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Structure–Activity Relationship of Chloramphenicols†

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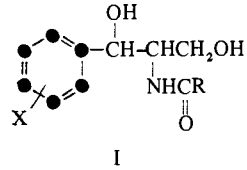
To obtain greater insight into the structure–activity relationship of chloramphenicol, 37 derivatives (including the parent drug) have been tested *via* the microbial kinetic technique against *Escherichia coli*. Two quantitative structure–activity relationships have been formulated: one for the side chain modified derivatives and one for the derivatives modified in the 4 position of the ring. From these results it is apparent that inductive effects of the acyl group in the side chain are important and that hydrophobic properties of the side chain are less important. For substituents in the 4 position, hydrophobic properties are most important. The first reported instance of a derivative more active than the parent drug is given for the compound having $NHCOCF_3$ instead of $NHCOCHCl_2$ of the natural drug. The trifluoro derivative is 1.7 times more active against *E. coli* than chloramphenicol.

Since the discovery[1-3] and synthesis of chloramphenicol by workers at the Parke Davis Co., it has been estimated[4] that about 40,000,000 people have been treated with this drug in the period of 1950–1970.

This antibiotic is an excellent inhibitor of protein synthesis by bacterial ribosomes.[5] The 50-S subunit appears to be the site of action and chain elongation beyond the first peptide bond is the process affected.

Although the use of chloramphenicol has been questioned recently because of rare cases of aplastic anemia where toxicity has been shown to be dose related,[6] chloramphenicol is still the drug of choice in certain diseases (*e.g.*, typhoid fever).

Chloramphenicol is a particularly interesting drug for modification and structure-activity study. It is well known[4] and it becomes more apparent in the results from this study that it is possible to introduce an interesting variety of structural variation in the side chain and the para position on the phenyl ring without destroying activity. As antibiotics go, chloramphenicol is easy to modify. Certainly it is highly desirable to find a derivative less toxic to bone marrow. Our own interest extends beyond the above in that chloramphenicol seems to be a fine molecule with which to study the use of substituent constants and regression analysis in correlating chemical structure with biological activity and the development of this technique for drug design. Preliminary studies[7-9] were the starting point in the design of the present study. In the present study variation of substituents in the 4 position and in the amide side chain of I have been tested *in vitro* against *Escherichia coli*.



I

Methods

The compounds of Table I were obtained *via* several methods. Those indicated by A under source in Table I were obtained directly from the Parke Davis Co. *N*-Acyl derivatives of D-*threo*-1-(4-nitrophenyl)-2-amino-1,3-propanediol were synthesized by the three different ways developed by Rebstock:[2] (I) amide forma-

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Table I. Structure-Activity Constants for X-C_6H_4CHOHCHCH$_2$OH
HNCOR

No.	X	R	Log k[a] Obsd	Log k[a] Calcd	$\lvert\Delta \log k\rvert$	Log P	σ_X	σ_X^+	σ_R^*	E_{S-R}	P_{E-X}	P_{E-R}	Source
1	NO_2	CF_3	2.24	2.74	0.50	1.07	0.78	0.79	2.61[f]	−1.16	7.30	5.69	I
2	NO_2	$CHCl_2$	2.00	1.82	0.18	1.15	0.78	0.79	1.94	−1.54	7.30	15.44	A
3	NO_2	$CHBr_2$	1.84	1.41	0.43	1.36	0.78	0.79	1.94[f]	−1.86	7.30	21.24	I
4	NO_2	CH_2Cl	1.71	1.44	0.27	0.59	0.78	0.79	1.05	−0.24	7.30	10.58	A
5	NO_2	CHClMe	1.47	1.29	0.18	0.98	0.78	0.79	1.00[f]	−0.47[f]	7.30	15.20	III
6	NO_2	CH_2Br	1.38	1.31	0.07	0.66	0.78	0.79	1.00	−0.27	7.30	13.48	III
7	NO_2	CHF_2	1.30[b]	2.16	0.86	0.42	0.78	0.79	2.05	−0.67	7.30	5.70	I
8	NO_2	CH_2F	1.16	1.24	0.08	0.15	0.78	0.79	1.10	−0.24	7.30	5.71	III
9	NO_2	CH_2I	1.10	1.03	0.07	1.03	0.78	0.79	0.85	−0.37	7.30	18.52	III
10	NO_2	$CHMe_2$	0.88	0.54	0.34	0.79	0.78	0.79	−0.19	−0.47	7.30	14.96	II
11	NO_2	CCl_3	0.75	1.06	0.31	1.97	0.78	0.79	2.65	−2.06	7.30	20.30	I
12	NO_2	CBr_3	0.72	0.20	0.52	2.17	0.78	0.79	2.65	−2.43	7.30	29.00	I
13	NO_2	Pr	0.71	0.60	0.11	0.87	0.78	0.79	−0.12	−0.36	7.30	14.96	II
14	NO_2	Me	0.48	0.26	0.22	−0.03	0.78	0.79	0.00	0.00	7.30	5.72	II
15	NO_2	CH_2CN	0.14	0.48	0.34	−0.22	0.78	0.79	1.30	−0.07[f]	7.30	10.09	I
16	NO_2	C_6H_5	−0.09	0.09	0.18	1.63	0.78	0.79	0.60	0.23	7.30	25.21	III
17	NO_2	$CH_2C_6H_5$	−0.10	−0.24	0.14	1.52	0.78	0.79	0.22	−0.38	7.30	29.83	III
18	NO_2	$CHCNC_6H_5$	−0.27	0.16	0.43	1.47	0.78	0.79	1.30[f]	−1.19[f]	7.30	36.60	I
19	NO_2	$CHEt_2$	−0.52	−0.48	0.04	1.71	0.78	0.79	−0.22	−1.98	7.30	24.20	III
20	NO_2	CMe_3	−0.53	0.12	0.65	1.33	0.78	0.79	−0.30	−1.54	7.30	19.58	III
21	$COCH_3$	$CHCl_2$	0.76	1.10	0.34	0.57	0.50	0.50	1.94	−1.54	10.35	15.44	A
22	SCH_3	$CHCl_2$	1.71	1.30	0.41	1.54[d]	0.00	−0.60	1.94	−1.54	13.69	15.44	A
23	I	$CHCl_2$	1.51[c]	1.25	0.26	2.22	0.18	0.13	1.94	−1.54	13.90	15.44	A
24	CN	$CHCl_2$	1.29	1.40	0.11	0.78	0.66	0.66	1.94	−1.54	8.61	15.44	A
25	3-NO_2	$CHCl_2$	1.28	1.62	0.34	1.15	0.71	0.67	1.94	−1.54	7.30	15.44	A
26	Br	$CHCl_2$	1.28	1.25	0.03	1.87	0.23	0.15	1.94	−1.54	8.86	15.44	A
27	OCH_3	$CHCl_2$	1.21[c]	1.07	0.14	0.92[d]	−0.27	−0.78	1.94	−1.54	7.36	15.44	A
28	Cl	$CHCl_2$	1.05	1.20	0.15	1.64	0.23	0.11	1.94	−1.54	5.96	15.44	A
29	*i*-Pr	$CHCl_2$	1.03[c]	1.25	0.22	2.24	−0.15	−0.28	1.94	−1.54	14.96	15.44	A
30	H	$CHCl_2$	0.80	0.86	0.06	0.94	0.00	0.00	1.94	−1.54	1.10	15.44	A
31	SO_2CH_3	$CHCl_2$	0.75	0.67	0.08	−0.27	0.72	0.72	1.94	−1.54	17.54	15.44	A
32	C_6H_5	$CHCl_2$	1.01	1.09	0.08	2.86	−0.10	−0.18	1.94	−1.54	25.21	15.44	A
33	NH_2	$CHCl_2$	0.50	0.30	0.20	−0.29	−0.66	−1.30	1.94	−1.54	5.41	15.44	A
34[g]	$-C_5H_4N$	CF_3	0.30										
35	NHCO-c-C_3H_5	$CHCl_2$	−0.04[b,e]	1.18	1.22	0.75	0.00	−0.60	1.94	−1.54	20.76	15.44	A
36	$CONH_2$	$CHCl_2$	−0.13[e]	−0.31	0.18	−0.55	0.36	0.36	1.94	−1.54	9.48	15.44	A
37	$NHCONH_2$	$CHCl_2$	−0.66	−0.39	0.27	−0.60	−0.24	−0.60	1.94	−1.54	12.85	15.44	A

[a] k values are in l. mol^{-1} sec^{-1} and equal $10^3 \times$ mol wt $\times$ k in ml/μg/sec. The negative slopes of the linear plot of $k_{app} = k_o - kC_x$ against concentrations of chloramphenicols, C, where k_o is the generation rate constant in the absence of antibiotic and k_{app} is the apparent generation rate constant for microbial generation of *E. coli* B/r at 37.5° in the presence of various concentrations of chloramphenicol. The k_{app} was determined from the positive slope of plots of logarithms of numbers of microorganisms, N, against time in accordance with $\log N = (k_{app}/2.303)t$ + constant; values for compounds 1–20 calculated using eq 5; values for compounds 21–37 calculated using eq 8. [b] These values not used in deriving regression equations. [c] The k values were determined on DL mixtures. The obtained values were doubled to give k for the D configuration on the premise that the L configuration has no activity. [d] Log P calculated using π from the benzene system. [e] Because of solubility limitations, microbial kinetic studies were conducted in media containing a few per cent ethanol. [f] Estimated values. [g] See Methods.

tion *via* esters; (II) amide formation *via* anhydrides; and (III) amide formation *via* acyl halides. The method we have used to make the variations in the chloramphenicol side chain is indicated under source in Table I. The following derivatives and their melting points have not previously been reported: CH_2CN, 126–127°; $CH_2CH_2CH_3$, 104–105°; $C(CH_3)_3$, 127–128°; CH_2Br, 109–110°; $CH(Et)_2$, 138–139°; $CHClCH_3$, 143–144°; CH_2I, 145–146°; $CH(CN)C_6H_5$, 193–196°; CBr_3, 128–129°.

Preparation of 1-[4-(2-Pyridyl)phenyl]-2-trifluoroacetamido-1,3-propanediol. 1-[4-(2-Pyridyl)phenyl]-2-trifluoroacetamido-1,3-propanediol was prepared according to Rebstock.[10] This nitro compound (5 g) was dissolved in 50 ml of ethanol and hydrogenated in a Parr apparatus using Pt on charcoal catalyst. The solution was then filtered and evaporated to dryness at room temperature on a spin evaporator. The resulting white powder was extremely hydroscopic and was not purified before use. The crude amino compound was dissolved in 10 ml of water and 1.5 ml of concentrated HCl and diazotized with 0.3 g of sodium nitrite dissolved in a few milliliters of water. The cold solution of diazonium salt was added during 1 hr to 500 ml of pyridine at room temperature.[11] After standing for 1 hr, the pyridine was removed on the spin evaporator, a few milliliters of dilute NaOH were added, and the mixture was extracted with ten small portions of ether. Evaporation of the ether yielded 1.1 g of solid. Recrystallization from 2-propanol-water yielded a product of melting point 225–227°. *Anal.* Calcd: C, 56.07; H, 4.34. Found: C, 56.47; H, 4.44.

Structure Proof of 1-[4-(2-Pyridyl)phenyl]-2-trifluoroacetamido-1,3-propanediol. In addition to yielding the proper carbon-hydrogen analysis, the 2-pyridyl compound possessed the expected octanol-water partition coefficient: obsd log P 1.31; calcd log P 1.38.

To establish the position of attachment of the pyridine ring, 200 mg of the pyridyl derivative was refluxed for 2 hr with 186 mg of $KMnO_4$ in 20 ml of water. The excess $KMnO_4$ was reduced with $NaHSO_3$ and the mixture filtered. Acidification with dilute HCl precipitated a white powder of mp 232–234°. Haworth, *et al.*,[11] report a melting point of 232° for 4-(2-pyridyl)benzoic acid.

The microbial kinetic technique used in our earlier work[8] was employed to determine the log k values of Table I for those compounds whose values were not previously reported. Except as noted, the log P values for the derivatives were measured in the octanol-water system as usual.[12] We have found that π values for R obtained from the chloramphenicol system serve to correlate the inhibitory power of amides on alcohol dehydrogenase[13] and the chaotropic properties[14] of $RCOO^-$ derivatives in several systems.

The total numbers of *E. coli* B/r obtained on generation at 37.5° in peptone broth USP were obtained by use of the Coulter counter with and without the presence of graded amounts of the variously modified chloramphenicols in the subinhibitory range. In addition, selected combinations of various chloramphenicols were studied to determine their combined effects on the inhibition of microbial generation.

The apparent generation rate constants, k_{app}, were determined from the slope of the initially linear portions of the plots of logarithms of numbers of organisms against time during the drug-affected steady-state generation phase in accordance with

$$\ln N = k_{app}t + \ln N_0 \quad (1)$$

where N_0 is the number of organisms at some time t_0 taken as zero, and N is the total number of organisms per milliliter after time t.

Linear plots were obtained when these k_{app} values were plotted against the concentration of the substituted chloramphenicol, c, in accordance with

$$k_{app} = k_0 - kc \quad (2)$$

where k_0 is the generation rate constant in the absence of drug and k is the inhibitory rate constant for the particular antibiotic.

Typical plots of k_{app} values against concentrations in μg/ml are given in Figures 1–4. The k values are determined from the slopes and would be in units of ml/μg/sec as given in the footnotes of Table II. The k values in Table I are given in units of l. mol^{-1} sec^{-1} and may be obtained from the former by

$$k \text{ (in l. mol}^{-1}\text{ sec}^{-1}\text{)} = 10^3 \times \text{mol wt} \times k \text{ (in ml } \mu\text{g}^{-1}\text{ sec}^{-1}\text{)} \quad (3)$$

Equipotent combinations of various modified chloramphenicols were prepared (see Table II) on the premise that the k_{app} value was a linear function of the separate concentrations in accordance with

$$k_{app} = k_0 - \Sigma k_i c_i \quad (4)$$

where the k value is for the ith modified chloramphenicol in the combination at its c_i concentration. Two sets of such combinations were prepared so that each set was *a priori* equipotent. These are given in Table II and the experimentally determined k_{app} values agreed with the *a priori* predictions based on eq 4. Thus the bacteriostatic inhibition of microbial generation from combinations of chloramphenicols modified in the acyl side chain is an additive process. This had been found previously for studies of combinations of chloramphenicols modified with various substituents in the phenyl ring.[8]

Results

The degree of variation present in the congeners in Table I is large. It is interesting that such large structural changes can be made in the side chain and in the 4 position without completely destroying the *in vitro* activity against *E. coli*. There is about an 800-fold difference between the most active and least active molecules.

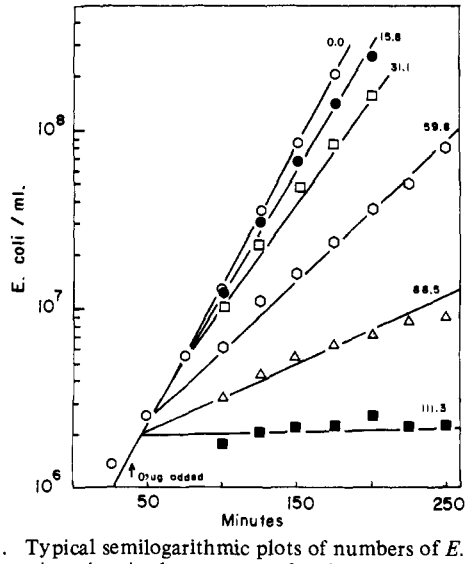

Figure 1. Typical semilogarithmic plots of numbers of *E. coli* per milliliter against time in the presence of various amounts of 1-[4-(2-pyridyl)phenyl]-2-trifluoroacetamido-1,3-propanediol at 37.5°. Each curve is labeled with the drug concentration in μg/ml.

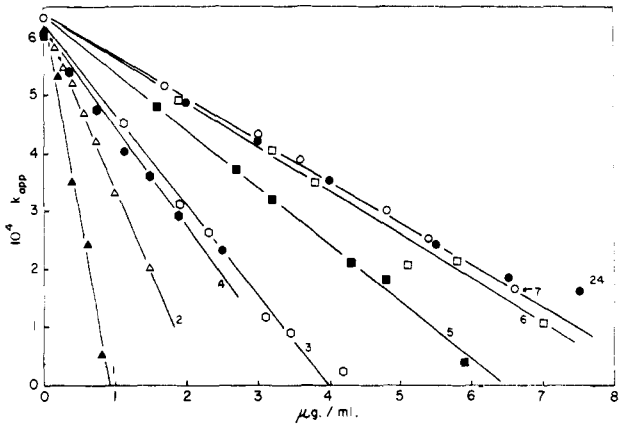

Figure 2. The linear dependencies of apparent generation rate constants, k_{app} in sec^{-1}, on concentrations of variously modified chloramphenicols. The curves for the compounds with X 4 substituents and R groups on the carbonyl of the side chain amine are: (1) NO_2, CF_3; (2) NO_2, $CHCl_2$; (3) NO_2, $CHBr_2$; (4) NO_2, CH_2Cl; (5) NO_2, $CHClCH_3$; (6) NO_2, CH_2Br; (7) NO_2, CHF_2; (24) CN, $CHCl_2$.

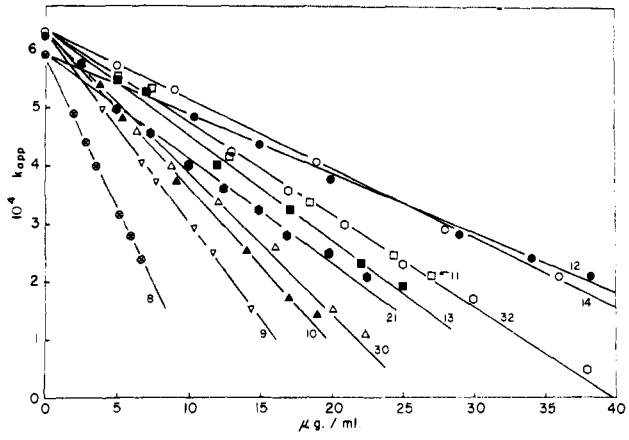

Figure 3. The linear dependencies of apparent generation rate constants, k_{app} in sec^{-1}, on concentrations of variously modified chloramphenicols. The curves for the compounds with X 4 substituents and R groups on the carbonyl of the side chain amine are: (8) NO_2, CH_2F; (9) NO_2, CH_2I; (10) NO_2, $CH(CH_3)_2$; (30) H, $CHCl_2$; (21) $COCH_3$, $CHCl_2$; (13) NO_2, $CH_3CH_2CH_2$; (11) NO_2, CCl_3; (32) C_6H_5, $CHCl_2$; (14) NO_2, CH_3; (12) NO_2, CBr_3.

At this stage in the development of quantitative structure-activity studies when the importance of different variables is not clearly understood, as many independent variables as possible should be considered.[15] For substituents in the 4 position, the following were studied: σ, σ^+, π, P_E. The polarizability as estimated by atomic refractivities is represented by P_E. In earlier work[9] it was found that the radical substituent constant E_R was the best predictor of the electronic effect of substituents in the 4 position. Since values of this constant are not available for a number of the substituents used in the present study, σ and σ^+ were used to obtain a less exact measure of the electronic effect of the ring substituents.

The changes in R could result in more complex interactions between drug and receptor. For example, if the oxygen of the carbon atom of the carbonyl function plays a critical part in binding the drug to the receptor, then the steric effect of the adjacent R could be important. To explore this possibility, the effect of Taft's steric parameter E_S was studied. An attempt to factor the steric effects of R was also made. Since R is free to rotate, it was considered possible that only the smallest group of the three possible functions

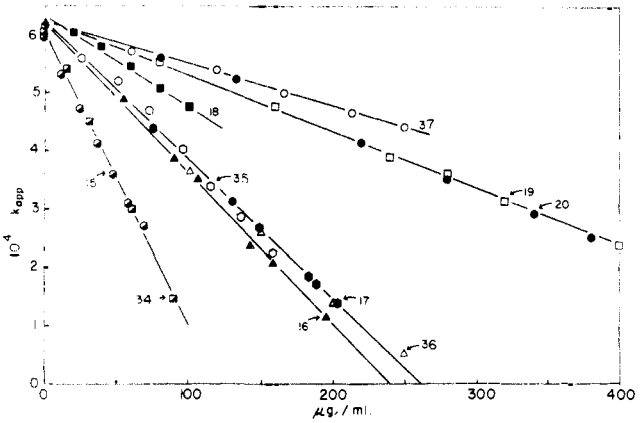

Figure 4. The linear dependencies of apparent generation rate constants, k_{app} in sec^{-1}, on concentrations of variously modified chloramphenicols. The curves for the compounds with X 4 substituents and R groups on the carbonyl side chain amine are: (**15**) NO_2, CH_2CN; (**34**) C_5H_4N, CF_3; (**16**) NO_2, C_6H_5; (**17**) NO_2, $CH_2C_6H_5$; (**18**) NO_2, $CHCNC_6H_5$; (**35**) NHCO-c-C_3H_5, $CHCl_2$; (**36**) $CONH_2$, $CHCl_2$; (**19**) NO_2, $CH(C_2H_5)_2$; (**20**) NO_2, $C(CH_3)_3$; (**37**) $NHCONH_2$, $CHCl_2$.

attached to the α carbon might be significant sterically; that is, for functions such as CH_3, CF_3, CCl_3, CBr_3, and $C(CH_3)_3$, $E_{s\text{-}S}$ refers to H, F, Cl, Br, and CH_3, respectively. The remainder of the steric effect is contained in $E_{s\text{-}2l}$ where this refers to ΣE_s for the two larger functions attached to the α carbon atom. In studying activity with respect to variations in R, 19 compounds (compound 7 was not included) where only the 4-NO_2 was present on the ring were considered separately. For this set the eight variables, E_s, log P, $(\log P)^2$, σ^*, $(\sigma^*)^2$, $E_{s\text{-}S}$, $E_{s\text{-}2l}$, and P_E, were considered in all possible linear combinations. This generated a set of 255 equations ($2^n - 1$ where n is the number of variables). Of these, eq 5 had the lowest standard deviation. In this equation the numbers in parentheses are 95% confidence intervals, n is the number of data points used in deriving the equation, r is the correlation coefficient, and s is the standard deviation. The optimum partition coefficient for a drug in this series is defined by setting $\partial \log k/\partial \log P = 0$ and solving for log P. This parameter is defined as log P_0. The confidence intervals on log P_0 indicate that there is some uncertainty about the ideal degree of lipophilicity for the side chain. This uncertainty is less than the lipophilicity of a CH_3 group ($\pi = 0.50$). The coefficient with $(\log P)^2$ is unusually large. The hundreds of examples in our present data bank that correlate "nonspecific" types of biological activity have coefficients with the $(\log P)^2$ term that are normally in the range 0.1-0.4. Equation 5 indicates that activity rises rapidly as lipophilic character increases and then falls rapidly after log P_0 is reached. This suggests that the lipophilic character of the side chain may induce a conformational change in the receptor which favors inhibition up to the point of log P_0. This effect would, of course, be superimposed on the role of relative hydrophobicity in drug movement to the site of action.

	n	r	s	
$\log k = -0.942\ (\pm 0.42)\ (\log P)^2 + 1.757\ (\pm 0.85) \log P + 0.623\ (\pm 0.22)\ \sigma^* - 0.0490\ (\pm 0.04)\ P_E + 0.595\ (\pm 0.56)$	19	0.925	0.369	(5)
$\log P_0 = 0.93\ (0.65\text{-}1.27)$				
$F_{4,14} = 20.9$				
$F_{4,14;\,\alpha\ 0.005} = 6.0$				

Table II. Predicted and Experimental Apparent Generation Rate Constants, k_{app} in sec^{-1}, for the Action of Combinations of Modified Chloramphenicols on *E. coli* at 37.5° in Peptone Broth USP

Concn (μg/ml) of modified chloramphenicols[a] in cultures								$10^5 k_{app}$	
A	B	C	D	E	F	G	H	Predicted[b]	Exptl
									61.5[c]
0.4									48.8
0.2	5.55								51.8
0.2		3.65							49.9
0.2			2.45						51.4
0.2				65.5				49.0	49.8
0.2					1.65				51.3
0.2						4.20			48.6
0.2							14.2		48.7
1.2									24.5
0.6	16.15								24.3
0.6		10.4							24.9
0.6			7.0						24.2
0.6				190					24.3
0.6					5.1			24.0	23.9
0.6						12.1			23.8
0.6							41.2		24.1
0.3	8.0	5.0	3.5						24.2
0.3			3.5	95	2.6				24.0
0.3					2.6	6.0	20.6		22.9
0.3	8.0	5.0					20.6		

[a] The chloramphenicols chosen were modified at the acyl side chain. The assigned symbols, the acyl side chain substituent they represent, and the obtained $10^5 k$ in ml/μg/sec values for that substituent were: A (chloramphenicol), $-CHCl_2$, 31.2; B, $-CH_3$, 1.18; C, $-C_3H_7$, 1.83; D, $-CH(CH_3)_2$, 2.68; E, $-C(CH_3)_3$,, 0.10; F, $-CF_3$, 3.67; G, $-CCl_3$, 1.57; H, $-CN$, 0.45. The k values given here for the $-CF_3$ substituent were determined on an impure sample and are not representative of the final values determined on the completely pure and characterized compound. This impure sample was used in the combinations. [b] The predicted $k_{app} = k_0 - \Sigma k_i c_i$ where k_0 is the generation rate constant in the absence of any chloramphenicol and the k_i is the specific inhibitory constant for the ith concentration of a particular chloramphenicol. For example, $k_{app} = k_0 - k_A c_A - k_B c_B - k_C c_C - k_D c_D$ where the k_A, k_B, k_C, and k_D are the specific inhibitory rate constants, k, given in the above footnote for the stated modified chloramphenicols and the c_A, c_B, c_C, and c_D are the respective concentrations of these chloramphenicols in μg/ml. [c] k_0 in sec^{-1}, the apparent generation rate constant for *E. coli* in the absence of drug.

Since the steric-polarizability nature of R as modeled by P_E seemed more important than log P, P_E and $(P_E)^2$ were substituted for log P and $(\log P)^2$ and all combinations examined as outlined above. Optimizing P_E in this way did not yield correlations that were as good.

The positive coefficient with σ^* shows that electron withdrawal by R increases the inhibitory power of the drug. The negative coefficient with P_E is more difficult to interpret. P_E is closely related to the volume of the substituent. It also is a measure of the firmness with which the electrons in the substituent are held. The negative coefficient with this term may be a correction factor on the $(\log P)^2$ and log P terms (see Discussion). The best three-variable result was eq 6 and, dropping the σ^* term from eq 6 yields eq 7. The low correlation with eq 7 (about 16% of the variance in log k is rationalized by eq 7) reveals that activity in the side chain is not heavily dependent on hydrophobic character. Considering the variables one at a time, σ^* and P_E are the most important. Each of these linear relations accounts for about 30% of the variance. However, r^2 for the relationship between P_E and σ^* is 0.000 so that the sets under consideration are truly independent. The electronic and steric character of the side chain is most important. Log P_0 from eq 7 is 0.73; however, the correlation is so

	n	r	s	
$\log k = -1.063\ (\pm 0.49)\ (\log P)^2 + 1.429\ (\pm 0.97)\ \log P + 0.737\ (\pm 0.25)\ \sigma^* + 0.144\ (\pm 0.53)$ $\log P_0 = 0.67\ (0.37\text{-}0.85)$	19	0.881	0.444	(6)
$\log k = -0.630\ (\pm 0.86)\ (\log P)^2 + 0.919\ (\pm 1.8)\ \log P + 0.777\ (\pm 0.90)$ $\log P_0 = 0.73\ (\pm\infty)$	19	0.412	0.829	(7)
$\log k = -0.339\ (\pm 0.24)\ (\log P)^2 + 1.131\ (\pm 0.40)\ \log P + 1.072\ (\pm 0.84)\ \sigma^2 + 0.022\ (\pm 0.05)\ P_E + 0.071\ (\pm 0.44)$ $\log P_0 = 1.67\ (1.2\text{-}3.8)$	16	0.936	0.270	(8)
$\log k = -0.468\ (\pm 0.26)\ (\log P)^2 + 1.256\ (\pm 0.48)\ \log P + 0.039\ (\pm 0.05)\ P_E + 0.236\ (\pm 0.53)$ $\log P_0 = 1.3\ (1.1\text{-}2.1)$	16	0.888	0.338	(9)
$\log k = -0.323\ (\pm 0.18)\ (\log P)^2 + 1.048\ (\pm 0.40)\ \log P + 0.566\ (\pm 0.27)$ $\log P_0 = 1.62\ (1.3\text{-}2.6)$	16	0.863	0.356	(10)

poor that confidence limits cannot be placed on this figure.

The examples of Table I where R is constant ($CHCl_2$) and functions (X) on the ring vary were treated as a group. For this group of 16 drugs (compounds **34** and **35** were not included) all combinations of the variables σ, σ^2, σ^+, $\log P$, $(\log P)^2$, and P_E yielded a set of 63 equations. The following (eq 8-10) are the most significant. Of the 63 equations, eq 8 had the lowest standard deviation of all equations having up to four variable terms and all terms significant as judged by the stepwise application of the F test at the $\alpha = 0.1$ level. Equation 10 accounts for 75% of the variance in the data, while eq 8 accounts for a total of 88%. Viewing the variables one at a time, the most important single-variable equation is that in $\log P$ ($r^2 = 0.45$); the most important two-variable equation is eq 10. Thus, the most important property of 4 substituents is their hydrophobic character. The least influential property is σ^2. Since our earlier work indicated that the important character of 4 substituents was their ability to delocalize an odd electron, it is not surprising that we cannot find high significance for σ^2. Replacing σ^2 with the two terms σ and σ^+ does not yield as good a correlation ($r = 0.929$).

It is not unexpected that σ^2 is significant in the present study. Cammarata and Yau[16,17] have shown that σ^2 correlates well with parameters for radical reactions. It seems likely that σ^2 is mimicking E_R. It is well known[18] that almost all substituents appear to delocalize radical electrons better than hydrogen.

One of the interesting differences between substituent effects in the side chain and those in the para position on the ring is their hydrophobic effect. $\log P_0$ for the set of side-chain modifications is defined by eq 5 as 0.93 (0.6-1.3) while the best definition of $\log P_0$ for ring substituents is given by eq 9 as 1.3 (1.1-2.1). Equation 8 gives a higher value of 1.7, but the 95% confidence intervals are not as tightly set by eq 8 as by eq 9. In any case, $\log P_0$ is about 0.5 higher for the para derivatives and one must consider the reason for this. The hydrophobic property of a drug is critical in the movement of the drug from the site of application to the receptor and again in the binding of the drug to receptor. This can be expressed[19] by the following equation:

$$\log 1/C = -k_1(\log P)^2 + k_2 \log P + k_3 \log P + k_4\sigma + k_5 \quad (11)$$

The first two terms on the right of eq 11 are associated with the random walk of the drug molecule to the receptor site and the third term models the role of hydrophobic character in the interaction of drug and receptor. We cannot derive eq 11 directly; what we obtain is the result where k_3 and k_4 have been combined. In fact, the situation is probably more complex than indicated by eq 11. The interaction of a set of drugs with a receptor is not indefinitely linearly dependent on $\log P$. It is reasonable to assume that the two different $\log P_0$ values reflect different values for k_3 of eq 11 and that functions in the 4 position have higher values for k_3 because of stronger hydrophobic interactions with the receptor. The most important single-variable equation for the side chain derivatives is the one in σ^* ($r = 0.553$); this is a better correlation than eq 7. This also indicates a less important role for the hydrophobic character of the side chain.

Discussion

The object of this study was to obtain insight into the structure-activity relationship which would permit the design of better derivatives. What useful information can be gained from the above regression studies? Turning first to variations in the side chain, it is clear that electron withdrawal is a most desirable property and that the hydrophobic character of the substituents is not as important. Bulky substituents are undesirable, as is indicated by the negative coefficient with the P_E term. Since E_s has no role, the bulk effect is not directed toward the adjacent carbonyl function. The steric effect can be better rationalized on the basis that it is intermolecularly rather than intramolecularly oriented. The lack of importance of E_s would indicate that the carbon of the carbonyl group is not involved in the binding of chloramphenicols to the active site. To what end is the prominent electron withdrawal of R directed? It would certainly affect the acidity of the amide nitrogen proton. From this point on in the side chain the inductive effect would fall off rapidly. The field effect of R could of course electronically influence the other oxygen atoms in the side chain or the benzene ring. The fact that the use of a term in $(\sigma^*)^2$ for R did not result in improved correlations suggests that more activity can be obtained by the use of stronger electron-withdrawing groups on the amino nitrogen. The size of R should be kept to a minimum.

The lower than expected activity for compound **7** where $R = CHF_2$ deserves comment in view of the fact that other cases where R contains halogens are well predicted. The fact that derivatives where R = CH_2F, CH_2Cl, CH_2Br, and CH_2I are all well predicted along with cases where R = CH_3 and $CH_2CH_2CH_3$ would indicate that significant loss of the halogen compounds by side reactions with nucleophiles is not occurring. It may be that the H in CHF_2 is so activated by electron withdrawal by F and so unhindered sterically by the small F atoms that metabolic dehydrohalogenation occurs with this function.

Hydrophobic substituents in the 4 position are beneficial up to $\log P_0$ of about 1.3-1.5. At least this holds for *E. coli in vitro*. Other systems would no doubt set other limits on this parameter.

The results in this paper should not be taken as invalidating our earlier finding that an important role of the 4 substituent is delocalization of a radical electron resulting from removal

of an α H. Further support for this idea comes from the work of Kutter and Machleidt[20] who synthesized α-deuteriochloramphenicol. Garrett found this derivative to have only 80% of the activity of the natural isomer. This isotope effect points to the chemical reaction of an α H being critical in action of chloramphenicols on bacterial generation. The fact that σ^2 appears to be important in correlating electronic effects of X supports the idea of radical delocalization. It is well known[18] that the introduction of almost any substituent increases the rate of radical attachment on aromatic rings. For example, CN and $N(CH_3)_2$ have opposite signs for σ, although both have E_R values of 0.24.

In our earlier analysis[9] based on eight derivatives it appeared that hydrophobic effects of 4 substituents were less important than electronic effects. For the present analysis using 16 derivatives and experimentally determined (rather than calculated) log P values, hydrophobic effects appear to be more important. We do not wish to leave the reader with the impression that electronic effects of 4 substituents are of little importance. For example, compare the activity of 4-NO_2 and 4-H. For these two compounds there is only 0.21 difference in log P values; yet, there is a difference of 1.20 in log k. The nitro derivative is about 16 times as active. While this activity could be the result of some unique property of the NO_2 function (*e.g.*, sensitivity to reduction), it seems more likely that this derivative acts in qualitatively the same fashion as the others. It is felt that proper electronic parameters will eventually establish the importance of this effect of 4 substituents.

Although the present set of compounds was selected mainly to formulate correlation equations for future work, it is of interest that for the first time we have found a derivative more active than the natural drug. Compound **1** of Table I with the side chain of CF_3 is 1.7 times as active as chloramphenicol. This is no doubt the ideal acyl moiety for the side chain, at least as far as can be ascertained from *in vitro* work. To obtain more potent drugs, one would want to start with this side chain and introduce into the 4 position good radical-delocalizing functions whose values would not push log P for the drug beyond about 1.5. Unfortunately, since so little is known in quantitative terms about the radical-delocalizing ability of various functions, one will have to proceed intuitively or *via* MO calculations in the selection of suitable substituents. One point favoring the search for better 4 substituents is that the 4-phenyl derivative (compound **32**) is well predicted by either eq 8 or 10. This indicates that its relatively low activity is the result of excessive lipophilicity rather than steric character.

Since the 4-phenyl derivative was well predicted, it was decided to make the 4-pyridyl analog (compound **34** of Table I). This derivative has a better log P value and it was felt that it would be as good a radical delocalizer as phenyl.[18] Its low activity is surprising. The reason for such low activity is not apparent to us.

The hydrophobic and steric effects deduced above are in line with our earlier[7] analysis of data from Shemyakin. Using his data for a different set of derivatives, we found a π_0 for *E. coli* to be 0.2. When this is added to the measured value of 0.94 for the parent compound, a log P_0 of 1.14 results for this study. This is close to the value found in eq 8. In Shemyakin's study the largest function in the 4 position was $C_6H_5N{=}N{-}$. Since it was well predicted, this also indicates the lack of steric sensitivity to rather large functions.

Equations 8-10 suggest a definite role for polarizability of substituents in the 4 position although it is small compared to hydrophobic character. This conclusion has also been reached by Cammarata.[21] It is not possible to compare his results with ours since he employed adjusted P_E values.[9]

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