Homolytic Constants in the Correlation of Chloramphenicol Structure with Activity

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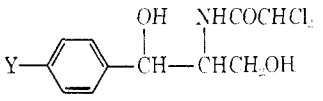
Received February 27, 1969

Homolytic constants are found to give a better structure–activity correlation for chloramphenicol derivatives than either the usual Hammett constant or polarizability constants. Evidence is presented indicating that a variety of aromatic methanol derivatives which are active as drugs are involved in interference at the molecular level with free-radical enzymes.

There has been considerable interest in structure–activity studies of chloramphenicol derivatives because of the particular effectiveness of the parent compound in fighting infections caused by gram-negative bacteria which are resistant to many other antibiotics.[2] Except for structural variations in the acylamino group, variation in the side chain of chloramphenicol results in a large decrease in activity.[3-5] On the other hand, variations in the *para* position of the aryl ring seem to be possible without such great loss in activity.[3] For this reason we shall be primarily concerned with variations in Y of structure I. The first quantitative correlation[6] of variations of I confirmed the qualitative observation of Shemyakin that electron withdrawal by substituents increased activity. Equation 1 for *Escherichia coli*, although not a very high correlation, showed[6] that activity depended on both electronic and hydrophobic properties of the substituent. In eq 1, σ_m represents the



I

	n	r	s	
$\log BR = -0.74\pi^2 + 0.36\pi + 1.82\sigma_m + 0.62$	10	0.824	0.555	(1)

electronic effect of the substituent on the *ortho* position. The σ_m constants were employed because they yielded a slightly better correlation than σ_p. For eq 1, n represents the number of data points, r is the correlation coefficient, and s is the standard deviation.

Using a much more accurate method of assay, termed *microbial kinetic*, Garrett, *et al.*, reinvestigated a set of chloramphenicols using *E. coli*.[7] Garrett's choice of substituents gave a better spread of electronic values although not as great a range of hydrophobic character as Shemyakin's. His data, as well as physicochemical constants of interest, are given in Table I. Garrett's attempt to use eq 1 to rationalize his results gave poor correlations.

Cammarata[8a] has analyzed Garrett's results using electronic polarizability (P_E) to explain the variance in the data. Agin, *et al.*, have shown this to be a significant parameter in structure–activity studies.[9] Although Cammarata showed a highly significant linear relationship between activity and P_E, his results are compromised because of a variety of assumptions he found necessary. Most significant, in order to obtain a good correlation, the most active compound, chloramphenicol itself, was omitted. A special postulate was necessary to include SCH_3, and the P_E values assumed for SO_2CH_3 and $CH(CH_3)_2$ are quite different from those normally employed. The values used by Cammarata, along with those we have calculated according to Bauer and Lewin,[10] are given in Table I. Cammarata[8b] assumed that the substituent portion of the aromatic moiety of chloramphenicol would come into contact with a flat surface. He further assumed that only the portion of the substituent in contact with the surface could contribute in a binding interaction unless the possibility existed for free rotation about the C_{AR}–X bond. He reasoned that the *i*-Pr group is less susceptible to steric factors when it is perpendicular to the plane of the aromatic ring. In this configuration only one methyl group would come into contact with the flat surface and P_E for a single methyl was employed. He also assumed that only one S–O bond would come into contact with the surface and therefore used P_E for a single S–O rather than that for a methyl group and two S–O bonds for CH_3SO_2. He considered it likely that the CH_3SO_2 cannot rotate freely because of resonance interaction with the aromatic ring. Using the normal values for P_E in a linear relation of the type employed by Cammarata, we obtain eq 2. In eq 2, the methyl-

	n	r	s	
$k_I = 0.296P_E + 10.960$	7	0.145	10.69	(2)

mercapto and 4-NO_2 functions are not included. Including these gives an even poorer correlation (r = 0.088). Substituting log K_I in eq 2 gives no better result.

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TABLE I
STRUCTURE-ACTIVITY PARAMETERS FOR CHLORAMPHENICOL

Function	P_E[a]	P_E[b]	E_R[c]	σ_p[d]	π[e]	Log A Obsd[f]	Log A Calcd[g]	Δ log A
SO_2CH_3	4.90	17.54	0.12	0.73	−1.26	0.80	0.851	0.05
I	14.55	13.90	0.12	0.28	1.26	1.56	1.423	0.14
$CH(CH_3)_2$	6.32	14.96	0.03	−0.15	1.40	1.08	1.179	0.10
Cl	6.53	5.96	0.10	0.23	0.70	1.10	1.234	0.13
OCH_3	7.83	7.36	0.11	−0.27	−0.04	1.26	1.097	0.16
Br	9.37	8.86	0.12	0.23	1.02	1.32	1.368	0.05
NO_2	8.37	7.30	0.41	0.78	0.24	2.00	2.081	0.08
SCH_3	11.72	13.69	0.24	−0.05	0.62	1.76	1.646	0.11
NH_2	3.66	5.41	...	−0.66	−1.63	0.55	...	...
3-NO_2	8.37	7.30	0.35	0.71	0.11	1.32	...	...

[a] From ref 8. [b] From ref 10. [c] From ref 14 and 15. [d] From H. Jaffé, *Chem. Rev.*, **53**, 191 (1953). [e] From the phenoxyacetic acid system. [f] From ref 7. [g] Calculated using eq 6.

Recent success[11-13] with homolytic substituent constants prompted us to consider these in the chloramphenicol problem. Two types of homolytic constants have proved of use. Using the results of Hey and Williams on homolytic aryl phenylation, $\sigma^{\cdot}$, analogous to σ, was formulated.[11] The parameter E_R of Yamamoto and Otsu[14] has also been shown to be valid for biochemical systems.[11-13] For the present work we have used E_R since more values of this parameter are available. Even so, two important values of E_R were lacking, p-SCH_3 and p-SO_2CH_3. Otsu, *et al.*, have shown[15] that there is a linear relation between E_R values and the Q parameter formulated by Price[16] for radical reactions. Using the Q values obtained for the copolymerization characteristics of substituted vinyl derivatives and E_R values of Table II, eq 3 was derived *via* the method of

$$E_R = 0.428Q + 0.091 \qquad n = 4 \quad r = 0.986 \quad s = 0.014 \qquad (3)$$

least squares. From eq 3 and the Q values of p-SCH_3 (0.34) and SO_2CH_3 (0.07) of Price and Oae[17] are calculated the E_R values in Table I. Unfortunately, no E_R or Q value is available for the NH_2 function.

TABLE II
E_R AND Q VALUES USED IN DERIVING EQUATION 3

Function	Q[a]	E_R[b]	E_R[c]	ΔE_R
OCH_3	0.015	0.11	0.097	0.013
Cl	0.028	0.10	0.103	−0.003
Br	0.10	0.12	0.134	−0.014
CN	0.34	0.24[d]	0.236	0.004

[a] See ref 16 and R. G. Fordyce, G. E. Ham, and E. C. Chapin, *J. Am. Chem. Soc.*, **70**, 2483 (1948). [b] See ref 14. [c] Calculated using eq 3. [d] The value of 0.24 for CN has been employed rather than the earlier reported value of 0.52 [T. Yamamoto, *Bull. Chem. Soc. Japan*, **40**, 642 (1967)]. The value of 0.24 is a corrected value which has been found to give correlations in a variety of systems.[14]

From the data in Table I are derived eq 4–8. Comparing the two single variable equations (4 and 5), one

$$\log A = [2.744\ (\pm 1.9)]E_R + 0.931\ (\pm 0.37) \qquad n = 8 \quad r = 0.820 \quad s = 0.243 \qquad (4)$$

$$\log A = [0.145\ (\pm 0.43)]\pi + 1.289\ (\pm 0.41) \qquad n = 8 \quad r = 0.317 \quad s = 0.403 \qquad (5)$$

$$\log A = [3.069\ (\pm 1.2)]E_R + [0.227\ (\pm 0.16)]\pi + 0.769\ (\pm 0.25) \qquad n = 8 \quad r = 0.954 \quad s = 0.140 \qquad (6)$$

$$\log A = [3.419\ (\pm 1.3)]E_R - [0.235\ (\pm 0.46)]\sigma + [0.187\ (\pm 0.18)]\pi + 0.786\ (\pm 0.25) \qquad n = 8 \quad r = 0.970 \quad s = 0.127 \qquad (7)$$

$$\log A = 2.865E_R - 0.053\pi^2 + 0.231\pi + 0.846 \qquad n = 8 \quad r = 0.957 \quad s = 0.151 \qquad (8)$$

sees that the electronic parameter is much more important than the hydrophobic. While neither correlation is high, the linear combination of the two gives an excellent result in eq 6. An F test indicates that eq 6 is significant at the 0.995 level. Addition of a term in π^2 to eq 6 does not result in an improved correlation (eq 8). From this it is concluded that only substituents of suboptimal lipophilic character were studied. *In vitro* studies[18] on gram-negative bacteria have indicated that ideal lipophilic character (log P_0) for the *in vitro* test is near 4. The most lipophilic compound in Table I is the iodo derivative having a calculated log P of 2.76 (based on the experimental value of 1.74 for chloramphenicol). It is of interest that although eq 8 is not statistically significant, π_0 calculated from it, when added to log P for the parent molecule, yields a log P_0 of 3.7 for the *in vitro* situation with chloramphenicols. Thus it appears that more *in vitro* activity could be achieved by more lipophilic derivatives. It is unlikely that much more lipophilic compounds would be of use in human therapy[19] because of the *in vivo* and *in vitro* difference in log P_0.

For structure–activity studies in homogeneous systems with simple organic molecules, Yamamoto and Otsu recommended an equation of the form

$$\log k_X = aE_R + b\sigma + c \qquad (9)$$

For the general analysis of substituent effects on homolytic reactions, eq 9 gives better results than E_R alone. For this reason we have derived eq 7. However, this is not a significant improvement over eq 6. Since in certain situations[12] there is a correlation between σ^+ and

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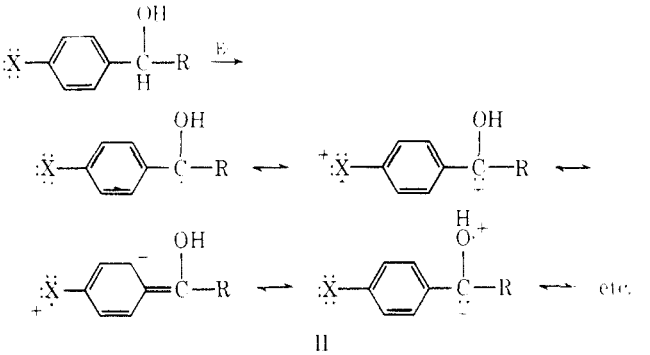
E_R, we also tested σ^+ in an equation of the form of 6. However, this combination gave a much poorer correlation than eq 6 ($r = 0.338$).

While no E_R value is available for NH_2, we can take an estimate of its value and in this way compare it with the observed value in Table I. E_R for $N(CH_3)_2$ is known to be 0.24. If we assume that the difference between $N(CH_3)_2$ and NH_2 in E_R parallels that of σ^+ for the two functions, we obtain an estimated value of E_R for NH_2 of 0.17. Substituting this along with the proper π value in eq 6 yields a calculated value of 0.92 compared with the observed value of 0.55. This is reasonable considering the assumptions involved, and especially considering the fact that the amino group, partly because of its basic character, is known to have a good deal of fluctuation in its σ and π constants.[20] The *m*-nitro derivative was not included because of considerable disagreement about its activity and about the activity of *meta* derivatives in general.[3,21,22] It seems probable that there may be some steric effect of *meta* substituents since the calculated value for 3-NO_2 using eq 6 is 1.86 while the observed value is 1.32.

Chloramphenicol has proved to be a strong inhibitor of protein synthesis. The syntheses of a variety of specific proteins have been shown to be inhibited by concentrations as low as 10 μg/ml. Several hypotheses have been developed to explain the mode of action of this antibiotic in bacterial cells.[2,5,23,24] Of special interest is the recent discovery of Mathison[24] who showed that chloramphenicol does not affect the full cycle of biosynthesis in *E. coli* cells up to the point of cell division. Only after cell division starts does the death rate increase exponentially. He concluded, therefore, that the synthesis of proteins essential in cell division is prevented. In connection with this finding, the paper by Firkin and Linnane[25] on the effect of chloramphenicol on the growth and respiration of mammalian cells deserves attention. They discovered that at concentrations between 10 and 40 μg/ml. the growth of HeLa cells ceased abruptly after two cell divisions. The mitochondria of the cells which had become arrested contained a markedly lower level of cytochromes. In addition, Freeman and Haldar recently reported[26] that the antibiotic and its methylmercapto analog are able to inhibit certain enzymes of the respiratory chain. Our results, taken together with those above, lead us to believe that chloramphenicol and its derivatives inhibit an important free-radical process involved in mitochondria at the critical juncture of cell division. It is well known that several steps of the electron-transport system involve free radicals.[27,28]

How might this occur? One would assume that ring substituents would not affect the side chain significantly beyond the first carbon atom. Thus the critical geometry of the side chain would appear to anchor the antibiotic to its site of action. The inhibitory effect could come about *via* a hydrogen-radical transfer to a mitochondrial enzyme as depicted in II. In II, R stands



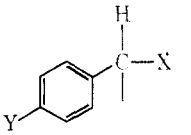
II

for the remainder of the chloramphenicol side chain and E· is an essential free radical or free-radical enzyme. Both the OH of the side chain and X in the *para* position stabilize the radical and thus aid in the loss of H.

The above hypothesis could be tested by preparing and testing the activity of α-deuteriochloramphenicol. The deuterium analog should have a lower activity if the rate-determining step is a deuterium-radical transfer.

The moderate selectivity of the antibiotic[2,25] is also in accord with this mechanism. Although chloramphenicol is quite toxic in general, one of the reasons it has an acceptable therapeutic index may be due to the higher rate of cell division in bacterial cells compared to mammalian cells.

If the above mechanism is correct, then one would expect compounds having the basic moiety of III to be toxic to microorganisms. Moreover, one would expect

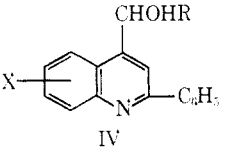


III

the activity changes due to Y to be a function of E_R of Y. Indeed, this appears to be true. It has recently been shown[13] that ring-substituted benzyl alcohols are toxic to *Aspergillus penicillium* and that the toxicity is a function of E_R. The result is shown in an equation analogous to (6).

$$\log (1/C) = 1.987E_R + 0.637 \log P + 0.772 \qquad n = 18 \quad r = 0.968 \quad s = 0.194 \qquad (10)$$

Equation 10 was derived[13] from the experimental work of Carter, *et al.*[29] Other such examples of exceptional toxicity of aromatic methanols are known.[13] One of current interest[30,31] is that of the antimalarials of type IV. The activity of the many known 4-quinolinemeth-



IV

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anols against malaria may also involve interference with biochemical radical processes. The unusual photosensitivity of these compounds could also have an explanation in terms of the ability of the ring system to stabilize a free radical. While attenuation of the radical-stabilizing ability of the ring may decrease photosensitivity, this might also reduce toxicity to the parasites. Quinine itself contains the benzyl alcohol moiety.

If the above picture of the structure–activity relationship in chloramphenicols is correct, some qualitative evidence should be apparent in the activity of some of the other derivatives which have been tested. An interesting example in this connection is that of the 4-phenyl derivative which has been shown to be very active.[32] High activity for this compound is not predicted by electron withdrawal by the phenyl group in terms of σ. However, the phenyl group has been shown to have considerable radical-stabilizing activity.[33]

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A point of great importance is that chloramphenicols inhibit strongly the division of HeLa cells. Since the analysis of this report implicates radicals in the process, it would seem worthwhile to study more lipophilic chloramphenicols as antitumor agents, particularly for rapidly growing tumors.

The results with the chloramphenicols underline the usefulness of the extrathermodynamic approach to medicinal structure–activity problems. They also show the difficulty of untangling substituent effects by means of regression analysis. One must be careful to consider a variety of different electronic and steric effects. In fact, it is quite difficult to know when one has exhausted the possibilities. The chloramphenicol series again points out the utmost importance of having highly precise measurements of biological activity if one is to uncover subtle structural features of importance.

Acknowledgment.—This work was supported by Grant CA-11110 from the National Institutes of Health.

Structure–Activity Relationships among Substrates for a Rabbit Kidney Reductase. Quantum Chemical Calculation of Substituent Parameters

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Received January 13, 1969
Revised Manuscript Received May 12, 1969

All-valence electron calculations have been made for a series of substituted acetophenones which are substrates for a rabbit kidney reductase. Substituent constants based on several different molecular parameters have been derived and compared with the relative substrate efficiency of the compounds in the series. Significant correlations using simple linear models for regression analysis have been obtained for properties as inclusive as the relative total energy differences between the ground-state and incipient-transition-state models of the compounds examined.

The isolation and partial purification of a TPNH-dependent carbonyl reductase from rabbit kidney tissue have been described earlier.[1] It was of interest to us to examine the substituent effects among substituted phenacyl derivatives employed as substrates for this enzyme. Some initial results of correlation attempts using the π–ρ–σ approach have been reported.[2] A relatively impure enzyme preparation was used to obtain V_{max} kinetic data for a series of *meta*- and *para*-substituted acetophenones in that study. A significant correlation was demonstrated for the relationship

$$\log V_{max} = k\pi + \rho\sigma + k'$$

where the π term has the meaning assigned by Hansch and Fujita[3] and the $\rho\sigma$ term is that of Hammett.[4,5]

In the present study, more definitive consideration is given to the reaction mechanism and an effort is made to calculate directly the substituent parameter most logically involved in substituent effects on substrate efficiency.

The reductions of the aromatic carbonyl compounds offer a unique opportunity to examine on a quantum chemical basis the relative effect of substituent variation on (a) molecular properties such as orbital charges at a reactive center in the molecule, (b) frontier orbital energies, and (c) electron density in space near a point of reactant attack. Relative energy relationships which can be approximated by molecular orbital methods may also have application in ranking reactivities.

The experimental results reported here represent the reaction situation wherein the elements of a hydride ion are transferred from TPNH to the substituted acetophenone at its carbonyl carbon atom position (C_c) (Scheme I). Such mechanisms for the reduction of carbonyl compounds have been studied both for DPNH- and TPNH-requiring enzymes.[1,6,7] Thus, it would appear intuitively that intramolecular electronic charge distributions which provide a lowered electron density near the carbonyl carbon atom would directly relate to improved substrate efficiency. The observed positive

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