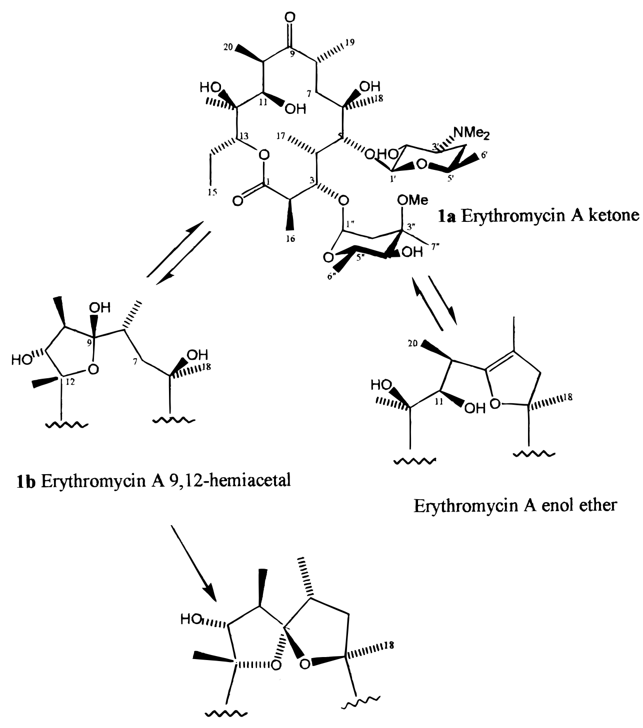
One of the major drawbacks in the use of the antibiotic erythromycin A is its extreme acid sensitivity, leading to degradation in the stomach following oral administration. The modern derivative clarithromycin degrades by a different mechanism and much more slowly. We have studied the pathway and kinetics of the acid-catalyzed degradation of clarithromycin and of erythromycin B, a biosynthetic precursor of erythromycin A which also has good antibacterial activity, using ^{1}H NMR spectroscopy. Both drugs degrade by loss of the cladinose sugar ring and with similar rates of reaction. These results suggest that erythromycin B has potential as an independent therapeutic entity, with superior acid stability compared with erythromycin A and with the advantage over clarithromycin of being a natural product.

Introduction

Erythromycin A (**1**) is an important antibiotic of the macrolide class and has been in clinical use for some 50 years. Despite long success, particularly in the treatment of deep-seated infections, it has a number of problems in use. Compliance in taking this drug is relatively poor. In children, this results from the vile taste; in adults severe gastric disturbance proves intolerable in a minority of patients.[1] In addition, erythromycin and its metabolites are processed by the same cytochrome P450 enzymes as a number of other drugs. Saturation of these enzymes can lead to overdose of theophylline, and even of common hay fever remedies, if they are taken with erythromycin. All these problems are exacerbated by the very large doses of erythromycin A required for effective antimicrobial action. Four doses of 250 mg or 500 mg per day for 7 or 14 days is typical. The large doses are necessary because of the extreme acid sensitivity of the drug. Below about pH 6.5 it is degraded to anhydroerythromycin (**2**), as shown in Scheme 1.[2,3]

Clarithromycin (**3**) is a modern derivative of erythromycin A with an improved range of antibacterial action and, in particular, with greatly improved acid stability. Daily oral doses of this drug are typically a quarter of the corresponding erythromycin doses, and the course is often shorter. Chemically, clarithromycin differs from erythromycin A only in the substitution of 6-OMe for 6-OH, but this gives two important changes in properties. First, clarithromycin is more hydrophobic than erythromycin A. This is the probable reason for its higher activity against certain Gram-negative organisms such as *Haemophilus ducreyi*.[4] Second, it is unable to cyclize in a 6,9-direction (and empirically does not cyclize in a 12,9-direction[5]) and so cannot degrade in acid via Scheme 1. The drug is much more stable to acid than is erythromycin A[6] and is even used as a component of triple therapy against *Helicobacter pylori*.[7] The mechanism of acid-catalyzed degradation of clarithromycin in aqueous solution has been explored[6] with the result shown in Scheme 2; clarithromycin degrades below pH 3 at 37 °C to 5-*O*-desosaminyl-6-*O*-methylerythronolide A (**4**), with loss of the cladinose sugar.[6]

Scheme 1. Decomposition Pathway for Erythromycin A in Acidic Aqueous Solution[2]



2 Erythromycin A anhydride

Erythromycin B (**5**) is a biosynthetic precursor of erythromycin A, lacking only the 12-OH group.[8] It is present as a minor component of commercial erythromycin. Erythromycin B has comparable antibacterial activity to erythromycin A[9] but has never been exploited as an antibacterial agent in its own right. The structure

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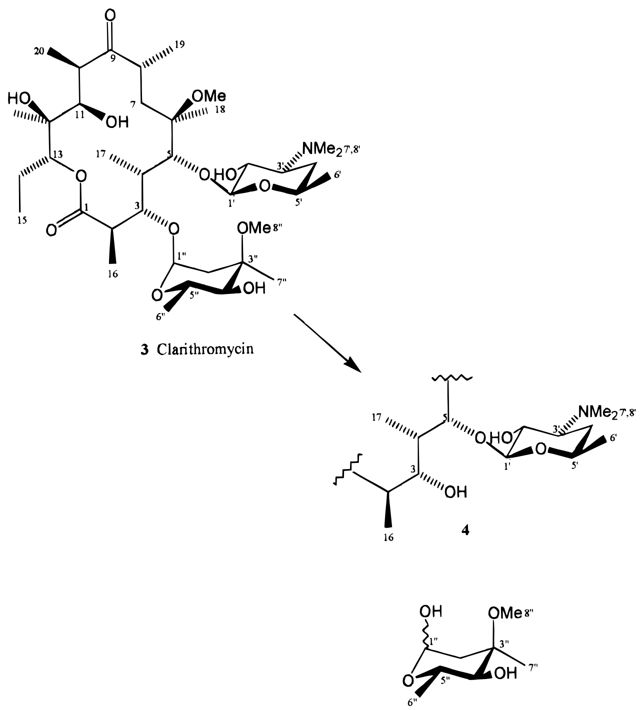
Scheme 2. Decomposition of Clarithromycin in Acidic Aqueous Solution[a]



[a] After ref 6.

of the drug suggests that it should possess some of the advantageous properties of clarithromycin. The lack of a 12-OH group is expected to increase hydrophobicity and will prevent 12,9-cyclization and the formation of inactive compounds analogous to anhydroerythromycin A. Erythromycin B also shares some of the advantages of erythromycin A. It is a fermentation product and can be produced relatively cheaply and with less adverse effect on the environment than the semisynthetic clarithromycin.

We now report a comparative study of the acid-catalyzed degradation of erythromycin B and clarithromycin, with a view to determining whether erythromycin B has potential as an independent therapeutic entity.

Results

Solubility Measurements. Clarithromycin is poorly soluble in aqueous media;[5] this property is expected to contribute to its activity against Gram-negative organisms. Erythromycin A, which has better water solubility, is almost inactive against most Gram-negative organisms, but it has better activity than clarithromycin against *Haemophilus influenzae*.[10] The relative solubilities of the three drugs at physiological pH were assessed by preparing saturated solutions in D_2O-based phosphate buffer containing 3 mM 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoic acid (TSP) and measuring the relative integrals in the ^1H NMR spectrum.

Table 1 shows that erythromycin B is of aqueous solubility intermediate between erythromycin A and clarithromycin. It is likely therefore to have a spectrum of action broadly similar to the other macrolides, with good activity against Gram-positive bacteria, but very limited activity against Gram-negative bacteria.

Table 1. Solubilities of Erythromycins A and B and Clarithromycin in Deuterated Phosphate Buffer (50 mM) at Apparent pH 7.0 and 25 °C

drug	solubility (mM)
erythromycin A	53.4
erythromycin B	19.9
clarithromycin	6.3

An NMR-Based Comparison of the Acid-Catalyzed Degradation of Erythromycin B and Clarithromycin. The aim of this study was to compare the pathway and kinetics of clarithromycin and erythromycin B decomposition under conditions which mimic the stomach (pH 2.5 and 37 °C). NMR spectroscopy was the preferred method because of the possibility of determining the pathways and the kinetics simultaneously. The two drugs were therefore degraded in the NMR tube at 37 °C and apparent pH 2.5 in Britton–Robinson buffer[11] over 48 h. Experiments were also carried out at 55 °C for clarithromycin in order to obtain good data for the slower phase of the degradation.

Some technical difficulties had to be overcome before these experiments could be implemented successfully. For sensitivity reasons it was necessary to work with solutions of the two drugs close to saturation under experimental conditions, leading to small amounts of transient precipitation during the early stages of the degradations. This caused severe disturbance of field homogeneity, rendering conventional NMR data acquisition methods unusable. A recently developed method for rapid and accurate automatic field homogeneity adjustment[12] was therefore used to maintain good field homogeneity throughout the experiments, using alternating periods of shimming and data acquisition. Residual shimming errors were corrected using the FIDDLE algorithm[13] for reference deconvolution.

(i) A Revised Decomposition Pathway for Clarithromycin. Figure 1 shows the region of the ^1H NMR spectrum of clarithromycin corresponding to methyl 15 as it changed over 48 h at 55 °C. It can be seen that the signals corresponding to clarithromycin (labeled **C**) disappear over less than 3 h, to be replaced by a second and then a third set of signals (labeled **F** and **G**). This shows that Scheme 2, proposed by Nakagawa et al.,[6] in which the macrolide degrades to a single product (**4**), is incomplete under these conditions.

The component corresponding to methyl signal **F** was identified by NMR and mass spectrometry. The degradation was run for 2 h, to the point where the concentration of **F** was maximized. A two-dimensional diffusion-ordered (DOSY[14,15]) spectrum of the mixture was then acquired.[16] In a 2D DOSY spectrum, NMR signals are separated according to chemical shift in one dimension and according to their self-diffusion coefficients in the other. The degradation was, of course, continuing during the DOSY experiment, which affected the signal-to-noise ratio slightly. (The alternative strategy, that of quenching the reaction by the addition of base, would have caused a more severe reduction in the signal-to-noise ratio because of the poor solubility of clarithromycin in aqueous media at neutral pH.) The DOSY spectrum showed clearly that the signals for the cladinose ring exhibit much faster diffusion than those due to the macrolide and the desosamine sugar, demonstrating that cladinose had been hydrolyzed from the macrolide.

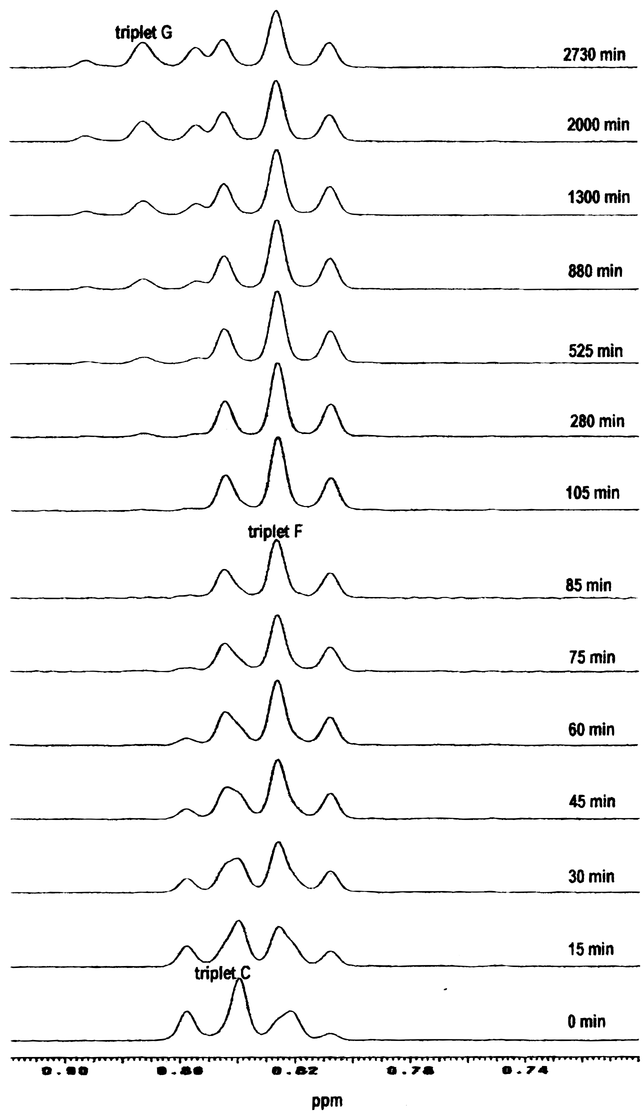


Figure 1. Degradation of clarithromycin in deuterated Britton−Robinson buffer at apparent pH 2.5 and 55 °C. The high field region of the 400 MHz NMR spectrum, corresponding to CH_3-15, is shown over the time course of the experiment (48 h).

The signals labeled **F** in Figure 1 therefore correspond to 5-*O*-desosaminyl-6-*O*-methylerythronolide A (**4**). This conclusion was supported by electrospray mass spectrometry; the major peak in the spectrum of the mixture appeared at *m*/*z* 590, corresponding to an MH^+ ion consistent with compound **4** and in agreement with Nakagawa et al.[6]

The material corresponding to methyl signal **G** was also identified by NMR and mass spectral methods. Clarithromycin was allowed to degrade at pH 2.5 and 55 °C for 4 days, and the ^{1}H NMR spectrum run. (The low field region of the spectrum is shown in the Supporting Information.) Extrapolation of the 48 h time course[16] revealed that by this point the **F** and **G** species were close to equilibrium, with approximately equal concentrations. An olefinic signal at 6.05 ppm follows the same time course as methyl **G**, and, on extreme resolution enhancement, shows quartet structure with a long range coupling constant of ca. 1 Hz. Electrospray mass spectrometry showed signals at *m*/*z* 590 (due to **4**) and 554. The MH^+ signal at *m*/*z* 554 suggests that **4** loses two molecules of water to give an olefinic species, with NMR signals **G**. Further signals, which grow more slowly than **G**, can be seen at 6.01 and 5.88 ppm; at the end of the experiment these were approximately 20 and 10% of the main olefinic signal, respectively.

Morimoto et al.[5] showed that it is possible to prepare **4** by acid treatment of clarithromycin in nonaqueous conditions, that prolonged treatment of **4** with acid yields **6**, and that **6** is capable of isomerization to **7**. Our NMR and mass spectral data are consistent with these reactions also occurring in aqueous acid, with clarithromycin **3** losing cladinose to form **4**, dehydrating to give **6**, and then isomerizing further, perhaps to **7**; the third olefinic signal suggests still further reaction. The signals labeled **G** would therefore correspond to **6**. In a further experiment, 5-*O*-desosaminyl-6-*O*-methylerythronolide A (**4**) was synthesized and incubated for 48 h at 55 °C in the same Britton−Robinson buffer at apparent pH 2.5. The NMR spectrum obtained at the end of the experiment was similar to that described above. The revised pathway for the degradation of clarithromycin in aqueous acid is shown in Scheme 3.

Several weak signals were observed at different stages of the degradation, suggesting the presence of small amounts of further species. A weak signal (ca. 5%) at 4.85 ppm followed the same time course as methyl **C**, suggesting a species in equilibrium with clarithromycin on the time scale of this experiment; this may be the elusive 9,12-hemiketal (analogous to **1b**). Anomeric equilibrium gives rise to two H1″ signals of the free cladinose at 5.1 and 4.96 ppm following its hydrolysis from **3**; the β:α ratio is ca. 6:1. The slow production of second and third olefinic signals suggests that, on a time scale of weeks, degradation to further products occurs.

(ii) Analysis of Rates of Reaction in the Decomposition of Clarithromycin. The principal rates of reaction in Scheme 3 were determined by iterative nonlinear least-squares fitting of integrated signal intensities extracted from the 55 °C proton NMR spectra. For each point on the time course, the spectrum was baseline corrected and the methyl region was

Scheme 3. Revised Pathway for the Decomposition of Clarithromycin in Acidic Aqueous Solution

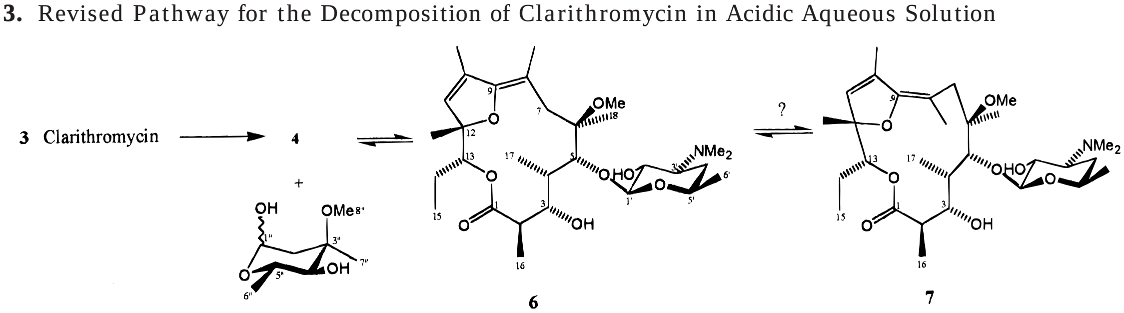


Table 2. Kinetic Parameters Obtained by Fitting Experimental Signal Intensities for the Degradation of Clarithromycin at 55 °C and 37 °C and Apparent pH 2.5[a]

	55 °C	37 °C
k_{CF}	$2.51 \pm 0.02 \times 10^{-2}$ min^{-1}	$2.229 \pm 0.004 \times 10^{-3}$ min^{-1}
k_{FG}	$2.28 \pm 0.02 \times 10^{-4}$ min^{-1}	$1.38 \pm 0.03 \times 10^{-5}$ min^{-1}
k_{GF}	$2.40 \pm 0.09 \times 10^{-4}$ min^{-1}	

[a] Uncertainties quoted are twice the standard deviations estimated in the Levenberg–Marquardt fit.

subjected to least-squares fitting using singular value decomposition[17] with a set of three basis functions. The basis functions contained the isolated methyl triplet signals **C**, **F**, and **G**, respectively, and were obtained by taking linear combinations of experimental spectra, adjusting the coefficients by eye to minimize cross-contamination of the signals. This fitting procedure gives close to optimum accuracy for the signal intensity and offers a significant improvement over simply measuring peak heights. The relative signal intensities were normalized to remove the effects of small drifts in spectrometer sensitivity, and finally the small baseline error in the intensities for **C** was corrected using the data for times greater than 250 min. The signal intensities thus obtained were fitted by the Levenberg–Marquardt algorithm using the reaction scheme

$$\mathbf{C} \xrightarrow{k_{CF}} \mathbf{F} \underset{k_{GF}}{\overset{k_{FG}}{\rightleftharpoons}} \mathbf{G}$$

with the program Mathematica 3.0[18] on an Apple Power Macintosh model 7600/132 computer, using the rate constants and initial time as variable parameters. The fitting was repeated for data obtained at 37 °C, but with the reverse reaction k_{GF} omitted from the reaction scheme since this reaction is not significant over the time scale of the experiment. Indeed the presence of the reverse reaction k_{GF} as 55 °C was detectable only by kinetic analysis. Direct evidence could, in principle, be obtained by measuring the incorporation of deuterium at C8 and C10 of **4**. The equilibrium is, however, so slow that further degradation takes place during the several days required to establish even a modest incorporation of deuterium. Table 2 shows the kinetic parameters and estimated standard errors obtained from the fitting process at the two temperatures. The experimental and calculated time courses of the integrals of signals **C**, **F**, and **G** at the two temperatures were compared,[16] and the quality of the fit obtained was very satisfactory.

The key step in terms of the use of clarithromycin as a drug is the initial loss of cladinose. At 37 °C, this is a first-order process with a rate constant of 2.23×10^{-3} min^{-1}, corresponding to a half-life for the drug of 310 min.

(iii) Mechanism of Acid-Catalyzed Degradation of Erythromycin B. A mechanism for the degradation of clarithromycin in acid has been published,[6] but that of erythromycin B has not appeared in the literature. Initially, therefore, we set out to determine the pathway of acid-catalyzed degradation of erythromycin B.

A solution of erythromycin B in D_2O-based Britton–Robinson buffer (containing TSP as a standard) was degraded in the NMR spectrometer at 37 °C. The reaction was followed by measuring ^{1}H NMR spectra at 10 min intervals. A typical time course is shown in Figure 2.

In Figure 2 the signals due to erythromycin B disappear, with concomitant emergence of a second set of signals (marked **J**). The product is essentially stable over this time scale in these reaction conditions. The DOSY spectrum of the mixture was similar to that obtained during clarithromycin degradation and is shown in Figure 3. Resonances assigned to the cladinose ring (H1″, H2″, H8″) showed considerably faster diffusion than those due to the macrolide ring and the desosamine sugar, showing that the cladinose sugar had been hydrolyzed from the macrolide. This result was confirmed by electrospray mass spectrometry of the mixture, in which *m/z* 561 (MH$^+$) indicated the presence of 5-desosaminyl erythronolide B as the principal component of the mixture. This compound, **8**, was now synthesized, isolated, and characterized. The full assignment of its ^{1}H NMR spectrum is given in the Supporting Information. The change in $J_{4,5}$ from 7 to 8 Hz to 2.6 Hz on loss of cladinose indicates a significant conformational change in this part of the macrolide ring. The same reduction in $J_{4,5}$ is observed in 3-mycarosylerythronalide B (a derivative of erythromycin B in which the desosamine sugar is missing). This suggests that interactions between the two sugars constrain the conformation of the macrolide ring in the C2–C6 region (Mordi and Barber, unpublished data). Conformational analysis of these derivatives is in progress.

Integration of the signals due to **5** and **8** over the time course of the acid-catalyzed degradation, and comparison with the standard, revealed that these two compounds did not account for all the macrolide present, especially in the early stages of the reaction. Traces of a third compound could be detected in the spectra (see Figure 2, in which these signals are marked **K**), but it could not be identified from these spectra alone. A clue to the identity of this intermediate came from the methyl 19 signal. During the course of the experiment this signal collapsed from a doublet to a singlet owing to incorporation of deuterium at C8. This suggested that erythromycin B enol ether (**9**) was a likely candidate for the intermediate compound.

The reaction was now followed by electrospray mass spectrometry, sampling at 5 min intervals. The early accumulation of a compound with an MH$^+$ at *m/z* 700 could be seen, followed by its disappearance between 50 and 250 min. Erythromycin B enol ether has a molecular weight of 699 Da, so this experiment provided further support for its presence in the reaction mixture.

Erythromycin B enol ether (**9**) was synthesized by the literature method[8] and fully characterized. Its ^{1}H NMR assignments at apparent pH 7.4 are shown in Supporting Information. It was then incubated at pH 2.5 and 55 °C in the NMR spectrometer. The ^{1}H NMR spectrum obtained was identical with that obtained when erythromycin B was treated in the same way. This demonstrates that erythromycin B enol ether is readily converted to erythromycin B at this pH, strongly supporting the suggestion that **9** is formed during the degradation of erythromycin B to **8**.

The data obtained by monitoring the degradation of erythromycin B at 37 °C were now subjected to the same type of kinetic analysis as the clarithromycin data. The erythromycin B spectra showed poorer signal-to-noise ratios in the methyl region than clarithromycin, but the 8″ methyl region of the spectrum, obscured in the

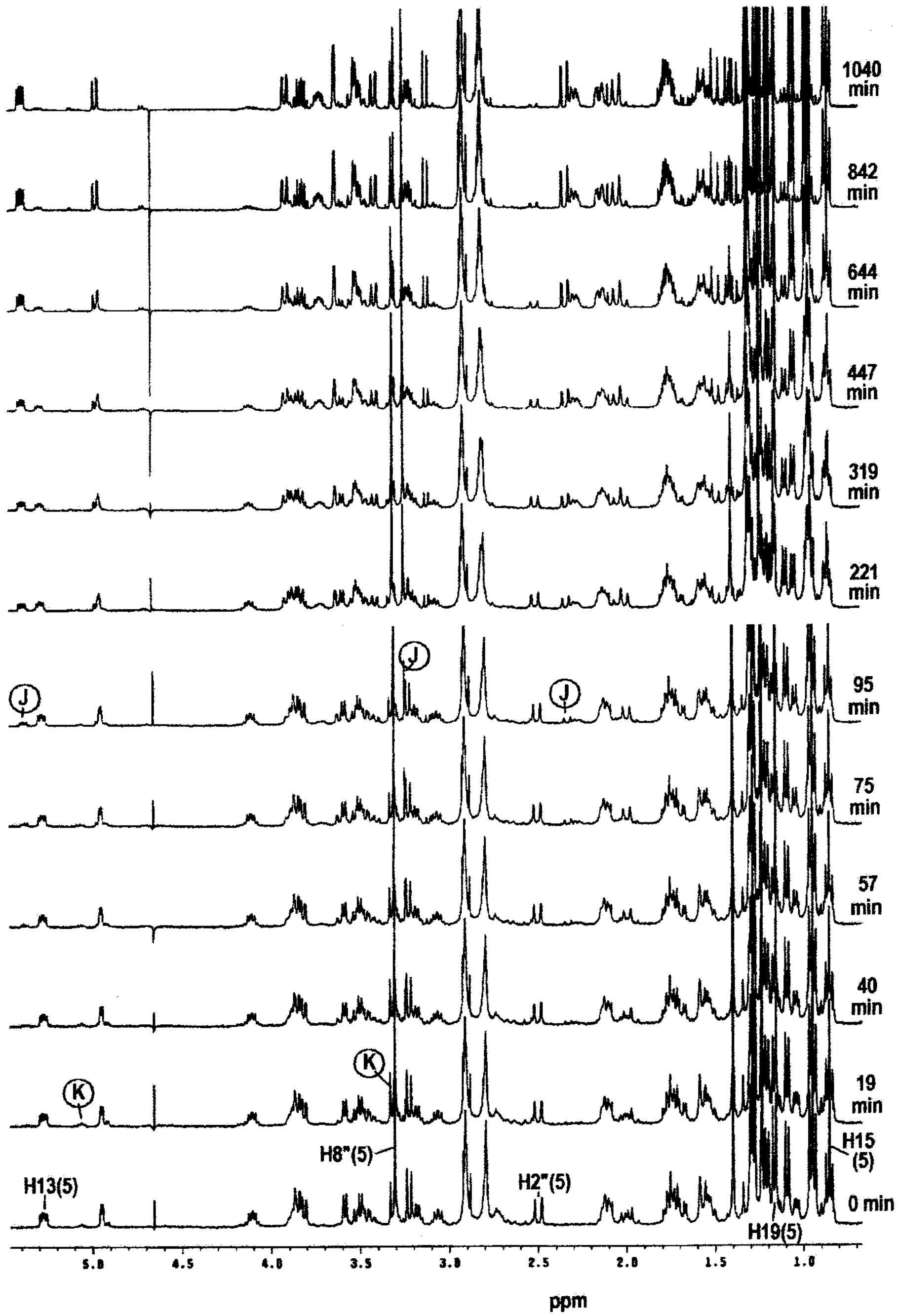


Figure 2. Degradation of erythromycin B in deuterated Britton–Robinson buffer at apparent pH 2.5 and 37 °C. Signals due to the degradation products are marked **J** and K.

clarithromycin samples, was well-resolved. The concentrations of the three species **5**, **9**, and **8** were therefore followed using the peak heights of the methyl 8″ signals rather than the fitted methyl multiplet integrals (which showed very similar time courses but more scatter). Experimental data were subjected to reference deconvolution, resolution enhancement, and baseline correction, and methyl 8″ peak heights were measured. The peak heights were multiplied by the experimental line widths to determine the relative integrals and normalized to remove the effects of any spectrometer gain drift. The 8″ methyl peak intensities for the two anomers of cladinose were combined to give a single value.

The time courses of the resultant three signals for species **5**, **9**, and **8**, shown in Figure 4, show some interesting features. The rapid initial fall in **5**, and concomitant rapid growth in **9**, are not consistent with the simple biexponential behavior expected for two sequential first-order reactions. The source of the unusual behavior here is the incorporation of deuterium from the solvent D_2O at C8 of erythromycin B, by the reverse reaction **9** → **5**. Only a single methyl 8″ signal is seen for **5**, deuteriation at C8 having a negligible effect on the chemical shift of this methyl group. Because the dehydration of **5** to the enol ether **9** requires the breaking of the C–H bond at C8, there is a substantial primary kinetic isotope effect on the reaction **5** → **9**. Thus at the onset of reaction, **5** rapidly forms **9**. The reverse reaction then forms 8-deuterio erythromycin B, $\mathbf{5_D}$, which dehydrates about five times more slowly than the parent compound **5**, rapidly pushing the limiting position of the equilibrium between **5** and **9** back toward **5**. Both compounds **5** and $\mathbf{5_D}$ hydrolyze at the same rate to **8** (which is, of course, partially

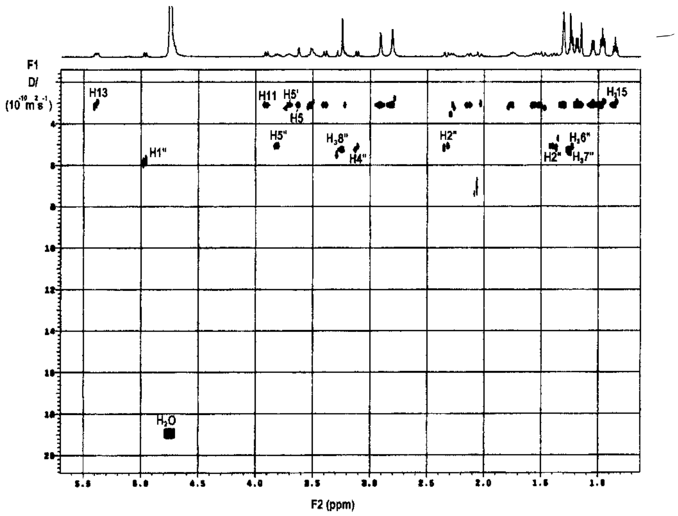


Figure 3. DOSY spectrum of the products of the degradation of erythromycin B in Britton–Robinson buffer at apparent pH 2.5 and 37 °C for 24 h.

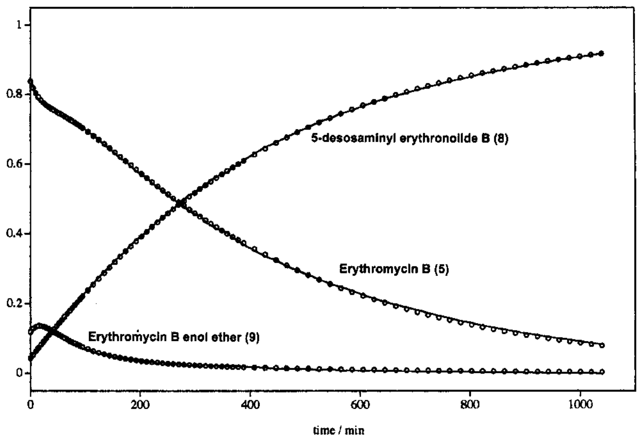


Figure 4. Seventeen hour time course of the degradation of erythromycin B in deuterated Britton–Robinson buffer at apparent pH 2.5 and 37 °C. Experimental points are indicated by open circles, calculated fit by solid lines.

deuterated), since this reaction does not involve the C–H bond at C8. The net result is a steep initial decline in **5** and growth in **9**, which rapidly level off, while the growth in the product **8** remains close (but not identical) to that expected for single-exponential conversion of reactants to product.

The measured signal intensities were again fitted by the Levenberg–Marquardt algorithm with rate constants and initial time as variable parameters, but this time using reaction Scheme 4, with $k_{58} = k_{5D8}$. Because of the complexity of the reaction scheme it was necessary to reduce the size of the data set used in the fitting, so only 43 of the 84 time points available were used. Table 3 shows the kinetic parameters, including the ratio R_{HD} of the dehydration rate constants k_{59} and k_{5D9}, and estimated standard errors obtained from the fitting process. Once again, the quality of the fit, as evidenced both by the statistics and by Figure 4, is gratifying.

As with all analyses of kinetic data, there is an infinite set of possible models which will fit the experimental data, which the combined application of chemical knowledge and Occam's Razor generally reduces to a single kinetic scheme. Here, however, there remains at least one plausible variation on Scheme 4, in which

Scheme 4. Degradation of Erythromycin B in Acidic Aqueous Solution

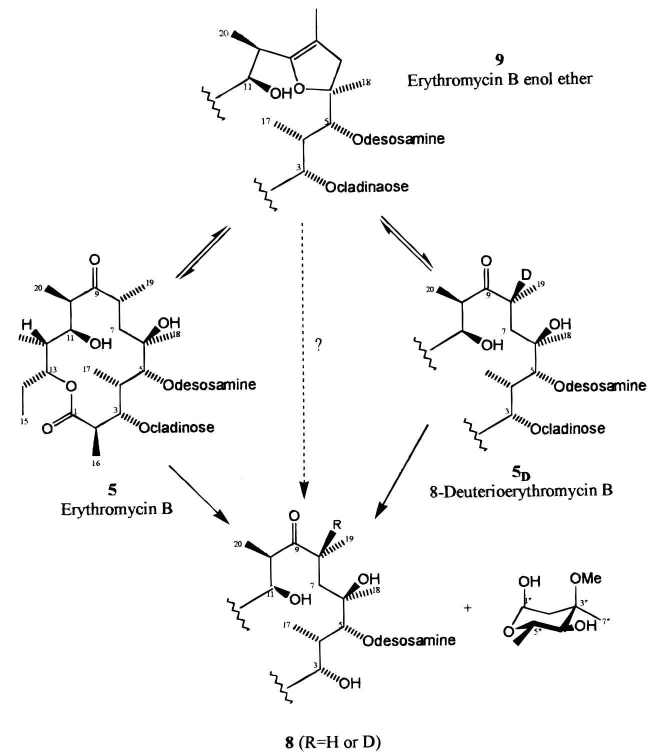


Table 3. Kinetic Parameters Obtained by Fitting Experimental Signal Intensities for the Degradation of Erythromycin B at 37 °C and Apparent pH 2.5[a]

k_{59}	$1.17 \pm 0.05 \times 10^{-2}$ min^{-1}
k_{95D}	$5.55 \pm 0.19 \times 10^{-2}$ min^{-1}
k_{5D9}	$2.36 \pm 0.19 \times 10^{-3}$ min^{-1}
k_{58}	$2.50 \pm 0.01 \times 10^{-3}$ min^{-1}

[a] Uncertainties quoted are twice the standard deviations estimated in the Levenberg–Marquardt fit.

an extra pathway is added from the enol ether (**9**) to 5-desosaminyl erythronolide B (**8**). (The descladinose enol ether would be a transient intermediate.) Attempts to fit the experimental data using a reaction scheme which includes this pathway also gave acceptable, albeit slightly poorer, fits for values of the rate constant k_{98} less than or equal to k_{58}. The presence or absence of this pathway has implications for the behavior of erythromycin B in H_2O as opposed to D_2O, since in H_2O, the unfavorable isotope effect will no longer act to reduce the concentration of the enol ether (**9**), emphasizing the importance of any side reactions involving **9**. The half-life for erythromycin B reduces from 235 to 200 min as k_{98} changes from zero to k_{58}. However, in medicinal terms, the enol ether **9** is expected to serve as a reservoir for **5**. The half-life of (erythromycin B + enol ether) varies from 335 min ($k_{98} = 0$) to 277 min ($k_{98} = k_{58}$). Thus, whether or not the extra pathway **9** → **8** is significant, the half-life of erythromycin B under conditions mimicking those in the stomach is similar to that of clarithromycin.

The Acid-Catalyzed Degradation of Erythromycin A. In a control experiment, erythromycin A was dissolved in Britton–Robinson buffer at apparent pH 2.5 and incubated in the NMR spectrometer at 37 °C. Under these conditions, the degradation of erythromycin A to anhydroerythromycin was complete before a spectrum could be acquired, that is, within 10 min of the beginning of the experiment.

Discussion

Erythromycin A is one of the most important and successful drugs of all time. It has excellent activity against a wide range of Gram-positive pathogens. It is particularly effective because it inhibits bacterial protein synthesis, a metabolic process which is necessary for cellular survival, not just for cell division. The second-generation macrolides, clarithromycin and azithromycin, have slightly different spectra of antibacterial activity from erythromycin A,[4,7,10,19] and azithromycin shows activity against *Toxoplasma gondii* in experimental animals.[20] The prediction of complex in vivo characteristics, such as metabolism and tissue absorption, from in vitro chemical data is in general a perilous undertaking. Among such similar compounds, however, it is fair to point to the large differences in acid stability as being crucial to the success of the modern macrolides. Acid stability is a key factor in allowing shorter courses and lower doses of these drugs to be given, reducing adverse reactions in both adults and children.

It would clearly be advantageous to have available an acid-stable macrolide with a therapeutic profile similar to that of erythromycin A. Erythromycin B has been known for almost as long as erythromycin A, but until very recently it has not been available in large quantity. (Interest in the large scale production of macrolides other than erythromycin A has been stimulated by the identification and modification of the genes responsible for macrolide synthesis in actinomycetes.[21]) In vitro studies have shown that this drug has a similar therapeutic range and activity to erythromycin A.[9] Our solubility data suggest that an exhaustive survey of the therapeutic profile would lead to a position intermediate between erythromycin A and clarithromycin.

Structurally, erythromycin B differs from erythromycin A only in having no hydroxyl group at C12. It is therefore unable to cyclize in a 12,9-direction and unable to form a spiroketal analogous to **2**. The formation of **2** from **1** is a facile process in aqueous acid, as indicated by the failure even to obtain an NMR spectrum of erythromycin A (**1**) under near-physiological conditions mimicking the stomach. We have now demonstrated that erythromycin B is indeed much more stable in acid than is erythromycin A. Like clarithromycin, it degrades in acid by loss of the cladinose sugar. The rate constants for loss of cladinose from clarithromycin and erythromycin B have been determined as 2.23×10^{-3} min^{-1} and 2.50×10^{-3} min^{-1}, respectively. These rates are very similar, as would be expected for processes involving the same mechanism, and indicate that erythromycin B has comparable stability in acid to clarithromycin. Both drugs show considerably higher stability than erythromycin A. Like clarithromycin and other modern macrolides such as azithromycin, erythromycin B is expected to survive oral administration without elaborate formulation. Depending upon the results of in vivo studies, it could therefore find use in the clinic as an inexpensive, but acid-stable, macrolide antibiotic.

Experimental Section

General Procedures. ^{1}H NMR spectra were acquired using a Varian INOVA 400 spectrometer operating at 400 MHz or a Varian Unity 500 spectrometer operating at 500 MHz. Electrospray-ionization mass spectra were acquired using a PLATFORM mass spectrometer. The sample solution (10 μL) was analyzed by ESI-MS using acetonitrile or 0.3% aqueous trifluoroacetic acid as the running solvent. Data were analyzed using the program PLATFORM (Micromass) with a Masslynx data system. All degradation experiments were carried out in deuterated Britton–Robinson buffer[11] (40 mM) unless otherwise stated. Fine adjustments to the pH of the buffer were made using sodium deuterioxide or deuterium chloride solutions in D_2O.

Aqueous Solubilities of Erythromycin A, Clarithromycin, and Erythromycin B. The aqueous solubilities of **1**, **3**, and **5** were determined at room temperature (25 °C) in deuterated phosphate buffer (50 mM, apparent pH 7.0) with a known amount of TSP (3 mM) as an internal standard. An excess of each compound was added separately to the phosphate buffer, and the suspensions were continuously stirred for 24 h. The pH of each suspension was determined at intervals during the solubilization and adjusted if necessary. After 24 h, the suspensions were filtered through filter paper and analyzed by ^{1}H NMR spectroscopy.

Measurement of the Time Course of the Degradation of 5 by ESI-MS. The degradation reaction of **5** (4 mM) in deuterated Britton–Robinson buffer (apparent pH 2.5) was monitored at 55 °C. At intervals of 0.5 min, 50 μL of the degradation mixture was aliquoted into 0.5 mL of cooled boric acid buffer (pH 7.5), to quench the degradation reaction and also to dilute the mixtures for mass spectrometry. Samples were analyzed by ESI-MS using 0.3% trifluoroacetic acid in water as the mobile phase.

Kinetic Measurements by NMR Using Real-Time Autoshimming. Compounds **3** and **5** were dissolved in deuterated Britton–Robinson buffer at apparent pH 2.5 to give concentrations of 4 mM, and the solutions were degraded at 55 °C or 37 °C for a total time of 42 h. The experiment was performed by switching at regular intervals between 1D ^{1}H NMR spectroscopy, using presaturation for water suppression, and a deuterium spin–echo-based autoshimming experiment.[13] The degradation was performed using an array of 30 transients for the first 20 spectra, 80 transients for the next 30 spectra, 180 transients for the next 40 spectra, and 380 transients for each of the remaining 40 spectra. The experiment was arranged so that the sample was automatically autoshimmed at the end of each block of transients. Autoshimming was used to compensate for the local field disturbance caused by variation in sample composition during the course of the reaction. The ^{1}H spectra were recorded with a spinning sample, with a preacquisition saturation delay of 2 s, a 90° pulse of width 5.2 μs, and a spectral width of 5000 Hz. The spectra were processed with reference deconvolution using the FIDDLE algorithm for line shape regularization using the TSP signal as the reference, with a Gaussian time constant of 0.25 s, followed by cubic spline baseline correction. The deuterium spin–echo autoshimming experiment was performed on a static sample as described in ref 13, with a spectral width of 1000 Hz, a 90° pulse of width 84 μs, and a preacquisition delay of 3 s.

DOSY Spectroscopy of the Degradation Products of 3 and 5. Compounds **3** and **5** were dissolved in deuterated Britton–Robinson buffer at apparent pH 2.5 to give concentrations of 4 mM, and the solutions were degraded at 55 °C for 2 and 24 h, respectively. The degraded sample of **3** was cooled to ambient temperature and subjected to a ^{1}H high resolution DOSY NMR experiment using the GCSTESL pulse sequence.[22] Ten spectra were acquired, with gradient pulses of 2 ms ranging in strength from 1 to 30 G cm^{-1} and a diffusion delay of 0.2 s. The degraded sample of **5** was subjected to a ^{1}H high resolution DOSY NMR experiment at 30 °C using the BPPSTE pulse sequence.[22] Twelve spectra were acquired, with gradient pulses of 2.9 ms ranging in strength from 1 to 15 G cm^{-1} with a diffusion delay of 0.1 s. FIDDLE reference deconvolution was employed to correct line shapes, using the TSP line as reference, and baseline errors and systematic errors in apparent gradient strength were corrected. The 2D HR-DOSY spectra were constructed by taking the first echo spectrum and distributing the intensities of the individual signals in the second dimension according to diffusion coefficient, using

Gaussian line shapes with widths determined by the standard errors obtained from the fitting process.[15]

Two-Dimensional NMR Spectroscopy (TOCSY and DQF-COSY). Solutions of **8** and **9** (4 mM) were prepared in deuterated buffer at apparent pH 2.5 and 7.4, respectively. Both TOCSY and DQF-COSY spectra were run at ambient temperature. For both experiments, the water resonance was suppressed by presaturation. Data matrices of 2048 $\times$ 1024 complex points were acquired. NMR spectra were processed using negative line broadening and Gaussian multiplication for enhancement of resolution.

Synthesis and Characterization of 5-Desosaminyl Erythronolide B (8). Erythromycin B (0.50 g) in 175 mL of protiated Britton−Robinson buffer (pH 2.5) was degraded at 55 °C for 16 h. The solution was allowed to cool to room temperature, and its pH was adjusted to 9.5. The aqueous solution was extracted with chloroform (3 $\times$ 175 mL), which then was concentrated on a rotary evaporator and dried over anhydrous sodium sulfate. Finally, the organic solvent was removed in vacuo. The colorless crystalline product was recrystallized from acetone to give 0.30 g (70% yield) of 5-desosaminyl erythronolide B (**8**), mp 102−104 °C. Full assignment of the ^{1}H NMR spectrum was achieved using a 4 mM solution of **8** in deuterated Britton−Robinson buffer (apparent pH 2.5) at ambient temperature. A TOCSY spectrum was used to distinguish the different spin groups of **8**, and a DQF-COSY spectrum allowed signals within each spin group to be distinguished. The isolated methyl signals were assigned by analogy with the ^{1}H NMR spectrum of erythromycin B (P. Tyson & J. Barber, unpublished data).

Synthesis and Characterization of Erythromycin B Enol Ether (9). Erythromycin B enol ether was prepared by an adaptation of the published procedure for the preparation of erythromycin A enol ether.[3] Erythromycin B (200 mg) was dissolved in glacial acetic acid (5 mL) and was allowed to stand at room temperature for 4 h. Saturated sodium bicarbonate solution (50 mL) was added, and the enol ether was extracted using dichloromethane (3 $\times$ 100 mL). The organic layer was concentrated under reduced pressure, washed with saturated sodium bicarbonate solution, and then was dried over anhydrous sodium sulfate. The solvent was removed in vacuo. The colorless crystalline product was recrystallized from acetone to give 177 mg of **9** (91% yield), mp128−130 °C (lit. 126−128 °C[23]). Full assignments of the ^{1}H NMR spectra were achieved in deuteriochloroform and deuterated phosphate buffer (apparent pH 7.4) using TOCSY and DQF-COSY spectra in the same way as for **8**. All spectra were measured at ambient temperature.

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Supporting Information Available: Complete ^{1}H NMR data for 5-desosaminyl erythronolide B (**8**) and erythromycin B enol ether (**9**) in aqueous buffer and the following figures: (a) low field region of the 400 MHz ^{1}H NMR spectrum of clarithromycin after degradation for 4 days at apparent pH 2.5 and 55 °C, (b) 400 MHz DOSY spectrum of clarithromycin after approximately 2 h degradation in the same buffer at 37 °C, (c) and (d) 48 h time courses of the degradation of clarithromycin in the same buffer at 55 °C and 37 °C. This material is available free of charge via the Internet at http://pubs.acs.org.

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