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Erythromycin A as a supramolecular receptor: Association with dyes

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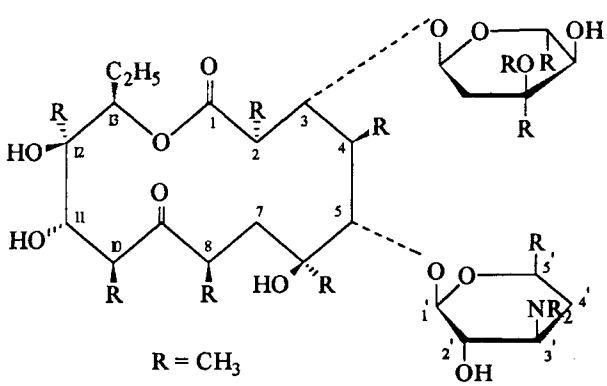
Several organic compounds, such as p-methyl red (1), o-methyl red (2), p-nitroaniline (3), sulforhodamine B (4), and rhodamine 6G (5) form molecular complexes in non-aqueous solutions with the antibiotic erythromycin A (E). The association constants (K_{Ass}) for these complexes were determined by spectrophotometric titrations. Enthalpies and entropies of complexation in chloroform were calculated from the temperature dependence of K_A for 3, 4, and 5. The association process appears to be mainly entropy controlled. In chloroform solution, p-methyl red associates with E while o-methyl red does not. On the other hand both compounds show a weak interaction in dioxane. The differences in behavior in the two solvents are attributed to a change in conformation of E which allows a favorable interaction with 2 in dioxane but not in chloroform. Rhodamine 6G forms dimers in dioxane and its association with E destroys them.

INTRODUCTION

The ability of biochemical compounds to recognize and selectively bind guest species represents one of their most important properties. Some antibiotics have been characterized as receptors for charged hydrophilic species such as Na^+, K^+, Ca^{+2}, etc., and thus have been studied as natural ionophores.[1] However, there are few reports in the literature on the complexation of these compounds with organic molecules. In this sense, the antibiotics ristocetin and vancomycin and synthetic analogs have received considerable attention in recent years because they form complexes with small peptides, and these complexes provide an excellent system for studying substrate-receptor interactions.[2] The role of molecular complexation in the activity of actinomycin D in the presence of heterosteroid-type substances has also been investigated.[3] The antibiotic vancomycin has been shown to catalyze the hydrolysis of carbamates.[4]

Erythromycins are widely used antibiotics of the macrolide family whose structures are very well defined not only in the solid state but also in solution.[5] These compounds have a poly-functionalised 14-membered lactone ring substituted with sugar units; erythromycin A (**E**) has a neutral sugar (cladinose) and an amino sugar (desosamine). Because of the macrocyclic nature of its structure, **E** may be a good receptor for organic guests. An analytical method for the determination of erythromycin is based on its association with organic dyes such as Alizarine red and Tropaeolin OO.[6] In previous studies it has been shown that erythromycin A (**E**) can act as a supramolecular receptor.[7,8] The study of the interaction of organic dyes with natural and synthetic hosts as well as with other organized assemblies like micelles, is common in host-guest complex chemistry because the absorption and/or the emission spectra of dyes are usually sensitive to changes in the microenvironment which allows for their use as sensors for the properties of



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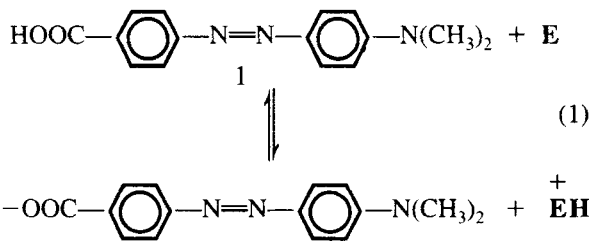
cavities and interfaces.[9] In addition, the knowledge of the equilibrium constants for the interaction of dyes with potential host may be useful for the determination of the interaction of this host with other guests using the competitive method.[10]

We found that **E** is capable of interacting with several organic molecules and that it can recognize subtle changes in the structure of the guest.

RESULTS AND DISCUSSION

The addition of **E** to solutions of 4-dimethylamino azobenzene-4'-carboxylic acid (p-methyl red) **1** in chloroform results in a small hypsochromic shift of the wavelength of the maximum absorption and a decrease in intensity. It is noteworthy that the spectrum of compounds of similar structure such as azobenzene or 4-dimethylamino-4'-methoxy azobenzene do not change upon addition of **E**. In order to determine whether the interactions were mainly related to the presence of the dimethyl amino group or to the carboxyl group, we studied the effect of the addition of **E** on the spectrum of 4-phenylazobenzoic acid and 4-dimethylamino azobenzene (methyl yellow). The spectrum of these two compounds did not change up to the maximum concentration of **E** used (1.65×10^{-2}M). Since the pKa of **E** is 8.8[11] and that of **1** is 3.3[12] in water, the changes in the spectrum could be attributed to the acid-base equilibrium between these two compounds, eq 1.



Therefore the effect of 1,4-diazabicyclo [2.2.2] octane (DABCO) (pK_a = 9.2)[13] on the spectrum of **1** was studied. The addition of DABCO to a solution of **1** produces similar changes in the spectrum as those produced by **E**, indicating that the interaction between **E** and **1** involves some degree of proton transfer. On the other hand, the spectrum of 4-dimethylamino azobenzene-2'- carboxylic acid (o-methyl red) **2** does not change upon addition of **E** but when DABCO is added the absorption intensity at the wavelength maximum decreases in intensity and there is an isosbestic point at 428 nm. These results show that the interaction between **1** and **E** involves the p-carboxyl group and the p-dimethylamino groups. It should be noted that neither 4-phenyl azobenzoic acid nor 4-dimethylamino azobenzene show any changes when **E** is added, although the pKa of the carboxyl and dimethylamino groups, respectively, should be similar to the corresponding pKa of **1**. CPK molecular models indicate that if the hydrogen of the carboxyl group of **1** is placed at the hydrogen bond distance of the dimethyl amino group of **E**, the dimethyl amino group of **1** can be placed at the hydrogen bond distance of the OH bonded to carbon 12 of the macrocycle of **E**. Therefore hydrogen bonding may be one of the major driving forces for the recognition of **1** by **E**. Other forces besides hydrogen bonding should also contribute to the interaction as it is common in other host-guest complexes.[14] There is also a weak interaction between p-nitroaniline **3** and **E** in non-polar solvents (Table 1) which manifests itself through modifications in the UV spectrum, but p-nitrophenol does not interact with **E** in protic nor in non-protic solvents. This points to the specificity of the interaction since the latter two compounds have quite similar structure.

In dioxane, the interaction of **1** with **E** is significantly weaker than in chloroform (see Table 1) but in this solvent the ortho derivative **2** also interacts and the equilibrium constant can be determined. The different behavior in the two solvents may indicate that the more stable conformation of **E** is different in the two solvents. It is known that **E** exist in several conformations and their relative energy depends on the solvent.[15]

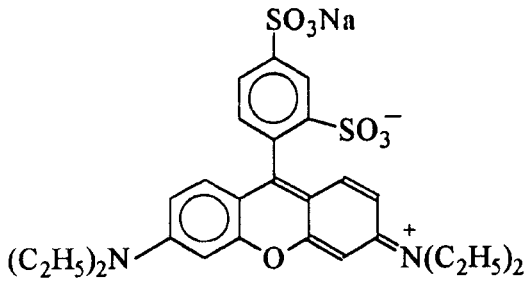
Since it was found that **E** forms inclusion compounds with rhodamine B,[8] the interaction of other xanthene dyes, sulforhodamine, **4**, rhodamine 6G, **5**, and eosine Y, **6**, with **E** was studied. The spectrum of **4** and **5** in dichloromethane and chloroform, changes with the addition of **E** but that of **6** is not affected.

In dioxane solution, **4** and **5** also interact with **E** but **6** does not. The behavior of **5** has some peculiarities which will be discussed below.

Table 1 Association constants of erythromycin A with compounds **1–5**

Solvent (dielectric constant)[b]	K_{Assoc}, M^{-1} (λ, nm)[a]				
	1	**2**	**3**	**4**	**5**
Cl_2CH (9.08)			24±3 (360)	179±9 (555)	467±32 (520)
Chloroform (4.8)	480±70 (440)	c	4.0±2.0 (383)	64±7 (553)[d]	110±10 (522)[e]
Dioxane (2.2)	8±5 (431)	37±18 (480)	4.0±0.1 (378)	80±7 (556)[d]	180±15 (536)[e,f]

[a]Wavelength at which the change in optical density was measured. [b]Dielectric constants taken from *Handbook of Chemistry and Physics*, 72nd Edition, Ed. D. R. Lide, CRC Press, USA, 1991–1992. [c]The changes observed in optical density were negligible. [d]The solvent contains 0.6% ethanol. [e]The solvent contains 0.4% ethanol. [f]Apparent association constant, see text.



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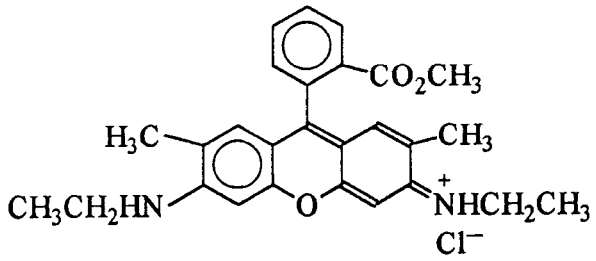

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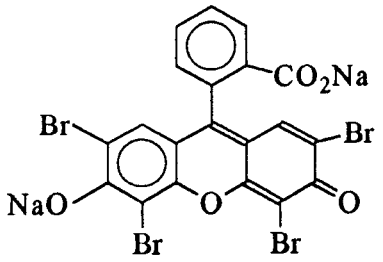

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The changes in the UV spectrum produced by the addition of **E** can be attributed to the formation of a host-guest complex where the dye is the guest and **E** is the host. Assuming a 1:1 type of interaction, eq. 2, the change in absorbance at a fixed wavelength is given by eq 3[16] where ΔA is the change in absorbance, $\Delta\epsilon_1$ is the difference in extinction coefficients of the guest and the complex, and G_0 is the initial concentration of the guest.

$$\mathbf{E} + \mathbf{G} \underset{\mathbf{G} = 1\text{–}5}{\overset{K_{Assoc}}{\rightleftharpoons}} \mathbf{EG} \qquad (2)$$

$$\Delta A = \frac{G_o \Delta\epsilon_1 K_{Assoc}[E]}{1 + K_{Assoc}[E]} \qquad (3)$$

Non-linear fitting of the measured absorbance to eq 3[17] yields the equilibrium constants collected in Table 1. Alternatively, eq 3 can be rearranged as in eq. 4 and the value of K_{assoc} is obtained from a plot of the left hand side of eq. 4 vs **E**.[18] Both methods of treatment of the data give results in good agreement, within experimental error.

$$\frac{[G_o]\,[E]}{\Delta A} = \frac{1}{K_{Assoc}\Delta\epsilon_1} + \frac{[E]}{\Delta\epsilon_1} \qquad (4)$$

The spectrum of **1** in chloroform in the presence of **E** does not show an isosbestic point indicating that there is more than one type of interaction, nevertheless the data can be fitted by eq 3 and 4. This may mean that complexes of higher stoichiometry, 2:1 or 1:2, are formed, but they are weaker than the 1:1 type of complexes. If complexes of 1:2 stoichiometry are formed, the expression for the absorbance change vs. concentration of **E** is given by eq. 5,[19]

$$\Delta A = \frac{G_0\,(\Delta\epsilon_1 K_1\,[E] + \Delta\epsilon_2 K_1 K_2\,[E]^2)}{1 + K_1\,[E]\,(1 + K_2\,[E])} \qquad (5)$$

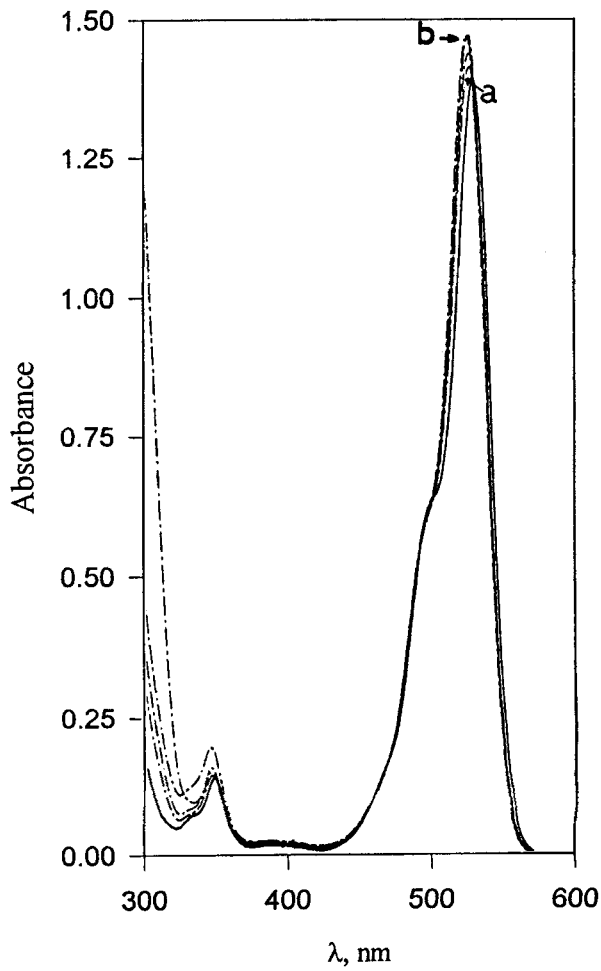

Figure 1 Spectrum of **5** (1.5×10^{-5}M) in chloroform at variable concentrations of **E** (4.0×10^{-3}M to 4.0×10^{-2}M, from a to b).

where $\Delta\epsilon_1$ and $\Delta\epsilon_2$ are the difference in extinction coefficients for the substrate and the 1:1 and 1:2 complexes, respectively. If $\Delta\epsilon_1 \approx \Delta\epsilon_2$, eq 5 simplifies to eq 6.

$$\Delta A = \frac{G_0 \Delta\epsilon_1 K_1 [E] (1 + K_2 [E])}{1 + K_1 [E] (1 + K_2 [E])} \quad (6)$$

The second order term in **E** becomes significant if K_2 [E] > 1. The fact that the data can be fitted by eq. 3, and 4 indicates that $K_2 < 61$, since the higher concentration of **E** used is 1.6×10^{-2} M. In dioxane, isosbestic points were obtained in all cases indicating that there are only 1:1 complexes.

It is known that xanthene dyes tend to aggregate[20] and that they form dimers or higher aggregates[21] in solution and a great number of studies has been concerned with the understanding of the spectral effects produced by dimerization.[22] The absorption spectrum of **5** in dioxane (Figure 2, solid line) corresponds to the dimer form[22] and as **E** is added, the spectrum changes to the form of that of the monomer (Figure 2, broken lines). Thus, the equilibria involved in dioxane may be represented by eq. 7 and 8, where **R** and $\mathbf{R}_2$ stand for the monomer and dimer of **5**, respectively.

$$R + E \rightleftharpoons E.R \quad (7)$$

$$2\,R \rightleftharpoons R_2 \quad (8)$$

The isosbestic point observed at 516 nm (Figure 2) indicates that the concentration of the monomer (**R**) is negligible compared to that of the dimer ($\mathbf{R}_2$) and the erythromycin complex (**E.R**). Therefore the equilibrium constant measured in dioxane is given by eq. 9.

$$K_{Assoc} = \frac{[E.R]}{[R + 2R_2]\,[E]} \approx \frac{[E.R]}{[2R_2]\,[E]} \quad (9)$$

Considering the dimerization equilibrium, the observed absorbance of Rhodamine 6G in dioxane is given by eq. 10.

$$\Delta A = \frac{P_1 [E]_o}{1 + P_2 [E]_o}$$

$$P_1 = \frac{\Delta\epsilon}{4K_D} (-1 + \sqrt{(1 + 8K_D [R_o])})\, K_{Ass}$$

$$P_2 = \frac{K_{Assoc}}{\sqrt{(1 + 8K_D [R_o])}} \quad (10)$$

Non-linear fitting of the data to this equation yields the parameters P_1 and P_2 from which an apparent association constant can be calculated; the value is reported in Table 1. The NMR spectrum of a solution containing **5** and **E** in Cl_3CD was compared with that of the isolated compounds at the same concentration. There were shifts of the signals corresponding to the proton bonded to C-1' and the two methyl groups of the desosamine moiety in the E molecule (Table 2) and those of hydrogens of the dibenzopyran ring and their substituents in the substrate. The changes in chemical shift are very small but out of the range of experimental error. This may indicate that the interactions between the host and the guest are weak and they take place mainly through hydrogen bonding between the dimethylamino group of the desosamina and one of the NH groups in **5**.

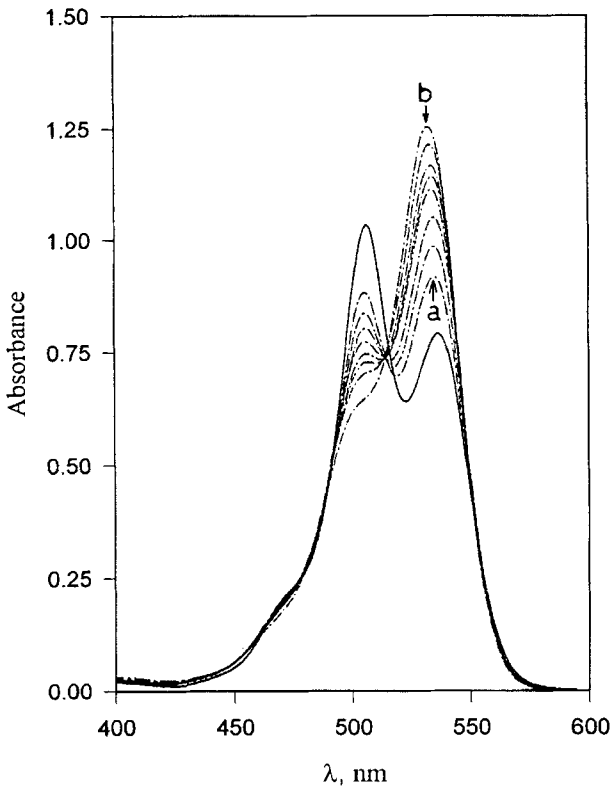

Figure 2 Spectrum of **5** (1.5×10^{-5}M) in dioxane at variable concentrations of **E** (2.5×10^{-3}M to 1.0×10^{-1}M, from a to b).

Unfortunately, solubility reasons preclude determinations in dioxane where the changes in the UV-visible spectrum from the dimer of **5** to the monomer is certainly

Table 2 ^{1}H NMR chemical shifts of selected protons in Erythromycin A and its complex with Rhodamine 6G[a]

	δ(Hz)		Δδ(Hz)
	Erythromycin A[b]	*Complex*	
H-1'	884.1	881.6	2.5
H-11	762.5	763.4	−0.9
H-5	708.6	710.0	−1.4
$-OCH_3$	662.0	661.9	0.1
$-N(CH_3)_2$	488.7	480.4	8.3
$-CH_3$-6	291.9	291.5	0.4

[a]In $CDCl_3$. [b]Chemical shifts assigned as indicated in the literature: Majer, J.; Martin, J. R.; Egan, R. S.; Corcoran, J. W. *J. Am. Chem. Soc.* *1977*, **99**, 1620 and ref 5.

a result of the association of **5** with **E** and should lead to bigger changes in chemical shift.[23]

Additional evidence for the association of **5** and **E** was obtained from the determination of the solubility of **5** in pure dioxane and in a solution containing 0.1 M erythromicin where **5** is 40 times more soluble.

From the temperature dependence of K_{Assoc}, the thermodynamic parameters (ΔH_{inc} and ΔS_{inc}) for the complexation of **3**, **4** and **5** were determined in chloroform. The range of temperature investigated (15–30°C) was limited by the low boiling point of the solvent and the solubility of the species of interest. Under these conditions, we only observed dependence of K_{Assoc} with temperature when **4** is the guest, ΔH_{inc} = 15 kJ/mol, and the change in energy involved is almost completely entropy controlled. The ΔS_{inc} are 13, 38 and 88 J/deg.mol for **3**, **4** and **5**, respectively.

The values obtained show one of the most important aspects for complexation: the entropy loss due to the "freezing" of motional freedom of the guest molecule is compensated by the entropy gain from the loss of the arrangement of molecules of solvent around the guest molecule in the bulk solution. This "balance" of entropies depends on the nature and the size of the guest.

The results reported here are in agreement with the general observation that host-guest interactions are achieved by hydrogen bonding and dipole-induced forces. The balance between these types of forces could be a critical factor in determining the ability of **E** to differentiate two structurally related compounds such as p-nitrophenol and p-nitroaniline, and hence in determining its ability to bind guest species selectively.

EXPERIMENTAL

Materials

Water was purified in a Millipore apparatus. DMSO was distilled under vacuum and stored over molecular sieves (4Å). Dioxane was purified by the Fieser method.[24] Ethanol and chloroform were purified by distillation.

Erythromycin A (Sigma) was used as received. Its purity was checked by UV and IR spectroscopy and by thin-layer chromatography. p-Nitroaniline (Mallinckrodt) was recrystallized from ethanol:water; p-nitrofenol (Mallinckrodt) was sublimed at 4 mmHg. Eosine Y (Schimd & Co.), Rhodamine 6G and Sulforhodamine B (Exciton) were used as received and their identity was checked by UV, IR and NMR spectroscopy. The azo compounds were samples of this laboratory from previous work.[25]

Association constant determinations

Spectral titrations: For the determination of the association constants of erythromycin A complexes, absorption spectra of solutions containing a constant organic guest concentration and increasing concentrations of **E** were recorded on a Shimadzu 260 recording spectrophotometer. The change in optical density (ΔA) was measured at the wavelength where the difference spectrum has its maximum (Table 1).

The concentrations of **1** and organic guests used are indicated in Table 3.

Thermodynamic parameters

For the determination of the temperature dependence of K_{Assoc}, spectral titrations were carried out at 15, 20, 25 and 30°C, recording the absorption spectra on a Beckman 24 spectrophotometer. The temperature of the cell compartment was controlled with a Haake F3 circulator (±0.05°C).

NMR determination

NMR spectra were done on a Brucker 200 NMR spectrometer 2.2 mg of **5** and 3.8 mg of **E** were dissolved in 0.5 mL of deuterated chloroform (Sigma). The separate spectrum of **E** and **5** were done at the above indicated concentration for the sake of comparison.

ACKNOWLEDGEMENTS

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Table 3 Experimental conditions for the determination of the association constants of **E** with organic guests

Solvent	[**E**],[a] 10^{-3}*mol/dm*^{3}	[**G**],[b] *mol/dm*^{3}
		p-nitroaniline
Chloroform	25 to 40	1.03×10^{-4}
Dioxane	40 to 160	1.09×10^{-4}
		Sulforhodamine
Chloroform[d]	3.98 to 40.0	7.32×10^{-6}
Dioxane[d]	4.22 to 52.7	9.96×10^{-6}
		Rhodamine 6G
Chloroform[c]	2.56 to 41.0	1.52×10^{-5}
Dioxane[c]	2.52 to 100	1.52×10^{-5}
		p-methyl red
Chloroform	0.30 to 17.0	4.84×10^{-5}
Dioxane	5.50 to 35.9	1.66×10^{-5}
		o-methyl red
Dioxane	3.27 to 29.9	2.97×10^{-5}

[a]Total concentration of Erythromycin A. [b]Total concentration of the organic guest. [c]The solvent contains 0.6% ethanol. [d]The solvent contains 0.4% ethanol.

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