the failure to induce polymerization of acrylonitrile and the reduction of mercuric chloride. In most hydrogen abstraction reactions, the reaction constants have small magnitude.[17b] In the present investigation, the large negative value of the reaction constant, together with the substantial kinetic isotope effect, would suggest considerable carbonium ion character in the transition state. The experimental results would point to a hydride ion transfer in the rate-determining step. The hydride ion transfer can occur directly (Scheme I) or may involve the prior formation of a chromate ester (Scheme II). The similarity in rate laws with chromic acid oxidations[12] would suggest the formation of a chromate ester.

Registry No. QDC, 56549-24-7; $PhCH_2OH$, 100-51-6; *m*-$MeC_6H_4CH_2OH$, 587-03-1; *p*-$MeC_6H_4CH_2OH$, 589-18-4; *p*-$OMeC_6H_4CH_2OH$, 105-13-5; *p*-$ClC_6H_4CH_2OH$, 873-76-7; *m*-$BrC_6H_4CH_2OH$, 15852-73-0; *m*-$NO_2C_6H_4CH_2OH$, 619-25-0; *p*-$NO_2C_6H_4CH_2OH$, 619-73-8.

Erythromycin A as a Supramolecular Catalyst: Effect on Rhodamine B Lactonization

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The intramolecular lactonization reaction of rhodamine B base in chloroform solution is remarkably accelerated by the antibiotic erythromycin A. The rate increases by a factor of ca. 10^{10}, a value significantly higher than typical enzymatic factors. This effect is ascribed to the formation of a host–guest complex between the dye and the antibiotic, a process that induces a conformational change conducive to the rehybridization of the central carbon atom of the dye.

Introduction

The study of reactions that involve noncovalent interactions between two molecules, one acting as a host and the other acting as a guest, is a subject of great current interest in fundamental as well as in applied research.[1,2]

The ability of biochemical compounds to recognize and selectively bind guest species and eventually alter chemical reactivity represents one of the most important properties of these molecules. Thus, certain antibiotics have been characterized as receptors for charged hydrophilic species such as Na^+, K^+, Ca^{2+}, etc., and have been studied as natural ionophores.[2] However, there are few reports in the literature on the complexation of antibiotics with organic molecules,[3,4] and, as far as we know, no kinetic studies have been conducted on such systems.

Erythromycins are widely used antibiotics of the macrolide family whose structures are well defined in both the solid state and in solution.[5] These compounds consist of a polyfunctionalized 14-membered lactone ring bonded to sugar units, a structure that suggests that these macrocycles may be good receptors for organic guests.

We recently initiated studies using erythromycin A (E) as a supramolecular receptor, with the finding that E forms molecular complexes with several organic dyes in nonaqueous solutions.[6] We further found that ring-opened erythromycin A inhibits the alkaline hydrolysis of *p*-nitrophenyl esters through the formation of a host–guest complex.[7]

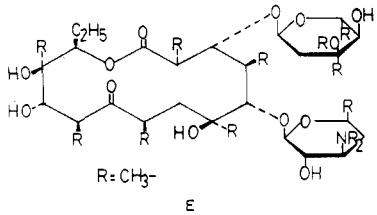

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Table I. Rate and Equilibrium Constants for the Lactonization Reaction of Rhodamine B Base in Chloroform[a]

T, °C	k_1, s^{-1}	K_1, M
50	1.70×10^{-3}	0.211
40	8.33×10^{-5}	0.125
25	6.2×10^{-7} [b]	0.054[b]

[a] Initial concentrations of Z = 1×10^{-5} M. [b] Extrapolated values.

In a previous communication we pointed out the remarkable effect of E on the lactonization reaction of rhodamine B base in aprotic or weakly polar solvents.[8] We wish to report herein the enormous rate enhancement of the intramolecular ring closure reaction of rhodamine B base in chloroform solution in the presence of E.

Results and Discussion

The xanthene dye rhodamine B base exists in solution as an equilibrium mixture of a colored zwitterion Z and a colorless lactone L (Eq 1).

The equilibrium constant for this system was found to depend on both the solvent hydrogen-bond donating ability (favoring the zwitterion) and the solvent dielectric/polarizability properties.[9]

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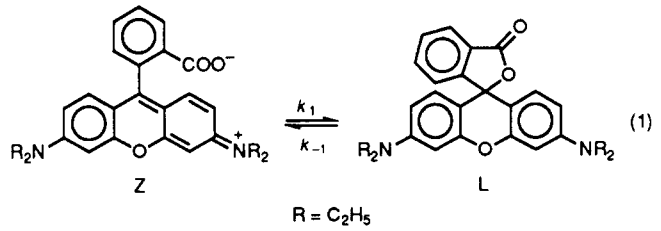

(1)

In $CHCl_3$ solution at room temperature, rhodamine B exists almost exclusively as the zwitterionic form, as confirmed by IR and NMR spectroscopy. Therefore it is not possible to measure k_1 at 25 °C. However, in order to estimate this value we determined the rate of ring closure at 40 °C and 50 °C by measuring the rate of decrease of absorbance at the maximum wavelength of Z in chloroform (λ = 551 nm).

From the absorbances resulting after establishment of the equilibrium, we determined the equilibrium constants at these two temperatures. These values, together with those for the rate and equilibrium constants extrapolated to 25 °C, are collected in Table I.

Effect of Erythromycin A. The addition of increasing amounts of erythromycin A to a solution of Z in chloroform results in an instantaneous bleaching of the solution (Figure 1). A similar behavior was observed when Z was dissolved in dimethyl sulfoxide or dioxane.[8] The observed decoloration is reversible, since the addition of ethanol to the solutions in chloroform or dioxane leads to the original spectrum. Furthermore when water is added to a colorless solution initially containing equimolecular amounts of Z and E in dioxane, the equilibrium is shifted toward Z. This effect is more pronounced than that resulting from the addition of the same number of molecules of ethanol,[8] reflecting the fact that the hydrogen-bond donating ability of water is better than that of ethanol.[10]

The NMR and IR spectra of the solid isolated from a colorless solution containing equimolecular amounts of Z and E in chloroform are similar to those of L and E, with only some of the signals perturbed (see Experimental Section). The fluorescence spectrum of L in chloroform shows the characteristic dual emission at 470 nm and 555 nm, which has been attributed to the decay of a relaxed form of the excited lactone and to the decay of the excited zwitterion.[11] The dual fluorescence spectra of (Z + L) mixtures in chloroform are very similar to those corresponding to (Z + E) mixtures. These results indicate that in the presence of E the zwitterion yields the lactone.

Thus, the decoloration observed when the antibiotic is added to solutions of Z in chloroform (as well as in DMSO or dioxane) may be ascribed to the formation of a host–guest complex between E and Z, followed by the conversion of Z into L. The stoichiometry of this complex was established by the molar ratio method.[12] The absorbance of solutions at variable concentrations of E and constant concentration of Z was plotted vs the ratio [E]/[Z]. In the three solvents analyzed, the stoichiometry of the interaction was 1:1, and the line broke abruptly at this ratio, indicating a very stable complex.[8]

The bleaching process could be represented as shown in Eq 2:

$$Z + E \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Z{:}E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} L{:}E \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} L + E \qquad (2)$$

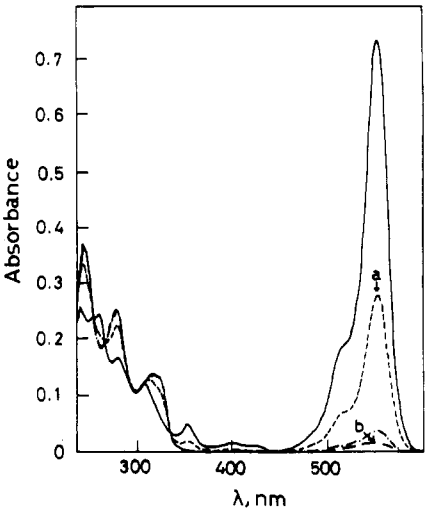

Figure 1. Spectrum of Z in chloroform at varying concentrations of E (4.8×10^{-1}–^{6}, 9.5×10^{-6}, and 1.9×10^{-5}M, from a to b; $[Z]_T = 9.6 \times 10^{-6}$M).

Scheme I

$L \longrightarrow S_1$

$S_1 \longrightarrow T_1$

$S_1 \longrightarrow Z$

$T_1 \longrightarrow Z$

The dissociation of the L:E complex is a slow process as evidenced by the fact that only a very slow decoloration is observed when 1 equiv of Z is added to a solution of L:E. Furthermore, when E and L are mixed and 1 equiv of Z is added after 1 h, the absorption of the final solution indicates that only 5% of E is complexed with L, since 95% of Z reacts, indicating that the association of L with E is also a slow process.

Photochemistry of L:E. Consistent with the fact that the thermal ring-opening of the lactone to give the zwitterion is a slow process ($k_{-1} = 1.2 \times 10^{-5}$ s^{-1}, value extrapolated at 25 °C), the lactone can be dissolved in chloroform and its properties studied without interference of the zwitterion.

There are only a few reports in the literature on the photochromism of rhodamine B lactone, wherein it has been shown that upon photoexcitation, the lactone yields the ionic form as a result of the dissociation of the C–O bond and subsequent formation of Z.[13] It has also been shown that the photodissociation of the neutral form of some rhodamine derivatives in solvents not containing heavy atoms (such as acetonitrile and ethanol) proceeds from the singlet state.[14]

Irradiating a deoxygenated solution of L in chloroform at 315 nm leads to the formation of the zwitterion with a quantum yield of (0.16 ± 0.01), a value that decreases to (0.08 ± 0.01) in the presence of oxygen. Under similar conditions of irradiation, L:E or (L + E) solutions lead to short-lived transients as described below.

It has been reported that the presence of heavy atoms ($CHBr_3$) produces an increase in the quantum yield of intersystem crossing to the triplet state of some rhodamines.[15] Accordingly (and taking into account the influ-

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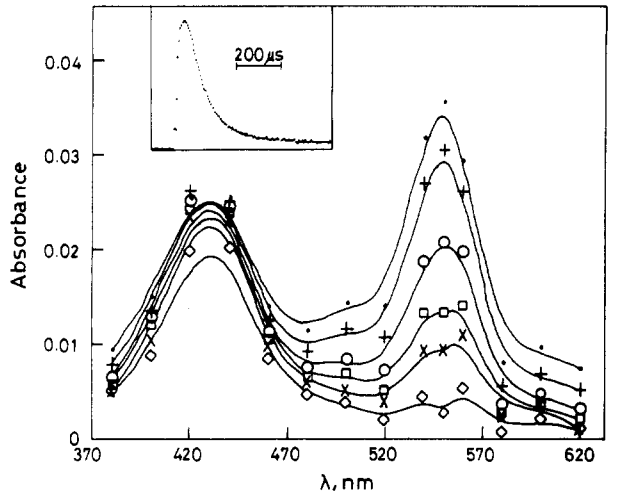

Figure 2. Transient absorption spectra of Z (2×10^{-4}M) and E (8.3×10^{-4}M) in chloroform [(●) 50 μs, (+) 80 μs, (○) 120 μs, (□) 160 μs, (×) 200 μs and (◇) 400 μs] after the flash. Inset: growth and decay signal for the transient absorption at 550 nm.

ence of oxygen on the quantum yield for the photodissociation of L in chloroform), we suggest that the phototransformation of L into Z occurs from both the S_1 and T_1 states (Scheme I).

The $S_1 \rightarrow Z$ and $T_1 \rightarrow Z$ processes are not necessarily elementary steps, since the heterolytic bond fission might involve the transition from the excited states of L to the excited states of Z, as well as the transition from the excited to the ground states of Z.[11]

The photochemical behavior of colorless solutions containing Z and E in chloroform ($Z_0 \leq E_0$) was investigated through laser flash photolysis techniques using a nitrogen laser.[16]

Flashing a degassed colorless solution containing Z (2×10^{-4}M) and E (8.3×10^{-4}M) in chloroform, leads to the appearance of two transient absorptions: one with $\lambda_{max} \approx 550$ nm and the other long-lived with $\lambda_{max} \approx 430$ nm (Figure 2).

The transient absorption observed at 430 nm in chloroform solution is completely quenched by oxygen. As we have mentioned before, the irradiation of L:E leads to an excited form of Z, and it is known that the irradiation of Z in alcoholic media leads to the formation of the half-reduced form of the dye (R) by reaction of the triplet state (T) with the alcohol molecules (Eq 3).[17]

$$R'CH_2OH + T \rightarrow R'\dot{C}HOH + R^{\bullet -} + H^+ \qquad (3)$$

Since in the complex with E the lactone is in an environment with several hydroxylic groups, we suggest that the transient absorption with $\lambda_{max} = 430$ nm may be due to the formation of $R^{\bullet -}$. This transient species, however, was not investigated in further detail.

The growth and decay signal for the transient absorption at 550 nm is shown in the inset of Figure 2. The visible absorption spectrum of this transient is in good agreement with that of Z. The decay of the absorption signal at 550 nm follows first-order kinetics with a lifetime of ≈95 μs. This lifetime does not change in the presence of oxygen or anthracene, a result which indicates that the species absorbing at 550 nm is not a triplet state. However, the decrease in absorption intensity observed in the presence of oxygen would seem to indicate that the transient species proceeds at least in part from a triplet state.

We can then conclude that the photochemical intermediate responsible for the time-dependent growth and decay of the signal at 550 nm is the zwitterion generated by photodissociation of the lactone form, a process that involves both the excited singlet and triplet states. This zwitterion can only arise from associated lactone (L:E) since, as we have mentioned, the spontaneous lactonization of Z in chloroform and the dissociation of the L:E complex (generated by the association between Z and E) are very slow. Besides, the concentration of L is insufficient to compete with L:E upon laser excitation.

The rate constant of decay (k_{decay}) for the transient absorption at 550 nm increases as the concentration of E (defined as $[E] = [E]_T - [Z]_T$) increases (Table II), approaching a maximum saturation value, a behavior similar to that observed in enzyme kinetics (Figure 3). The dependence of k_{decay} on the concentration of E indicates that the zwitterion generated by photodissociation of L:E becomes uncomplexed before regenerating the neutral form. The fact that the signal intensity at 550 nm decreases as the concentration of E increases supports this hypothesis.

We suggest that the processes involved in the growth and decay of the absorption signal at 550 nm can be represented as shown in Scheme II.

Again, S_1:E → Z + E and T_1:E → Z + E may not represent elementary steps. Enough data has not been accumulated to determine whether the liberation of Z occurs

Table II. Rate Constants of Decay for the Transient Absorption at 550 nm in Chloroform Solutions, at Varying Concentrations of Erythromycin A

10^4[E], M^a	$10^{-3}k_{decay}$, s^{-1}	10^4[E], M^a	$10^{-3}k_{decay}$, s^{-1}
1.05	2.58	8.30	10.6
2.11	4.67	14.4	15.1
6.21	9.01	18.5	17.0

[a] Uncomplexed erythromycin A ([E] = $[E]_T - [Z]_T$).

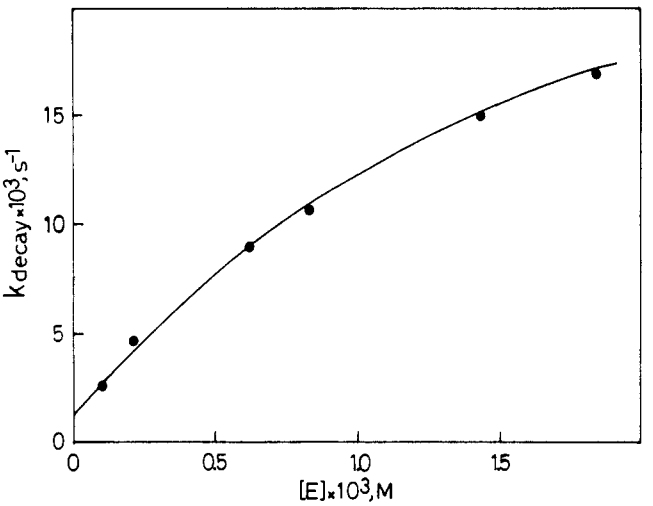

Figure 3. Rate constant of decay (k_{decay}) for the transient absorption at 550 nm vs [E].

Scheme II

growth

L:E → S_1:E

S:E → T_1:E

S_1:E → Z + Z

T_1:E → Z + Z

decay

$$Z + E \underset{}{\overset{K_2}{\rightleftharpoons}} Z{:}E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} L{:}E$$

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from the ground or the excited states.

The decay processes indicate that Z and E undergo a rapid association followed by the conversion of Z into L (rate-limiting step). The expression of k_{decay} is given by Eq 4:

$$k_{decay} = \frac{K_2 k_3 [E]}{1 + K_2[E]} \quad (4)$$

From the slope and intercept of the linear plot of $1/k_{decay}$ vs $1/[E]$ we were able to determine K_2 and k_3, these values being $(1.2 \pm 0.1) \times 10^3$ M^{-1} and $(2.3 \pm 0.4) \times 10^4$ s^{-1}, respectively. Thus, the resulting ratio between k_3 and k_1 (at 25 °C) is $\approx 10^{10}$, a value significantly higher than typical enzyme catalytic factors (10^4–10^6).

Conclusions

We have found that the intramolecular lactonization reaction of rhodamine B base (Z) in chloroform is remarkably accelerated by erythromycin A (E), through a mechanism that involves a rapid host–guest complex formation between Z and E. We suggest that the binding energy which results from this association acts to decrease the free energy of activation of the intramolecular reaction, probably inducing a change in the geometry of Z conducive to the rehybridization of the central carbon atom. Since the efficiency of an intramolecular nucleophilic attack depends on the geometry of approach to the electrophilic center, the carboxylate group must be directed toward the electrophilic central carbon in such a way as to stabilize it until it reaches an appropriate position to form the C–O bond. A similar interpretation has been advanced for the catalysis of the lactonization of phenolphthalein by β-cyclodextrin.[18]

The example of induced geometrical distortion presented herein is analogous to those found in enzyme–substrate complexes and, hence, represents a simple chemical model for an enzymic system.

Experimental Section

Erythromycin A (Sigma) and Rhodamine B base (Aldrich, mp 202–3 °C) were used as received. Rhodamine B lactone was prepared as reported.[8] The purity of these compounds was verified by IR, ^1H NMR, and thin-layer chromatography.

Malachite green leucocyanide (MGL) was prepared as previously described.[19] Chloroform was purified by distillation.

The UV–vis absorption spectra were recorded on a Shimadzu UV-260 recording spectrophotometer. The ^1H NMR and IR spectra were recorded on Bruker 80-FT and on Nicolet-FT 5SXC instruments, respectively. Fluorescence spectra and quantum yield determinations were carried out on a Farrand Mark I spectrofluorometer.

Laser flash photolysis experiments were carried out by using, for excitation, the pulses from a Laser Optics S.A. nitrogen laser (337 nm, 7 ns FWHM, 5 mJ/pulse). Transmitted light from a 150-W quartz–halogen lamp was resolved by a monochromator and detected with a photomultiplier, and the resulting signal was captured with a Hewlett-Packard digitizing oscilloscope interfaced to an IBM/PC.

Rhodamine B Lactone–Erythromycin A Complex. For isolation of the association product between rhodamine B base and erythromycin A, equimolar amounts of the dye and antibiotic were dissolved in chloroform at room temperature, and the solvent was then removed under reduced pressure. From comparison of the IR (KBr) and ^1H NMR spectra of E, Z, and L with those of the solid obtained as described above, and based on the displacement observed in the signals, we deduced that the neutral form of the dye is associated with the antibiotic; however, the data at hand are insufficient to deduce the structure of this complex. IR (carbonyl stretching): Z, 1344 and 1589 cm^{-1}; L, 1755 cm^{-1}; E, 1715 cm^{-1}; complex, 1728 cm^{-1} (shoulders at 1701 and 1754 cm^{-1}). The IR spectrum of a solution of L summed to the spectrum of a solution of E is exactly the same as the spectrum of a solution containing E + L. The ^1H NMR spectra are shown in Tables III and IV.

Table III. ^1H NMR Chemical Shifts of Selected Protons in Erythromycin A and Its Complex with Rhodamine B Lactone[a]

	erythromycin A		
	lit.[b]	obsd	complex
H-3	4.00	4.00	3.97
H-5	3.56	3.51	3.43
H-11	3.86	3.84	3.80
H-13	5.07	5.09	5.10
H-1′	4.41	4.46	4.54
H-1″	4.92	4.92	4.89
H-5′	4.01	4.05	3.99
6-CH_3	1.47	1.47	1.44
OCH_3	3.32	3.32	3.34

[a] In $CDCl_3$ δ (ppm). [b] Majer, J.; Martin, J. R.; Egan, R. S.; Corcoran, J. W. *J. Am. Chem. Soc.* **1977**, *99*, 1620.

Table IV. ^1H NMR Chemical Shifts of Rhodamine B[a]

	δ (ppm)		
	zwitterion	lactone	complex
CH_3	1.30	1.17	1.19
CH_2	3.58	3.36	3.38
xanthene ring	6.75–7.15	6.24–6.62	6.30–6.695
phenyl ring	7.68	7.59	7.59
	8.30	7.99	8.01

[a] In $CDCl_3$. [b] The solid complex, prepared as indicated in the Experimental Section, was used.

Flash Photolysis Experiments. All measurements were made on freshly prepared solutions in chloroform at room temperature. Solutions were degassed by bubbling with oxygen-free nitrogen for 15 min before flashing. The total concentration of the dye used to analyze the photodissociation in the presence of erythromycin A was 2×10^{-4} M.

Quantum Yield Determinations. The quantum yield of L in chloroform solutions was determined by using an ethanol solution of malachite green leucocyanide as actinometer, $\Phi = (0.91 \pm 0.01)$.[20] The solution of MGL was prepared in absolute ethanol as described by Calvert and Rechen.[21] The solutions were degassed by bubbling with oxygen-free nitrogen saturated with the corresponding organic solvent. Photolysis was carried out by irradiation at 315 nm. The yields of the photoproducts were determined by measuring the absorption at 551 nm (rhodamine B solutions) or at 620 nm (MGL solutions).

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Registry No. E, 114-07-8; Z, 81-88-9; L, 509-34-2.

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