(a) The product (IV, R = OH) was suspended in H_2O and acidified with dilute acetic acid; the solid was washed with hot H_2O, boiled with CH_3OH, dissolved in 2% aqueous–alcoholic NaOH, and precipitated on cooling as the sodium salt; after crystallization from CH_3OH, the product was acidified with dilute acetic acid and boiled with H_2O.

(b) The crude product (V, R = OH) was suspended in H_2O, neutralized with dilute acetic acid, and recrystallized from pyridine or N-methylformamide.

Sodium salts of IV were obtained in 2% H_2O-alcohol solution of NaOH and recrystallized from alcohol; they crystallize with 1 mole of alcohol.

Hydrochlorides of IV were prepared in hot concentrated HCl, washed with absolute ether and dried *in vacuo;* they lose HCl on heating.

1-Aryl-3-(4,6-dimethyl-2-pyrimidyl)ureas (IV, Y = O; R = CH_3) and 1-Aryl-3-(4,6-dimethyl-2-pyrimidyl)guanidines (V, Y = NH; R = CH_3).—Both compounds of type IV and V were prepared according to methods A and B using acetylacetone instead of ethyl acetoacetate and recrystallized from acetone–ethanol solution, 1-butanol, or pyridine.

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An Apparent Correlation between the *in Vitro* Activity of Chloramphenicol Analogs and Electronic Polarizability[1]

ARTHUR CAMMARATA

Department of Chemistry and Pharmaceutical Chemistry, Medical College of Virginia, Richmond, Virginia 23219

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An apparent correlation between the activity of chloramphenicol analogs, as determined by microbial kinetics, and the electronic polarizability of their aromatic substituents has been found which suggests the activity of chloramphenicol and its thiomethyl analog may arise, in part, by intramolecular charge transfer.

Recent attempts at correlating the biological activity of chloramphenicol analogs by means of the Hansch equation[2] suggest that the correlation, or lack of correlation, obtained by this equation depends markedly on the accuracy of the method used to evaluate biological activity. Hansch and associates[1a,2a] reported a fairly good correlation (correlation coefficient, $r = 0.824$; *Escherichia coli*) for chloramphenicol analogs whose activities were determined by a serial dilution method.[3] In contrast, Garrett and co-workers[4] were unable to correlate many of the same chloramphenicol analogs studied by the Hansch group when their activities were determined by a more accurate kinetic method.

We wish to present an apparent correlation between the activity of chloramphenicol analogs, as determined by microbial kinetics,[4] and the electronic polarizability of their aromatic substituents. In light of this new correlation, it appears that a Hansch treatment can provide a fairly good, but not necessarily significant, correlation for chloramphenicols whose activities are determined by kinetic methods, provided the limits imposed by the parameters employed in this treatment are not exceeded.

Results and Discussion

The molar electronic polarizability of a substance is given by the Lorentz–Lorenz equation[5] as follows where

$$P_E = \frac{n^2 - 1}{n^2 + 2}\frac{M}{D} = \frac{4}{3}\pi N\alpha_E$$

n is the refractive index of the substance, M is its molecular weight, D is its density, N is Avogadro's number, and α_E is the electronic polarizability. A useful property of molar electronic polarizability, alternatively known as molar refraction, is its additivity, *i.e.*, the molar refraction of a substance may be represented as the sum of atomic or group refractions.[5b] Further, since electronic polarizability is expressed in units of volume, molar, atomic, or group refractions are a measure of molar, atomic, or group volumes, respectively.

When Fisher–Hirschfelder–Taylor models are made of the substituted benzenes corresponding to the aromatic nucleus of chloramphenicol analogs, it is noted that the activity of a chloramphenicol appears proportional to the volume which its aromatic substituent presents to a surface. Using atomic and group refractions[5b] as a measure of this volume, an excellent linear correlation is obtained with the inhibition constants[4] of all chloramphenicols except chloramphenicol itself and its thiomethyl analog (Table I). The correlation which is obtained, while empirical in origin, does have some theoretical justification.[6]

From a consideration of the partition function for a population of electrically uncharged molecules confronted with both an electrically conducting surface and an adjacent solution, Agin, *et al.*,[6] derived the equation

$$\ln C_s = K'\alpha_E + \ln C^*$$

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TABLE I

CORRELATION OF CHLORAMPHENICOL ACTIVITY WITH ELECTRONIC POLARIZABILITY

$k_1 = 2.76P_E - 6.55$ ($r^a = 0.991$)

No.	Substituent	P_E, ml[b]	k_1 Obsd[c]	k_1 Calcd	Δk_1
1	p-NH_2	3.66	3.16	3.55	−0.39
2	p-SO_2CH_3	4.90[d]	5.60	6.97	−1.37
3	p-i-C_3H_7	6.32[e]	10.7	10.9	−0.2
4	p-Cl	6.53	11.2	11.5	−0.3
5	p-OCH_3	7.83	16.4	15.0	1.4
6	m-NO_2	8.37	19.0	16.5	2.5
7	p-Br	9.37	19.0	19.3	−0.3
8	p-I	14.55	32.6	33.6	−1.0
9	p-SCH_2^-	19.52	51.5	47.3	4.2
	p-SCH_3	11.72	51.5	...	...
10	p-NO_2	8.37	90.0	...	...

[a] The correlation coefficient for the fit cited. [b] Values obtained from tables and text of ref 5b. [c] Determined from the slope of a plot of the observed generation rate constants for *E. coli*, k (in sec^{-1}), against the chloramphenicol concentration, *i.e.*, $k = k_0 - k_1[I]$, where k_0 (in sec^{-1}) is the rate constant in the absence of antibiotic and k_1 (in l. $mole^{-1}$ sec^{-1}) is the inhibition rate constant for the D isomer of the chloramphenicol analog.[3] [d] Value for SO_2. [e] Value for a CH_3 group.

where C_s is the administered concentration, *e.g.*, minimum inhibitory concentration, and C^* is the concentration at the surface. The constant K' is assumed in the derivation to be represented by

$$K' = (E_L - E_C)/RT$$

where E_L is the induced dipole–induced dipole interaction energy and E_C is the interaction energy of a conducting surface for a neutral molecule. These energies are a function of distances separating the compound from the surface and of the ionization potentials of the compound and the surface. As a reasonable first approximation, the distances involved and the ionization potentials may be assumed constant.[7] Replacing the concentration terms by their rate equivalent[4] at unit time and substituting the expression for molar electronic polarizability affords an expression of the form found in this study

$$k_1 = KP_E + k^*$$

where now

$$K = 3K'/4\pi N$$

To correlate the activity of the thiomethyl analog it is necessary to use the electronic polarizability of a negatively charged thiomethyl group (P_E = 19.52 ml).[8] This suggests the neutral thiomethyl analog (P_E = 11.72 ml) may be undergoing a charge-transfer interaction which increases its inherent activity by increasing the polarizability of the thiomethyl group. A similar interaction may also, in part, account for the exceptionally high activity of chloramphenicol itself relative to the electronic polarizability of the "neutral" nitro group (P_E = 8.37 ml). Unfortunately, no refractivity data for the nitrite ion seem available for comparison. In this regard, it is perhaps significant to note that evidence has been presented which strongly suggests that nitrobenzene is capable of undergoing *intramolecular* charge transfer upon interacting with a hydrogen-bonding surface (silica gel).[9]

Since charge-transfer interactions are implied by the above correlation, an attempt was made to incorporate into the Hansch equation parameters which have been demonstrated to correlate donor–acceptor pairs in chemical systems.[10] This may be done by separating the Hammett substituent constant σ into its constituent inductive and resonance parameters,[11] σ_I and σ_R, respectively, and treating these as independent parameters in a modified Hansch equation.

$$\log A = a\pi^2 + b\pi + c\sigma_I + d\sigma_R + f$$

When the chloramphenicols are treated using this modified equation, none of the usual statistical tests indicate a correlation (Table II). If, however, it is assumed that the thiomethyl analog of chloramphenicol and chloramphenicol itself undergo an intramolecular charge transfer, and these compounds are omitted in a subsequent treatment, a fairly good correlation is obtained for most of the equations and π is indicated as contributing significantly (Table III). Unfortunately, an F test on the correlations indicates the points are widely scattered in the regression plane, thus no definitive statement can be made concerning any of the correlations obtained.

Conclusions.—The correlation of chloramphenicol activity with electronic polarizability observed in this study tends to support the suggestion that chloramphenicols may owe their bacteriostatic activity to binding at a protein, possibly a ribosomal, surface.[12] For most chloramphenicol analogs, secondary or van der Waals forces appear sufficient to describe the binding of the aromatic moiety. More specifically, dispersion and/or inductive forces are implied, since these attractive forces are directly related to electronic polarizability.[5] In the cases of chloramphenicol and its thiomethyl analog, binding could be enhanced through charge transfer, possibly of an intramolecular nature.

Since electronic polarizability is also a measure of volume, the observed correlation does not exclude the possibility that bulk effects also may lead to variations in activity. That is, a substituent, by virtue of its bulk, can modify the structure of the protein to which it is bound and thereby change the nature of substrate sites which are in the environs of the chloramphenicol or elsewhere on the protein. Those chloramphenicols which may function as a charge-transfer species would be expected to perturb the structure of a protein even more than would simple bulk or polarizability effects. In these cases, the correlation with polarizability could serve only as a good first approximation.

The attempts to correlate chloramphenicol activity by the Hansch equation, or the modified forms used in this study, point out some limitations of this approach.

(7) Inspection of the ionization potentials reported in ref 6 for a number of structurally different molecules suggests this assumption is not unreasonable as a first approximation.

(8) A referee suggested that biological oxidation of the thiomethyl group to either a methyl sulfoxide group or a methyl sulfone group could account for the divergence in activity. It must be pointed out that the thiomethyl analog is the most active of the analogs, whereas the methyl sulfone analog is one of the least active. Thus it would appear that a process other than oxidation must be taking place.

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TABLE II

ATTEMPTED CORRELATION OF CHLORAMPHENICOL ACTIVITY BY HANSCH TECHNIQUE

n[a]	log k_1[b,c] =				F[d]	r[e]
10	$-0.322\pi^2$ + 0.364π + 0.023σ			+1.326	2.409	0.739
	(−1.484) (2.136) (0.073)					
10	$-0.309\pi^2$ + 0.395π		+$0.252\sigma_I$ − $0.110\sigma_R$	+1.190	1.536	0.742
	(−1.277) (1.746)		(0.246) (−0.166)			
10		0.237π + 0.320σ		+1.083	2.143	0.616
		(1.485) (1.168)				
10		0.315π	+$0.793\sigma_I$ + $0.002\sigma_R$	+0.808	1.362	0.636
		(1.378)	(0.810) (0.003)			
10		0.316π	+$0.795\sigma_I$	+0.807	2.383	0.636
		(1.996)	(1.312)			
10		0.203π	+ $0.418\sigma_R$	+1.239	1.804	0.583
		(1.144)	(0.928)			
10			$-0.026\sigma_I$ + $0.636\sigma_R$	+1.367	0.968	0.465
			(−0.031) (1.158)			

[a] The number of compounds treated in each equation. [b] k_1 values are given in Table I. The values for π and for σ were obtained from ref 3. The values for σ_I were obtained from M. Charton, *J. Org. Chem.*, **29**, 1222 (1964), and were used to calculate σ_R by the equation $\sigma_R = \sigma - \sigma_I$.[7] [c] The quantity in parenthesis below each variable is the significance, as given by t test, that may be associated with that variable in the over-all correlation. [d] F test for the significance of the correlation. [e] The correlation coefficient for the fit cited.

TABLE III

ATTEMPTED CORRELATION OF CHLORAMPHENICOL ACTIVITY BY HANSCH TECHNIQUE

n[a]	log k_1[b,c] =				F[d]	r[e]
8	$-0.191\pi^2$ + 0.327π − 0.038σ			+1.133	3.250	0.842
	(−1.217) (2.746) (−0.158)					
8	$-0.152\pi^2$ + 0.388π		+$0.475\sigma_I$ − $0.319\sigma_R$	+0.821	2.405	0.873
	(−0.887) (2.680)		(0.703) (−0.748)			
8		0.245π + 0.150σ		+0.972	3.770	0.775
		(2.386) (0.763)				
8		0.346π	+$0.769\sigma_1$ − $0.267\sigma_R$	+0.609	3.110	0.836
		(2.599)	(1.343) (−0.651)			
8		0.288π	+$0.515\sigma_I$	+0.786	5.033	0.817
		(3.099)	(1.306)			
8		0.244π	+ $0.108\sigma_R$	+1.019	3.241	0.751
		(2.071)	(0.334)			
			$-0.078\sigma_I$ + $0.449\sigma_R$	+1.212	0.598	0.439
			(−0.114) (1.004)			

[a] The number of compounds treated in each equation. In these treatments, chloramphenicol and its thiomethyl analog were omitted. [b-e] See corresponding footnotes in Table II.

One obvious limitation is the result of a change in the nature of the substituent, in which case, the substituent constants employed in the equation for the compound administered are not the constants for the compound actually affording the biological response. Possible examples are provided by chloramphenicol and its thiomethyl analog. To include these compounds in a Hansch treatment would appear to require the constants for a negatively charged thiomethyl group and probably a negatively charged nitro group.

An additional limitation appears to depend on the range of values covered by π. Hansch and associates[1a] found a dependence on π for chloramphenicols whose activities were determined by the less accurate serial dilution method. In this study, we again find π to contribute significantly to the correlation for chloramphenicol analogs whose activities were determined by a more accurate kinetic technique.[4] However, the F test on these data designates the correlations obtained as insignificant. It would appear then that π is more heavily weighted in this type of regression analysis, which implies that correlations with π should be interpreted with caution.[13] This is substantiated by other systems we have studied. In these systems reasonably accurate activity data provide a correlation with only σ; but, if data are used which are known to be unreliable, a correlation with π can be obtained.

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(13) It is not intended that these observations be interpreted as implying that π is a useful constant for correlating random error. Rather, the intent is to point out that misleading information may at times be gained through this approach.