Chemical Modification of Erythromycin Antibiotics. 4. Structure-Activity Relationships of Erythromycin Esters

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The quantitative relationships between physical properties and antibacterial activity (*Staphloccus aureus* and *Klebsiella pneumoniae*) of 28 formyl, acetyl, and propionyl esters of erythromycin A and B have been studied by the Free-Wilson and extrathermodynamic techniques. Both analyses indicate that esterification decreases activity; this decrease increases with increasing chain length of the ester. The extrathermodynamic equations suggest that the partition coefficient is an important determinant of activity. *In vivo* several derivatives are equally active *vs. S. aureus* as erythromycin A (CD_{50} = 75 mg/kg) whereas the remainder are inactive (CD_{50} > 300 mg/kg). No SAR calculations were performed on these data.

This report includes data on the *in vitro* and *in vivo* antibacterial activity of the 28 erythromycin (E) esters (Figure 1) for which the synthesis was the subject of the accompanying paper.[1] An analysis of the structure-activity relationships is also included.

It has previously been shown that the relative antibiotic activity of members of a cogeneric series of molecules is often a function of the lipophilicity as measured by the octanol-water partition coefficient of the molecule.[2] EA is an antibiotic which binds to the 50S ribosome and thereby inhibits protein synthesis.[3] Ribosomal binding studies with selected E derivatives demonstrated that this binding is necessary for antibiotic activity.[4] The 3′-Me_2N and 2′-OH groups of E are necessary for binding, but preliminary work indicated that esterification of the 4″- and 11-OH would not eliminate activity but only modify it. Since ribosomal binding had been shown to involve some specificity we expected that our analysis of the structure-activity relationships of the esters would reveal a dependence of activity on steric or hydrogen-bonding factors as well as the partition coefficient.

Experimental Section

Octanol-Water Partition Coefficients.† The partition coefficients (P) were detd by shaking a soln of known concn of the antibiotic in octanol with pH 8.0 Tris buffer for 5 min. After sepn of the phases the concn in the aqueous phase was detn by the arsenomolybdate method.[5] Each compound was used as its own standard. The obsd P is the ratio of concns of antibiotic in the two phases. The P of the uncharged form was calcd from the obsd by multiplying it by the fraction of the total drug which is not protonated at pH 8 (calcd[6] using 8.6 as the pK_a). The corrected experimental values are listed in Table I.

From the log P values it was possible to calc π values,[7] or changes in log P, which result from given changes in structure. These are summarized in Table II. To calc the log P's of the esters values of 2.58 and 3.02 were used for EA and EB. These were adjusted from the obsd so that the difference is 0.44, the mean difference between corresponding EA and Eb derivatives (Table II). For the log P change produced by esterification the following values were used: Fo, 0.0; Ac, 0.23; EtCO, 0.46.

Assay for *in Vitro* Activity. Antibacterial potency was detd by a turbidometric[8] procedure. *Staphylococcus aureus* AT 9144 or *Klebsiella pneumoniae* ATCC 10031 were grown in antibiotic medium No. 3 broth,‡ pH 7.0. The activity is relative to EA as a standard of 1000, and adjusted for differences in molecular weight.

Chromatography. Bioautography.§ To examine the stability of 4″-formyl-EB during assay 5 μg was incubated in 20 ml of inoculated (*S. aureus*) broth as above. The reaction was stopped with CH_2O and the pH adjusted to 9.3. It was extd 3× with 20 ml of Et_2O; the exts were combined, evapd to dryness, reconstituted with 0.1 ml of MeOH, and chromatographed on silica gel with CCl_4–DMF–NH_4OH (70:5:1). The ester and EB were references. The antibiotics were detected by bioautography using *Bacillus subtilis* Illinois No. 10707 seeded on agar. There was no evidence of hydrolysis of this ester. Since it is the most chemically labile[1] of all the 4″ or 11 esters studied, it is assumed that all 4″ and 11 esters reported here are stable under the *in vitro* antibacterial assay condns.

The stability of the esters under condns of the log P measurements was examined in a similar manner. Portions of the aqueous phase were chromatogd in $CHCl_3$–MeOH–NH_4OH (95:5:1) and visualized by bioautography. There was no hydrolysis.

Mouse Protection Test for *in Vivo* Activity. Mice were infected ip with 10–100 LD_{50} *S. aureus* Smith. The esters were administered orally at 1 and 5 hr after infection. In the standard test 10 mice per level were used; where confidence limits were calcd 30 per level were used. The CD_{50} was calcd from the % survivors after 7 days as a function of the total amt of drug administered by probit analysis.

Table I. Experimentally Determined Partition Coefficients (Adjusted for Ionization as Indicated in the Text)

No.	Compound	log P
1	Erythromycin A	2.54
2	Erythromycin B	3.07
3	4″-Acetylerythromycin A	2.85
4	4″-Acetylerythromycin B	3.23
5	11-Acetylerythromycin B	3.30
6	4″-Formylerythromycin A	2.64
7	4″-Formylerythromycin B	3.05
8	4″,11-Diformylerythromycin B	2.79[a]

[a] Since this compound is difficult to dissolve its partition coefficient is considered unreliable and was not used for further calculations.

Table II. π Values Calculated from Observed Log P's

Change	Derivative (Table I)	Parent (Table I)	π
OH to formyl ester	6	1	+0.10
	7	2	−0.02
			mean +0.04
OH to acetyl ester	3	1	0.31
	4	2	0.16
	5	2	0.23
			mean 0.23 ± 0.08
Erythromycin A to erythromycin B	2	1	0.53
	4	3	0.38
	7	6	0.41
			mean 0.44 ± 0.08

Results

***In Vitro* Antibacterial Activity (Table III).** The antibacterial activities of these compounds vary by 100-fold. The

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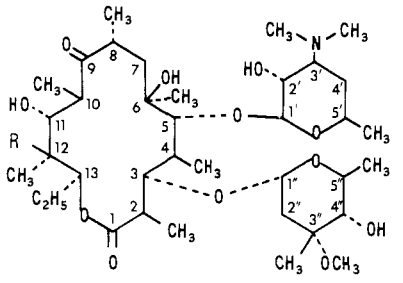

IA R=OH Erythromycin A

IB R=H Erythromycin B

Figure 1. Structure of erythromycin.

are summarized in Table IV. These numbers indicate that esterification usually decreases activity; that the larger or more lipophilic the group the larger the decrease; and that EA (12 OH) derivatives are more active than EB derivatives *vs. S. aureus*.

Free-Wilson Fujita-Ban Analysis. The first method of analysis of the quantitative structure-activity relationships of these derivatives was the modification of the Free-Wilson technique which has recently been described by Fujita and Ban.[9] In this technique the logarithm of the activity (A) is assumed to be "correlated with the mathematical sum of contributions of structural fragments to the total activity of the molecule." The unsubstituted molecule (EB in this case)

Table III. Antibacterial Activity and Partition Coefficients of Erythromycin Esters

Compound Number[a]	Structure 2'	Structure 4''	Structure 11	A or B	log P	Log potency *S. aureus* observed	Log potency *S. aureus* calcd (eq 4)	Log potency *K. pneumoniae* observed	Log potency *K. pneumoniae* calcd (eq 8)
A	OH	OH	OH	A	2.58	3.00	2.99	3.00	2.98
7A	OCOH	OH	OH	A	2.58	2.91	2.99	3.00	2.98
B	OH	OH	OH	B	3.02	2.82	2.80	3.00	2.95
7B	OCOH	OH	OH	B	3.02	2.78	2.80	2.90	2.95
2A	$OCOCH_3$	OH	OH	A	2.81	2.75	2.74	2.77	2.78
9A	OH	OCOH	OH	A	2.58	2.72	2.69	2.88	2.82
8A	OCOH	OCOH	OH	A	2.58	2.71	2.69		
16B	OH	OH	OCOH	B	3.02	2.71	2.59	2.75	2.70
10A	$OCOCH_3$	OCOH	OH	A	2.81	2.70	2.44	2.74	2.61
2B	$OCOCH_3$	OH	OH	B	3.25	2.54	2.55	2.78	2.74
9B	OH	OCOH	OH	B	3.02	2.54	2.50	2.65	2.79
8B	OCOH	OCOH	OH	B	3.02	2.50	2.50	2.79	2.79
15B	OH	OCOH	OCOH	B	3.02	2.42	2.28	2.63	2.53
14B	OCOH	OCOH	OCOH	B	3.02	2.39	2.28	2.63	2.53
12B	OH	OH	$OCOCH_3$	B	3.25	2.29	2.34	2.35	2.49
10B	$OCOCH_3$	OCOH	OH	B	3.25	2.28	2.25	2.69	2.58
4A	OH	$OCOCH_3$	OH	A	2.81	2.24	2.44	2.47	2.61
3A	$OCOCH_3$	$OCOCH_3$	OH	A	3.04	2.15	2.19	2.40	2.40
13B	$OCOCH_3$	OH	$OCOCH_3$	B	3.48	2.11	2.09	2.37	2.28
3B	$OCOCH_3$	$OCOCH_3$	OH	B	3.48	2.04	2.03	2.46	2.41
4B	OH	$OCOCH_3$	OH	B	3.25	2.02	2.25	2.47	2.58
18B	OH	$OCOCH_2CH_3$	OH	B	3.48	2.02	2.00	2.33	2.37
17B	$OCOCH_3$	$OCOCH_2CH_3$	OH	B	3.71	1.87	1.75	2.26	2.16
11B	$OCOCH_3$	OCOH	$OCOCH_3$	B	3.48	1.65	1.79	2.09	2.12
6B	OH	$OCOCH_3$	$OCOCH_3$	B	3.48	1.51	1.79	2.00	2.12
5B	$OCOCH_3$	$OCOCH_3$	$OCOCH_3$	B	3.71	1.39	1.54	1.82	1.91
20B	OH	$OCOCH_2CH_3$	$OCOCH_2CH_3$	B	3.94	1.38	1.29	1.65	1.70
19B	$OCOCH_3$	$OCOCH_2CH_3$	$OCOCH_2CH_3$	B	4.17	1.18	1.04	1.57	1.49

[a] See ref 1.

Table IV. Group Contributions to Antibacterial Activity

Group added	Number of examples	Mean % change in potency (± standard deviation) *S. aureus*	Mean % change in potency (± standard deviation) *K. pneumoniae*
4''-Formyl	7	−37.9 ± 12.4	−25.0 ± 16.0
4''-Acetyl	6	−79.0 ± 6.3	−62.7 ± 8.9
4''-Propionyl	2	−81.5 ± 3.5	−74.5 ± 6.4
11-Formyl	3	−25.3 ± 10.4	−32.0 ± 23.4
11-Acetyl	5	−71.2 ± 5.2	−71.4 ± 7.5
11-Propionyl	2	−78.0 ± 1.4	−79.5 ± 0.7
12-OH	7	+51.4 ± 14.4	+17.4 ± 30.2

least active is 2'-acetyl-4'',11-dipropionylerythromycin B; the most active is EA. In general, the activity decreases with increasing size and number of ester groups.

A preliminary analysis of the data indicated that each structural change produces a constant change in activity. Thus adding a 4''-Ac group to A, B, 2A, 2B, 13B, and 12B to produce 4A, 4B, 3A, 3B, 5B, and 6B, respectively, results in a 79.0 ± 6.3% decrease in activity *vs. S. aureus*. Similar values for each type of ester position and organism has group contributions (G) at each position equal to zero.

The results of these calculations are summarized in Table V. In this table n is the number of examples of the group listed, r is the multiple correlation coefficient, and s is the standard deviation. From the high r values and low standard deviations of the G's it is clear that an excellent statistical fit of the data is obtained by this technique. The only statistically significant relationship between the G values and physical properties is that between the Taft[10] E_S steric parameter and G at the 4'' position *vs. S. aureus*. The problem with this set of compounds is that there are so few types of substitution at each position. However, in any case this sort of analysis results in fewer degrees of freedom than the method discussed in the next section.

Semiempirical Structure-Activity Analysis. A more direct approach to quantitative SAR is that pioneered by Hansch and coworkers.[2a,2b] Equations 1 and 3 (Table VI) show the relationship between log P and activity. In these equations A is the activity, P is the partition coefficient, n is the number of compounds included in the analysis, r is the correlation coefficient, F is the variance ratio, and s is

Table V. Free-Wilson Calculated Group Contributions to Antibacterial Activity

Group	*n*	Contribution to log A (± standard deviation) *S. aureus*	*K. pneumoniae*
2'-Hydroxyl	12	0[a]	0[a]
2'-Formyl ester	5	0[b]	0[b]
2'-Acetyl ester	11	−0.149 ± 0.030	−0.097 ± 0.025
4''-Hydroxyl	9	0[a]	0[a]
4''-Formyl ester	9	−0.282 ± 0.035	−0.151 ± 0.029
4''-Acetyl ester	6	−0.680 ± 0.039	−0.433 ± 0.032
4''-Propionyl ester	4	−0.731 ± 0.061	−0.591 ± 0.048
11-Hydroxyl	18	0[a]	0[a]
11-Formyl ester	3	0[b]	−0.163 ± 0.040
11-Acetyl ester	5	−0.543 ± 0.042	−0.546 ± 0.032
11-Propionyl ester	2	−0.655 ± 0.074	−0.685 ± 0.060
12-Hydrogen	20	0[a]	0[a]
12-Hydroxyl	8	+0.228 ± 0.035	0[b]
r		0.991	0.991
s		0.074	0.059

[a]Reference activity. [b]Not significantly different from zero.

lationships between size and activity is obscure. All equations confirmed the negative dependence of activity on log *P*.

In another type of analysis we postulated that the consequences of esterification was not a simple steric block but actually an "all or none" change resulting from esterification. Such a change might produce a change in angle or strength of H bonds or a slight conformational shift. We therefore used dummy variables (D)[11] to account for such quantal changes. The variables for each position in a molecule were assigned a value of zero if that position was free, and one if it was esterified. Equations 2 and 4 (Table VI) summarize the results of this treatment. All terms are significant at the 99% confidence level.

The high negative dependence of activity on log *P* is again evident. The values of the corresponding coefficients are not significantly different between eq 2 and 4; however the greater activity of the 4'' esters *vs. K. pneumoniae* than *vs. S. aureus* is indicated by the larger negative coefficient of

Table VI. Equations Which Relate *In Vitro* Antimicrobial Activity to Physical Properties

Eq		*n*	*r*	*F*	*s*
	Activity *vs. S. aureus*				
1	$\log A = 5.74 - 1.09\ (\pm 0.10)^a \log P$	28	0.899	109.6	0.224
2	$\log A = 5.79 - 1.09\ (\pm 0.09) \log P - 0.306\ (\pm 0.056)\ D(4'') - 0.211\ (\pm 0.061)\ D(11) + 0.284\ (\pm 0.077)\ D(B)$	28	0.971	97.6	0.128
	Activity *vs. K. pneumoniae*				
3	$\log A = 5.23 - 0.856\ (\pm 0.092) \log P$	27	0.879	85.2	0.191
4	$\log A = 5.32 - 0.907\ (\pm 0.068) \log P - 0.164\ (\pm 0.041)\ D(4'') - 0.256\ (\pm 0.043) D(11) + 0.307\ (\pm 0.055)\ D(B)$	27	0.976	114.8	0.091

[a]Coefficients ± standard deviation.

Table VII. *In Vivo* Activity of Erythromycin Esters *vs. S. aureus* Infections

Compound No.	CD_{50}, mg/kg, oral (95% confidence interval)
A	77 (62-98)
7A	75[b]
B	82 (67-98)
9A	51 (44-60)
8A	76 (66-88)
16B	88[b]
9B	62[a]
8B	48[a]
15B	87 (75-100)
14B	110 (49-424)
12B	60 (29-125)
4A	123 (105-145)
10B	>300[b]
3A	85 (69-105)
4B	>300[b]
18B	69 (58-83)
11B	>300[b]
5B	>300[b]

[a]CD_{50} determined by graphical analysis. [b]Ten mice per level.

the standard deviation of the estimate. Thus in the case of both organisms approximately 80% of the differences in *in vitro* activity can be "explained" by differences in log *P*.

The relationship between the Taft[10] E_S parameter for "size" of the group at the various positions and activity is very difficult to analyze in this group of molecules. Specifically, no value for the E_S of -OH *vs.* -OCOR groups is available. Thus we were limited to comparing the activities of only three examples; R = H, CH_3, C_2H_5 within subseries in which positions of esterification were held constant. Although several statistically significant equations were generated from such analyses the physical meaning of the re-

$D(4'')$ in eq 2 than eq 4. The similarity of the intercepts of the two equations does not indicate a similar sensitivity of the two organisms to E derivatives since the activities are calculated on the basis that EA is 1000. Finally, the activity of the compounds was not dependent on the dummy variable for the 2' position in either case. Thus the principal result of this analysis is that the relative activity of these esters *vs.* the Gram-positive organism *S. aureus* is governed by the same factors which govern their activity *vs.* the Gram-negative bacterium *K. pneumoniae*.

It is known that 2' esters of E do not bind to ribosomes and are not active *per se* but must be hydrolyzed in order to be active.[12] In contrast, our 4'' and 11 esters are active as such and do not hydrolyze. Thus we must explain why all of our esters fit into the same equations; or why our 2' esters have activities predicted by these equations (on the basis of the log *P* of the original compound) when at the time they are active antibacterial agents their log *P* has been lowered as a result of the hydrolysis of the 2' ester. The simplest explanation is that the dependence of activity on log *P* is due to an event which occurs prior to hydrolysis and ribosomal binding. For example, the extra lipophilic character might hinder penetration through the bacterial cell wall or membrane. A slightly more complex explanation would be the fortuitous possibility that there is an identical negative dependence on log *P* of both antibacterial activity as such and rate of hydrolysis of the 2' ester group. It is not possible to distinguish between these possibilities with the present data.

In Vivo **Antibacterial Activity.** The results of the *in vivo* experiments are collected in Table VII in which the compounds are arranged in order of decreasing activity in the *in vitro* test. It can be seen that the derivatives are either inactive ($CD_{50} > 300$ mg/kg) or they are approximately

equally active as EA (CD_{50} = 75 mg/kg). The inactive derivatives are in general the least active *in vitro*. The equal *in vivo* potencies of the several E's which differ severalfold in *in vitro* activity may indicate either that the esters which are less potent *in vitro* are more easily hydrolyzed in the animal to the more active parent antibiotic or that the additional lipophilic character produced by esterification enhances penetration to the site of infection. The nature of the *in vivo* data prevented evaluation of quantitative structure-activity relationships.

Discussion

It is instructive to compare the π values for groups on erythromycin with those of other molecules. In particular, information on the environment of the 11-, 12-, and 4″-hydroxyls would be useful. The π value for the change -OH to $-OCOCH_3$ at either the 4″ or 11 positions is +0.23 whereas that for the change from $PhCH_2CH_2OH$ to $PhCH_2CH_2OCOCH_3$ is +0.89. Since these π values reflect mainly differences in π_{OH}, the 4″- and 11-OH's on E are more lipophilic than usual by a factor of 0.64. This is the increase in lipophilicity which results when an OH is H bonded. (The log *P* of *o*-OHPhCOOH is 2.21[13b] and that of *p*-OHPhCOOH is 1.58.[7]) In a similar manner, π for the 12-OH is −0.44; that of a normal OH ($CH_3(CH_2)_2OH$ *vs.* n-C_3H_8) is −1.16.[13b] The difference is +0.72. Thus it appears that all three of these OH's are H bonded in EA. A space-filling model of EA in the proper conformation supports the possibility of H bonding; the 11-OH to the 9-ketone and the 12-OH, the 4″-OH to the 3″-OCH_3, and the 12-OH to the 11-OH.[14] In addition, the ir spectra of certain erythronolide B derivatives show that the 11-OH can be H bonded to the 9-carbonyl. The multiplicity of hydroxyls in the parent antibiotic prevents the interpretation of the ir spectrum of the intact E. Finally, the π_{CH_3} calculated from E derivatives is approximately half that usually found. This suggests that the alkyl chains of the E esters may form an intramolecular hydrophobic bond with the nonpolar surface of the molecule. Thus the π's are useful indicators of various sorts of intramolecular interactions.

Antibacterial activity frequently is a function of log *P*. For example Lien, *et al.*,[2a] studied nonspecific toxic agents and found an optimum log *P* value of 6 for antibacterial activity *vs.* Gram-positive organisms. Hansch, *et al.*,[2b] reported an optimum log *P* of 3.7 for chloramphenicol analogs tested *vs. E coli*. Finally, Martin and Lynn[2c] reported an increase in antibacterial activity of members of the macrolide leucomycin complex with an increase in log *P*, as well as, an optimum log *P* of 1.1 for lincomycins. Thus it appears that for each structural type of antibiotic a different optimum log *P* will be found. This observation makes it more difficult to speculate as to the reason for the dependence of activity on log *P*.

The erythromycin analogs are instructive for the medicinal chemist to ponder since the mode of action has been studied in great detail. It is known that ribosomal binding is a prerequisite to antibacterial activity;[4,15] that is, the ribosome is the "receptor" for E. Studies of ribosomal binding of a series of E analogs indicate that although binding is a prerequisite for antibacterial activity, of compounds which bind there is no correlation between the extent of receptor binding and whole cell (*in vitro*) antibacterial activity. For example 4″-OAc EB binds equally as well as EB but is one-sixth as active; 11-OAc EB binds less than 4″-OAc EB but is more active.[15b] Similar lack of correspondence between relative activity at the receptor level and whole cell antibacterial activity has been observed with lincomycin and chloramphenicol analogs.[16]

At the next higher level of complexity the correspondence again fails: neither ribosomal binding nor *in vitro* activity predict *in vivo* potency.

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